Pragya Mishra
Raghvendra Raman Mishra
Charles Oluwaseun Adetunji *Editors*

Innovations in Food Technology

Current Perspectives and Future Goals

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*This book is dedicated to the **Vindhya Range of West-Central India,***
oldest part of the world with diverse fauna and flora having
*Goddess of Nature **Vindhyachal Temple**, Windom fall, Sirsi fall and Tanda fall.*

&
***Mahamana Pandit Madan Mohan Malaviya**, founder of Banaras Hindu University, the*

largest residential university in Asia and one of the largest in the world.

Foreword

I am delighted in writing a foreword on this very special achievement of Dr. Pragya Mishra and other editors in the form of this book that contains innovative information about food technology, processing and management. Microbial biodiversity is an ultimate source that may be utilized and applied to modern biology and food biotechnology and has the potential to be developed as innovative and sustainable solutions to a wide range of problems of human beings.

Food technology includes engineering, science and technology where we could evaluate the application of biological organisms, biomolecules, systems and bioprocesses by various industries to learn about the improvement of the value of materials and organisms. This book "Innovations in Food Technology: Current Perspectives and Future Goals" focuses on these aspects suitable for researchers in this field.

I appreciate their organized way of writing that focuses on major areas of food technology. Both national and international scholars have contributed to this book. In Part I: Food Processing and Technology Innovations, Prof. Kifordu comprehensively explains the scenario of food innovation and sustainable development in bioeconomics viewpoint. In Part II: Food and Industrial Microbiology, the work of Dr. Osahon Itohan Roli on shogaol (4, 6, 8, 10 and 12), an active constituent of ginger, opens new avenues of its application in food technology. In this part, Dr. Charles Oluwaseun Adetunji, Juliana Bunmi Adetunji and others unfold the significance of exopolysaccharides, which is very informative in antimicrobial, food and health aspects. Part III: Food Technology and Environmental Biotechnology deals with the current scenario of our society. Dr. Charles Oluwaseun Adetunji and Dr. Osikemekha Anthony Anani have written about *Trichoderma* biofertilizer and its application, which is a novel approach for ecological balance. This is a boon for agriculture production and management of soil-borne and root-borne plant pathogens. Part IV: Agriculture, Food, Nutrition and Health focuses on human welfare and betterment of our society. In this part, Prof. Ajayi, Prof. Chandana Haldar, Prof. Ravi S. Pandey, Dr. O. T. Olaniyan and Dr. G. E. Okotie gave their expert views on the application of food technology in various fields. In addition, the application of bacteriophages in food safety work by Dr. Raghvendra Raman Mishra is opening a new area of research in food industry.

It is my pleasure to endorse Dr. Pragya's experience in food technology and her collaborative interactions with world leaders in this particular field. As a result of this effort as a book, both Dr. Pragya and the institution are enriched and privileged to have an opportunity of working with such international laureates. I am delighted to see that contributors of this book have several years of collaborative experience and have signatory status in food research.

In short, Dr. Pragya's book is unique and surely a work to treasure for anyone who is interested in food technology innovation, research and development. My heartiest congratulations and wishes are always with Dr. Pragya and her team for producing such a nice piece of work that will guide young minds for a long time to come.

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Preface

Since the twentieth century, when five proteins from genetically engineered cells had been approved by the United States Food and Drug Administration (FDA), food technology has grown up to a height where we can say the upcoming decade would be the decade of food technology. The world is faced with the challenge to feed an estimated 9 billion people of the Earth by 2050. Therefore, the safety of food is an important health, social and economic issue that has become the topic of global discussion, particularly from the point of increasing population. Food safety refers to safety measures that should be taken throughout the supply chain, from the farmers/suppliers to the ultimate beneficiaries. At present, food technology has wider application in several areas such as crop production, agriculture, health care, non-edible crops and environmental issues. Considering these facts, we planned to write this book.

This book is a compendium of knowledge, discoveries and significant findings of several studies revealing the application of novel ingredients for the development of new value-added food products and food packaging materials. Though there are many conventional food products produced from different food sources, a wide variety of food products produced worldwide have not received sufficient scientific attention. Nowadays, developing new food products with enhanced health benefits is a trend which likely will continue in future. A strategic research is needed to determine the relationship between processing and reduction of toxic compounds, to understand the dynamics of different processing methods and to manipulate food products to increase nutritional level and shelf life. It is imperative that modern biotechnological techniques be integrated into traditional processing procedures to upgrade their production methods. In addition, the book mentions health benefits of different nutraceuticals as well as biosorption of metals and their positive impacts on living systems. Chapters of this book briefly describe the main nutritional implications, in particular reducing food wastage, preventing losses in nutritive value and conserving or enhancing palatability. Several ongoing research projects have gained importance for their potential applications in every field. However, a combined effort from all sectors related to food, health and nutrition and their shared knowledge with prodigious expertise are helpful to overcome major existing challenges and appear as a remarkable milestone from the prospects of the nation's health and security.

The book mainly comprises four parts. Part I: Food processing and Technology Innovations discusses recent research involving innovative processing and technological methods for novel food products development and evaluation of their health benefits. Part II: Food and Industrial Microbiology explains microbial functions, biotechnology and their application for industrial utilization. Part III: Food Technology and Environmental Biotechnology reveals the role of metals and biosorption of metals along with water quality issues with regard to environmental safety. Part IV: Agriculture, Food, Nutrition and Health deals with nutraceuticals, their therapeutic and pharmacological properties and their potential application for food safety and security.

As a whole, the book contains 31 chapters. In these chapters, medical, food, pharmacy and agriculture experts evaluate the current status of food technology along with its implications in various interdisciplinary and translational fields because food sector is a major contributor to the Indian economy. For example, development in food engineering increases sustainable food products and boosts food security and crop production. Food processing industry, especially agro-horticulture based research, has been identified as a thrust area of development. Agricultural intensification will contribute to 80% increase in future yield and productivity in developing countries. Henceforth, researchers emphasize that smallholder farmers should procure improved cultivars, produce higher yields tolerant to various climatic conditions and develop suboptimal soil to enhance the productivity of crops. Moreover, there is an increasing interest to apply modern food technology to address food and nutrition problems. A thirst for healthier and wealthier lifestyle has always been there in humankind. Population explosion poses a serious challenge in meeting the demands of people to provide adequate supplies of nutrient-rich sources. Nutraceuticals due to their immense potential are found to be beneficial for protection and treatment of various diseases of modern times caused due to oxidative stress, suppressed immune system and other degenerative disorders. These nutraceuticals have a significant role in human nutrition so we need to explore their utilization via innovative technologies suitable to India's conditions and newer ventures in food industries to process and preserve food products. It will also create awareness among stakeholders about the efficiency and benefits of innovative technologies available for processing, preservation and value addition of foods that are essential for effectively managing our foods and for reducing post-harvest losses. The food microbiology part of the book examines various aspects of microorganisms, microbial enzymes and advances in the utilization of microbes for industrial as well as pharmaceutical applications for the benefit of teaching and research community. Biotechnological methods using microbes have enabled us to combat environment and human health problems worldwide in an ecofriendly manner. The book covers all living commodities from producers (such as plants, animals and microbes like bacteria and cyanobacteria) and their ultimate applications to health-conscious consumers. Nowadays, consumers prefer organic foods that are easy to process, ready to cook/eat and have longer shelf life, which may allow us to generate renewable food types with these characteristics. Therefore, it is a crucial and vital need for continuous exchange of shared knowledge among research contributors throughout the world. This book provides comprehensive information on the effective utilization of different constituents of food for increasing food security and nutritional health status. This also sheds some light on unknown facts about current research in various sectors of food technology, which may further be advantageous to farmers as well as small- and large-scale industries.

Great efforts have been made by all contributors in preparation of these chapters in this book to improve our lives through agriculture, food industry and public health. This book will be valuable for students, teachers, academicians and researchers related to food technology, chemistry, engineering, microbiology, dairy technology and environmental biotechnology.

Varanasi, Uttar Pradesh, India — Pragya Mishra
Varanasi, Uttar Pradesh, India — Raghvendra Raman Mishra
Om Auchi, Nigeria — Charles Oluwaseun Adetunji

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About the Editors

Pragya Mishra is presently working as Assistant Professor of Food Processing and Management Course, DDU KAUSHAL Kendra, Banaras Hindu University. Dr. Pragya completed her Master's degree in Food Technology in the year 2010 in Centre of Food Technology at the University of Allahabad with distinction. She did her dissertation research on "Feasibility studies for enhancement of sensory attributes of pearl millet weaning food by fermentation" under the supervision of Prof. Latha Sabikhi, National Dairy Research Institute, Karnal, Haryana. Dr. Mishra had qualified for the University Grant Commission National Eligibility Test Examination, and she was awarded Doctor of Philosophy in Food Technology by Centre of Food Technology, Institute of Professional Studies, University of Allahabad, India, in 2017, for her thesis titled "Isolation and molecular characterization of edible Spirulina species under different abiotic stress condition and evaluation of their food supplement" under the supervision of Prof. Sheo Mohan Prasad, University of Allahabad. She worked as Senior Research Fellow in the Uttar Pradesh Council of Agricultural Research funded project under the guidance of Prof. Neelam Yadav, Director-Center of Food technology, University of Allahabad. She had received an accession number (GenBank: KM407008.1) from National Center of Biotechnology Information, USA, for new isolates of *Spirulina maxima* species from cyanobacterial isolates collected from the paddy fields of Prayagraj (Soraon-Allahabad) region targeting specific 16sRNA gene. She had got A patent on potato flakes energy bar (Application no. –

201711014441). Dr. Mishra has published in more than 20 international and national papers in peer-reviewed journals and four edited books of Bharti Publication, New Delhi, and contributed five chapters in international books. She had received MFPA Technology, 2018, Young Scientist Award, organized by Banaras Hindu University. She is lifetime member of All India Medical Laboratory Technologists Association, New Delhi, and Society of Bacteriophage, Varanasi, and member of AFSTI. Dr. Mishra is also editor of *International Journal of Academic Research and Development* (ISSN.2395-1737).

Raghvendra Raman Mishra is working as Assistant Professor of Medical Lab. Technology Course, DDU KAUSHAL KENDRA, and Warden of Shivalik Hostel (received Best Hostel Award, from 2016 to 2019) of South Campus, Banaras Hindu University, from academic session 2016. Dr. Mishra received his Master's degree in Biomedical Technology in the year 2005 from Jiwaji University Gwalior. He had completed his project at Virology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi. He earned his Ph.D. (2009) in Biomedical Technology from Molecular Oncology Laboratory, Department of Surgical Oncology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. He then worked with Thyrocare Technologies Limited, Navi Mumbai, as Officer-Central Processing Lab (2010). In 2011, he joined as Postdoctoral Research Associate the Bacteriophage Research Lab, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

From 2012 to 2015, he was Assistant Professor in the Department of Biotechnology (Code 641) and Group Leader of Controller of Examination at Dr. APJ Abdul Kalam Technical University, Lucknow. With these 12 years of research and teaching experiences, his areas of specialization are biomedical technology, biotechnology, bioinformatics, cancer biology, microbiology, molecular biology and food biotechnology. He has published more than 50 national and international peer-reviewed papers. He had received Rapid Grant for Young Investigator (2010) from Department of Science and Technology, Government of India. He did a course on "General Course on Intellectual Property Right" from World Intellectual Property Organization (WIPO). He had received Fellowship of the University Grant Commission, Indian Council of Medical Research and Council of Scientific and Industrial Research, New Delhi, at various stages of his research. He is lifetime Member of The Cytometry Society, Hyderabad; All India Medical Laboratory Technologists Association, New Delhi; and *Society of Bacteriophage,* Varanasi. He is Associate Editor of *Journal of Applied Pharmaceutical Science and Research* (ISSN. 2581-5520) and Editor of *International journal of academic research and development* (ISSN.2395-1737).

Charles Oluwaseun Adetunji is Acting Director of Intellectual Properties and Technology Transfer Office and Senior Lecturer in the Department of Microbiology and Guide of Microbiology, Biotechnology and Nanotechnology Laboratory at Edo University Iyamho, Edo State, Nigeria. Previously, he did his duties in the Department of Biological Sciences at Landmark University Omu Aran, where he participated in the agrarian revolution through quality teaching, research and community development. He started his career at Nigerian Stored Product Research Institutes (NSPRI), Ilorin, Nigeria. As Principal Research Scientist at NSPRI, he developed a lots of novel, biodegradable edible coatings for postharvest management and extension of shelf life of fruits and vegetables. He has won a lot of scientific awards and grants from renowned academic bodies like Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology (DBT), India, The World Academy of Science (TWAS) and the Netherlands Fellowship Programme (NPF), among many others. He has published more than 80 papers in international refereed journals. The breadth of his scholarly contributions to research is evident from his contributions, which cover topics relating to food security and environmental sustainability. His research interest includes microbiology, biotechnology, food and nanotechnology. He is an editorial board member of many international journals and also serves as a reviewer to many double-blind peer-reviewed journals like Elsevier, Springer, etc.

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Part I

Food Processing and Technology Innovations

Food Innovation and Sustainable Development: A Bioeconomics Perception

1

A. A. Kifordu, Charles Oluwaseuni Adetunji, W. O. Odiwo, and R. S. Mishra

Abstract

Food innovation and sustainable development through biotechnology is a new and emerging world order. The Millenium Development Goals' cardinal objective is eradication of extreme poverty and promotion of good well-being of humanity. Nigeria having evolved a policy with boards to drive this science and technological advancements has not been able to achieve this target optimally as evidenced by other countries globally. There are two major areas of concern which are determining the extent of government involvement in production of food innovation and sustainable development and establishing the relationship between food innovation and sustainable development under a bioeconomy perception in Nigeria. Having regard for the forgoing, the study examines food innovation toward sufficiency and sustainable development. A descriptive paper that looked at experiences across the globe against Nigeria and possible inventions from Agricultural products it can leverage on. It was observed that the right framework, policy, implementation, and drive have not been adopted. It was then submitted that current boards should be strengthened and integrative culturally. Also, a proper application of the triple helix concept that drives new inventions should be upheld.

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Keywords

Food innovation · Sustainable development · Policy · Bioeconomy

1.1 Introduction

The Oxford Dictionary defined food as any nutritious substance that people or animals eat or drink or that plants absorb in order to maintain life and growth. When an organism uses those sources to make food, it starts the food chain. The process continues to the organisms that eat that plant or animal and then the organism that eats what ate it, all the way to the apex predator, an organism that eats others but is not eaten itself. Basically, there are seven kinds of food which are carbohydrates, proteins, fats, fiber, vitamins, minerals, and water. Food makes your body work, grow and repair itself. The kind of food you eat can affect the efficiency of these processes. Body function and the food that sustains it are infinitely complex. Food is in fact one of the most complicated sets of chemicals imaginable.

Getting to know which nutrients are in which foods can help you to understand something of this complex relationship between your food and your body. Food is composed of many different chemical substances – “macronutrients” (major nutritional components that are present in relatively large amounts, such as protein), “micronutrients” (major nutritional components that are present in relatively small amounts, such as vitamins), water, and roughage (dietary fiber). Many other components can also be present in food.

Food may contain colors (natural and synthetic), flavors, pharmacologically active substances (such as caffeine, steroids, and salicylates, which chemically affect the body), natural toxicants (naturally occurring poisons, such as cyanide), and various contaminants (substances resulting from a contaminated environment, such as pesticides). Even characteristic flavors such as those of oranges and passion fruit can depend on the presence of a dozen or more chemicals. The chemical nature of food is changed by storage, preservation, and, especially, cooking. Food chemicals can also interact among themselves within the body. For example, the availability to the body of iron from plant sources depends on the amount of vitamin C present in the food eaten. The way in which carbohydrate is absorbed from the bowel depends to some extent on the presence of dietary fiber, even though the fiber itself is not absorbed.

Food is also more than just the chemicals it contains. Its physical characteristics are important. The size of food particles can affect the extent to which nutrients are digested and made ready for absorption by the body. For example, eating an intact apple has nutritional value different from drinking all the same chemicals in an apple purée. Ground rice is more rapidly digested than unground rice. Nutrients can be more easily absorbed from peanut butter (paste) than from peanuts eaten whole.

The acidity and alkalinity of food are physical properties often thought to be important. In fact, they are only important insofar as they might alter the rate of emptying of the stomach, digestion in the small bowel, and the acidity or alkalinity of the urine. Our bodies can cope with a wide range in food acidity and alkalinity without much problem. Acid foods are generally sour, while alkaline foods often have a slightly soapy taste. The use of sodium bicarbonate (baking soda) can make foods alkaline. It can also cause loss of vitamin C and contribute to our intake of sodium. Simply put, innovation is the process of translating ideas into useful and used, new food products, processes, or services. The word innovation means different things to different people. It is clear from these examples that not all new products need to be highly technical.

Lopez and Garcia (2005) assert that innovation is the act of converting an information or discovering ways into a good or service that creates benefit or for which customers will pay for such idea. If something is to be called innovative, it means that such an idea must be comparable at an economical cost and must fulfill a specific need to which customers must pay for it. It requires a unique approach that is driven by customers focused on a product. Therefore, companies' innovative capability becomes a major issue in building a sustainable competitive advantage in both home and foreign markets (Marques and Ferreira 2009). Afuah (2003) asserts that innovation could be identified as incremental or radical depending on the level to which it affects a firm's capabilities. This is mostly referred to as the organizational way of classifying innovations (Gowdy and Mesner 1998). In a like manner, an innovation can be called radical if the knowledge expected to exploit is not the same from the knowledge that is profitable within the firm (Coviello 2006). In incremental innovations, the information that is needed to create a product is built on existing knowledge. Jones and Coviello (2005) suggest that the act of making a product suitable for foreign market can be viewed as either radical or incremental based on the geographic and cultural closeness to the domestic market. Internationalization as an incremental innovation is considered as the increase of firms from home country to neighboring countries or markets with only slight differences, which is similar with the traditional Uppsala model (Johnson and Vahlne 1977). The Uppsala views incremental innovation as those firms that gain market idea of the domestic market before gradually moving to foreign markets that are culturally or geographically close. Internationalization as radical innovation is viewed as the expansion firms to markets that are significantly different from the domestic market in regards to cultural and geographic qualities (Odiwo 2018).

The formation of a bioeconomy had its roots in the twentieth century but grew in lips and bounds during the twenty-first century and became a tool toward reshaping the developmental strides of a nation drawing special attention to scientists, politicians, and societies at large. Though linked earlier on in the fields of biology and biotechnology, its fast evolution has its dynamic relevance to the human environment particularly linked with environment, ecological development, and sustainability, but there is little consensus on what it is or what it does or does not include (McDonald 2018). In recent times, the innovative utilization of living organisms of plants, animals, and microorganisms and their integration into one large segment (complex) of real economy, which is the essence of bioeconomy, has been adopted by several countries to drive their developmental efforts (Adamowicz 2017).

The term bioeconomy, according to Commission of Europea Communities (2009), is defined as “the production of renewable biological resources and the conversion of these resources and waste streams into value-added products, such as food, feed, bio-based products, and bioenergy”. Its sectors and industries have strong innovation potential due to their use of a wide range of sciences, enabling industrial technologies, along with local and tacit knowledge. Bioeconomy is considered to be an alternative economic model to our present fossil-dependent model which is based on high consumptions of rare materials, often low economic returns, and extensive consumption of natural and labor resources (Gemenne et al. 2013). As a new wave of economic system, the bioeconomy combines in a synergic way both natural resources and technologies, together with markets, people, and policies to provide a solid and realistic foundation for achieving the sustainability need worldwide (Maciejczak 2017).

In a similar way, innovation comes from different results and ways, such as internal research and development and imitation of the innovation of other firms which improvement has been done. The Research and development is especially added to the opening of recent markets and the rediscovery of firm’s transformation in ways that allow the firms to better serve those new markets. The creativity of such firms includes the masterful leveraging of knowledge and organizational capabilities despite scarce financial, human, and tangible resources. Knight and Cavusgil (2004) assert that firms are inherently entrepreneurial and innovative firms, displaying a specific pattern of idea and capability management that bring up early internationalization with renewable, quality performance in foreign markets.

The society today is characterized with a high rate of poverty. In spite of the numerous natural endowments and the kind of natural resources that every nation both developed and developing seeks to possess, poverty, hunger, and related social menace have no place in countries like Nigeria. That poverty is recorded in most communities in Nigeria where two-third of the population cannot afford basic needs of life, especially food. The basic needs has led to increases in poverty, social vices among others that impedes development of a nation. Given the very importance of food within the context of bioeconomy, it becomes imperative to address issues around innovation, sustainable development, and sustainable development goals in a developing nation like Nigeria. The objective is to:

1. Determine the extent of government involvement in production of food innovation and sustainable development: a bioeconomics perception in Nigeria
2. Establish the relationship between food innovation and sustainable development: a bioeconomics perception in Nigeria

1.2 The Sustainable Development Goals at NHSMUN

"Half our world is female. Half our world is under 25 years of age. We cannot meet the Sustainable Development Goals without drawing on the power of women and the enormous energy of young people." *Secretary-General António Guterres, addressing the UN General Assembly on September 19, 2017*

The Sustainable Development Goals (SDGs) and the 2030 Agenda are the transformative goals adopted by the United Nations General Assembly to achieve peace, equality, and prosperity. Improving the Millennium Development Goals (MDGs) that guided many organizations' work from 2000–2015, the SDGs place a new emphasis on not just development but sustainable development. This places the need for action not only on developing states but rather on all states, as injustices toward people, the planet, and joint prosperity exist all across the globe. These goals are ambitious, but with the full force of the UN system behind them and the support of passionate civilian advocates everywhere, we can achieve these goals and put the world on the path toward peace and justice for all.

As one of the leading platforms for youth to engage with the UN's agenda, National High School Model for United Nations (NHSMUN) is passionate about connecting our delegates and students all over the world with the SDGs. All of our committee's debate topics are directly related to one or more of the SDGs, and delegates will find discussions of the SDGs integrated throughout NHSMUN's research materials. Our staff, the advocates of the SDGs, are also trained in various ways to incorporate the SDGs into debate and conference activities and will ensure that the resolutions adopted by each committee reflect the values of the SDGs.

The SDGs, however, are far more important than just authentic MUN simulations. The SDGs have the potential to reshape our world for the better, but that change will only take place if we all contribute as global citizens. This is why we encourage all Model UN delegates to advance the SDGs in their regular MUN meetings and in their daily lives. To promote this, we regularly host awareness campaigns and contests related to one or more of the SDGs to allow students to showcase their work. At NHSMUN, we believe in and have experienced (as Secretary-General Guterres says) "the enormous energy of young people," and we are humbled to be just one of many avenues for youth to unlock that energy. To learn more about the SDGs, the Eight Millennium Development Goals are to eradicate extreme poverty and hunger, to achieve universal primary education, and to develop a global partnership for development.

However, MDGs are derived from earlier development targets, where world leaders adopted the United Nations Millennium Declaration. The approval of the Millennium Declaration was the main outcome of the Millennium Summit held in Rio de Janeiro in 1988. The MDGs originated from the United Nations Millennium Declaration. It is therefore important for sustainable development to meet up present-day development without compromising the ability of future generations to meet their own development. It is on this note that the paper is hinged on Goals 1–8 of the MDGS to eradicate poverty and health challenges in Nigeria at large.

Goal 1. Eradicate extreme poverty and hunger.

- Target 1. Halve, between 1990 and 2015, the proportion of people whose income is less than 1 dollar a day.
- Target 2. Halve, between 1990 and 2015, the proportion of people who suffer from hunger.

Goal 2. Achieve universal primary education.

- Target 3. Ensure that, by 2015, children everywhere, boys and girls alike, will be able to complete a full course of primary schooling.

Goal 3. Promote gender equality and empower women.

- Target 4. Eliminate gender disparity in primary and secondary education, preferably by 2005, and to all levels of education no later than 2015.

Goal 4. Reduce child mortality.

- Target 5. Reduce by two-thirds, between 1990 and 2015, the under-5 mortality rate.

Goal 5. Improve maternal health.

- Target 6. Reduce by three quarters, between 1990 and 2015, the maternal mortality ratio.

Goal 6. Combat HIV/AIDS, malaria, and other diseases.

- Target 7. Have halted by 2015 and begun to reverse the spread of HIV/AIDS.
- Target 8. Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases.

Goal 7. Ensure environmental sustainability.

- Target 9. Integrate the principles of sustainable development into country policies and programs, and reverse the loss of environmental resources.
- Target 10. Halve by 2015 the proportion of people without sustainable access to safe drinking water.
- Target 11. By 2020 to have achieved a significant improvement in the lives of at least 100 million slum dwellers.

Goal 8. Develop a global partnership for development.

- Target 15. Deal comprehensively with the debt problems of developing countries through national and international measures in order to make debt sustainable in the long term.
- Target 18. In cooperation with the private sector, make available the benefits of new technologies, especially information and communications.
- Target 19 Telephone lines and cellular subscribers per 100 population.

1.3 Methodology

The paper will look at the future of food innovation and sustainability in biotechnology, experiences across the globe, Nigeria and bioeconomy, and discussions and draw conclusions considering the potentials, drawbacks, and future of bioeconomy in Nigeria.

1.4 Selected Emerging Food Innovations in Biotechnology

The future of food production, innovation, and engineering could look very different from what we have taken for granted in the present day. The incorporation of several disciplines into the singular process of producing food could see the advent of a so-called post-animal bioeconomy. The adoption of things like 3D printing, lab-grown meat, the blockchain, vertical farming, and cellular culturing could see agriculture and animal husbandry (for food) extinct. Just think about that for a second – we could literally be on the brink of retiring the very innovation that made civilization possible. The identified sustainable innovative areas in biotechnology include the following:

1.4.1 3D Food Printing

The 3D food printing food technology is the work of institutes like TNO who are developing a means of 3D printing food. With the massed proliferation of 3D printing over the last few years, this development was probably an inevitability. The technology will work as you anticipate – by building the end product layer by minute layer. This solution will offer endless possibilities for the shape, texture, composition, and, ultimately, taste of food products in the future. 3D printing can probably be likened to the replicators in Star Trek, albeit a lot slower and more cumbersome. Like Star Trek, 3D printing will let you customize the final dish to your specific demands and tastes – just like cooking for yourself but without all the work. TNO believes that this technology will be popular with food producers, retailers, and consumers alike. Whether it will usurp the growing momentum in robotic chefs (more on them later) or compliment them – only time will tell. The technology will greatly reduce the waste produced from "conventional" cooking and could be used to promote healthy high-tech food and completely redefine how we produce "recipes."

1.4.2 High-Pressure Processing

High-pressure processing is a cold pasteurization process that introduces foods sealed in packaging into a high isostatic pressure environment (300–600 MPa) that is transmitted by water. That is more pressure that can be found at the base of the Mariana Trench. This technique effectively inactivates microorganisms to guarantee food safety. This combination of high-pressure and low-temperature environment safely maintains the taste, food, appearance, texture, and nutritional value of food. High-pressure processing respects the sensorial and nutritional properties of food, because of the absence of heat treatment, and maintains its original freshness throughout the shelf life. Another benefit of HPP is the fact that no irradiation or chemical preservatives need to be introduced in the process.

1.4.3 Automated Grading Systems

This system is used to grade and sort chickens (and potentially other animals) efficiently and accurately. AQS lets Aris' clients sort chickens by their shape, size, color, and any other characteristics desired. This relatively new system can manage an excess of 12,000 chickens in 1 h greatly improving food production efficiency. Aris' AQS-system is, by all accounts, the first of its kind. It uses a camera system and software program to detect a suite of variations (like color) on the examined specimen. This system registers many profile deviations like broken wings or missing parts, poor coloration, etc. and can even learn and improve itself over time.

The AQS-system also collects data from the products and product streams to feed and control the entire slaughterhouse operating system. Aris has also devised similar systems for grading plants like orchids, pot plants, and other seedlings at impressive rates per hour. These kinds of automation could completely replace human alternatives as they provide a greater level of accuracy and can operate tirelessly without needing to take breaks or holidays (Aris).

1.4.4 Insect Protein Could Replace Beef, Chicken, Pork, and Lamb

Although eating arthropods, like insects, is par for the course for many nations around the world, it is a bit rarer in the "West" – if we disregard things like lobsters and crabs of course. This is set to change with Kickstarter companies like Exo hoping to make insect protein bars and other foodstuffs commonplace in our diets. Exo is not the only kid on the block with our insect protein manufacturers quadrupling their revenue between 2014 and 2015 according to Fortune. Insect protein tends to contain about 60% protein, is packed with vitamin B12, and has more calcium than milk. It also has more iron than spinach and can supply you with all the essential amino acids your body needs. Insect meat is also better for the environment compared to its lumbering four-legged alternatives. It requires much less water (about 455 l to make 72 g of crickets compared to 6 g of beef) and requires much less physical space. If its popularity grows, it could spark an entirely new industry and create hundreds, if not thousands, of jobs.

1.4.5 Robo Chefs Could Change the Way We All Cook Food

It consists of a pair of fully articulated and automated robotic arms that can, for all intent and purpose, replicate the movement of humans' arms and hands. Moley believes that their robotic chef has the same level of dexterity as that of any human alternative – especially when it comes to speed and sensitivity. This robot chef takes its cue from famous chefs whose cooking skills are being followed to the letter by the robot. Each recorded "recipe" is not only a list of ingredients and a set of instructions but also a complete and accurate replay of the original chef's actual motions and movements. This technology could change the way we all cook food.

1.4.6 Lab-Grown Meat

Lab-grown meat, otherwise known as in vitro animals or "clean meat," could be on sale very soon indeed. It could also make meat production a new form of sustainable engineering. This kind of "meat" is grown from stem cells that are harvested by biopsy from donor livestock and then cultured in a lab for a few weeks. In vitro meat is very popular with environmentalists who believe it could greatly reduce the environmental impact of large-scale animal husbandry. Some estimates believe that "greenhouse gas" emissions, most notably methane, could be reduced by 96% if it were adopted large scale. The technology is being developed by companies like JUST who hope to bring its products to market at some point in 2018. Products like chicken nuggets, sausage, and even foie gras could be created by this technique. Of course, public opinion and the market's "invisible hand" will ultimately dictate the commercial success of this new industry. However, some polls indicate that a significant percentage of people are open to eating "clean meat" (Winfried Mosler/ Flickr).

1.4.7 Vertical Farming

Vertical farming could be the future of large-scale agriculture. With more and more people moving into cities and traditional agriculture requiring large tracts of land, the solution to future crop production could be to farm "upwards." The concept is nothing new and was first proposed by Dickson Despommier who noted that an upscaling of the concept of rooftop gardens could be the future of farming. He envisaged purpose built farming "towers" that could allow crops to be produced on every single level of the buildings, including the roof. These kinds of farms generally fall into one of the two categories – hydroponics (plants are grown in a basin of nutrient-enriched water) or aeroponics (roots are exposed and sprayed with nutrient-enriched mist). Neither requires any soil, and artificial lighting tends to also be incorporated unless sunlight is in abundance. These kinds of farms have some clear advantages over more traditional means of agriculture. Physical ground space is minimized, all-year round farming is possible, and agrochemicals are eliminated (SkyGreens).

1.4.8 Personalized Nutrition Could Be the Future of Eating Plans

Personalized nutrition is the concept of tailoring your diet to the specific means in which your genetic makeup predisposes you to react to different foods and other consumable products. The concept is not new, but some companies are already offering it to their clients. "Nutrigenomics" as it's called, is widely considered to be too far in its infancy for public consumption (Sserv27/Wikimedia Commons).

1.5 Lessons from International Experiences

1.5.1 European Commission

The European Commission (2010) combined a strategy and action plan document called Innovating for Sustainable Growth: A Bioeconomy for Europe which offers direction for research and innovation agendas in the bioeconomy sectors. This contributes to a more enabling policy environment and paves the way for a more innovative, resource-efficient, and competitive European society. The policy model brings together several stand-alone policy areas (e.g., climate change, agricultural and industrial policy, R&D and innovation, environmental policy, etc.), as an attempt to provide an integrated response to several broad challenges – i.e., climate change; food and energy insecurity; resource constraints with emphasis on the sustainable use of natural resources; competitiveness; and socioeconomic and environmental issues.

The European Commission's efforts have made bioeconomy the central component in shaping sustainable development in Europe. Countries such as Germany, Finland, Sweden, Russia, Austria, Netherlands, Belgium, and West Nordic countries are developing strategies and declare their intentions and visions for the development of a bioeconomy. Denmark has adopted the EU bioeconomy strategy via The Copenhagen Declaration for a Bioeconomy in Action 2012. Turkey, Estonia, Italy, Poland, and Spain are other countries where dedicated bioeconomy strategies are under development.

1.5.2 United States

The National Bioeconomy Blueprint for the United States describes the actions of the government in the area of bioeconomy based on the use of research and innovation in the biological sciences to create economic activity. The driving forces are economic growth, societal benefits, health, and environment, as well as the United States being a leading nation in the field. The strategic objectives encompass: supporting R&D investments, facilitating transition from laboratory or market, forming and reforming regulations, adapting training and aligning institutional incentives, and supporting public-private partnerships (The White House 2012).

1.5.3 Canada

There is no official strategic document for bioeconomy in Canada, rather, the *Canadian Blueprint: Beyond Moose and Mountains* was published by Biotec-Canada, an association representing the biotechnology sector in Canada. The document defines bioeconomy as biotechnology and used these terms interchangeably throughout the document. Success will be measured in at least three ways: the bioeconomy as percentage of GDP; growth in Canada's percentage of the world bio-based sector; and the world's adoption of Canadian biotechnology. An interesting development in bioeconomy development in Canada is the formulation of provincial policies. British Columbia (BC) and Alberta provinces have independent but complimentary polices (BioteCanada 2009).

1.5.4 Latin America

Sasson and Malpica reviewed how Latin America has embraced bioeconomy in the last two decades. The study showed that the transition toward knowledge-based bioeconomy in representative countries in the region, including Argentina, Brazil, Chile, Costa Rica, Colombia, Cuba, Mexico, and Peru, is highly dependent on the level of applicability of new technology developments in specific sectors of their economies as shown by high socioeconomic impact which is the implementation of GMO technology in agriculture.

1.5.5 China

China is pursuing a strong position in the bioeconomy with a special focus on biochemistry and life sciences. Malaysia also has a vision for the creation of a bioeconomy – the BioEconomy Initiative Malaysia (BIM) launched in 2011, in addition to the National Biomass Strategy to 2020. Only South Africa has published an official bioeconomy strategy in Africa. The majority of African countries are yet to develop any form of integrated bioeconomy development strategy. Countries such as Nigeria, Ghana, Namibia, Uganda, Ethiopia, South Africa, Kenya, Mozambique, Democratic Republic of Congo, Mali, Congo, Tanzania, and Zimbabwe have some bioeconomy development activities based on various crops and oil plants, but there is no evidence of any significant positive impact on the economy (Abass 2004).

The evaluation from countries drawn across the globe revealed that there is a strong nexus between research and bioeconomy development and growth and sustainability. The concepts of sustainability and sustainable development are used as selling points for bioeconomy agenda and goals to allow various actors to make related commitments without necessarily undertaking any significant changes to their policies, strategies, and actions. Across the different national and regional strategies, a range of visions and motivations for development of the bioeconomy is put forward. These include to address societal challenges, drive economic growth, mitigate climate change, reduce dependence on fossil fuel, have a transition to a more resource-efficient economy, and achieve national pride of becoming leaders in sustainable economy. The policy interventions driving bioeconomies across the world are research, development and innovation, stakeholder's engagement, and markets and competiveness. The specific strategies to achieve the bioeconomy visions include support to R&D activities, education (training and skills acquisition), knowledge enhancement and technology transfer, policy interactions and stakeholders engagement, commercialization, market development support, removal of regulatory barriers, public procurement mandates, and private-public partnership for business innovation (McCormick et al. 2015).

1.5.6 Nigeria and Bioeconomy Experience

The government of Nigeria through National Science Research, Technology and Innovation Fund (NSRTIF) for the development of science and technology in the country adopted a biotechnology policy and approved the establishment of the National Biotechnology Development Agency (NABDA) on April 23, 2001, to promote, coordinate, and deploy cutting-edge biotechnology research and development, processes, and products for the socioeconomic well-being of the nation. Conseqeuntly, in 2013, the National Biosafety Management Agency (NBMA) Act 2015 was subsequently enacted to support and consolidate the existing bodies.

However, the NSRTIF has lacked the considerable right framework for implementation adequately considering the growing population and diverse benefits biotechnology can bring to a developing nation and one of Africa's largest economies. Similarly the drive and orientation has not been able to hit its optimum capacity toward a realistic sustainable index since the past 18 years of its adoption.

The NBMA act of 2015 has continued to suffer funding and institutional challenges arising from its level of priority, whereas at the international level, nations are already at various levels of advancement through innovation by tapping into the advantages of biotechnology.

1.6 Government Involvement in Production of Food Innovation and Sustainable Development: A Bioeconomic Perception in Nigeria

Most developed countries from international experiences drawn from this work have evolved workable measures in the production of food. This innovation has continued to be sustained through biotechnological applications, thereby eradicating poverty and hunger, achieving funded education at basic school levels, promoting gender equality, and reducing child mortality. These cardinal goals of every economy are enshrined as part of the sustainable development goals. Nigeria as a nation should harness her rich natural endowment and leverage on her economic potentials to drive a bioeconomic-driven society. Presently, Nigeria is one of the counties in Africa classified under the food-deficient nation. There is a strong relationship between extreme hunger and basic education, whereas of 2015, Nigeria ranked 103 out of 118 countries in UNESCO's Education For All (EFA) Development Index which addresses issues of adult literacy, gender parity, universal basic education, and adult literacy. The idea of EFA is to address access to education, promote gender parity, and reduce infant mortality rates across affected countries. The Nigeria's weak alignment to national policy, measurements, and monitoring since the outset of the different education initiatives has been weak and improved only slightly over time; objectives are weak measurable targets and some less well-defined objectives.

1.7 Food Innovation and Sustainable Development: A Bioeconomics Perception in Nigeria

The Government of Nigeria has several attempts through National Science Research, Technology and Innovation Fund (NSRTIF) for the development of science and technology in the country, and NBMA act of 2015 has continued to suffer funding and institutional challenges arising from its level of priority, whereas at the international level, nations are already at various levels of development. We have left and reduced the innovative ideology driving biotechnology that would promote export, grow food sufficiency, and sustain the nation for a more import-substituted economy. Efforts made in the area of agriculture and entrepreneurship are still not driven national where comparative advantages are leveraged upon across states. Also, the manufacturing and policy flexibility by Nigerian government have not promoted the right environment to harmonize and encourage research in institutions anchored on the triple helix effect (industry, institution, and government) collaboration. The resultant issues of maternal, child health issues, insurgency, diseases and banditry. Environmental sustainability is also a major concern in Nigeria; deterioration of urban physical quality, deforestation, soil erosion, and flooding have continued to characterize the land space of Nigeria which is a major factor in achieving food sufficiency. That most developed countries have sustained and developed global partnerships for development.

1.8 Conclusions

Nigeria, as a nation, is endowed with human and natural resources and has the capacity to drive food sufficiency through innovative policies using biotechnology. The continuous neglect of biotechnology has not given her the opportunity to be reckoned in the comity of nations especially when she has the potential to drive the same. If the right framework, policies, and implementation are applied, food sufficiency and sustainability would be achieved. Also it will trigger the objectives of the MDGs toward poverty eradication and better well-being. Nations across the globe have taken a leap through innovations, and for over 14 years since the 2001 act, Nigeria is yet to achieve its optimum comparatively.

1.9 Recommendations

1. The National Science Research, Technology and Innovation Fund (NSRTIF) for the development of science and technology in the country should be sustained and strengthened that could trigger rural and urban value chain effect.

2. Government should prioritize inventions in biotechnology to the extent of filling the gap in areas of food sufficiency with the right framework for implementation adequately considering the growing population and diverse benefits biotechnology can bring to a developing nation and one of Africa's largest economies.
3. Government collaboration through research and related institutions under the triple helix (government, institutions, and industry) to enable the country to hit optimum capacity toward a realistic sustainable index since the past 14 years of its introduction has given little or nothing to that regard.

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2 Fermented Pearl Millet Weaning Food: An Innovation of Food Technology and Application in Food Processing and Management

Pragya Mishra and Latha Sabikhi

Abstract

In the present study, we evaluated the nutritional parameters of pearl millet to develop a special dish named as *pearl millet weaning food*. This is hypothesized that the addition of curd culture to *pearl millet weaning food* may enhance its flavor and digestibility and improves its palatability. Finally, the study may be subjected for its sensory attribute analysis. The data analysis concludes that the development of fermented *pearl millet weaning food* has opened up a newer opportunity to enhance the flavor and digestibility of traditional *pearl millet weaning food*. However, more work needs to carry out on process development to have a shelf-stable product with high acceptability. After that, fermented *pearl millet weaning food* may be used as a good quality nutritional food for human.

Keywords

Pearl millet · Fermented pearl millet weaning food · Nutritional food

2.1 Introduction

Millet is one of the oldest cultivated foods known to humans (Khailwal et al. 1997; Kumar 1989). Since prehistoric time, pearl millet (*Pennisetum glaucum*, family, Poaceae) is the most widely grown in Africa and the Indian subcontinent (Mc. Donough et al. 2000). It is one of the major staple food crops in the dry and

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rural regions of India (Burton et al. 1992). Consumption of pearl millet is very high, and it occupies almost 7.4% of the total food grain area and contributes to nearly 3.4% of the total food grain production of our country (Bhatnagar 2002). Pearl millet is also termed as "nutracereal" in view of its unique nutritional profile. The grain contains complex carbohydrates, proteins, high proportion of dietary fibers, and myriad of phytochemicals such as fat, vitamins, and minerals with nutraceutical qualities (Hulse et al. 1980).

Grains of pearl millet have high fat content as compared to other grains like wheat, rice, etc. The grain has about 5% fat, roughly twice the amount found in the standard cereals. It is composed of about 75% unsaturated and 24% saturated fatty acids. The unsaturated fatty acids making up the oil are oleic (20–31%), linoleic (40–52%), and linolenic (2–5%). The saturated fatty acids are palmitic (18–25%) and stearic (28%) (Mc. Donough et al. 2000). In carbohydrate, sucrose (66%) and raffinose (28%) make major constituents. Predominant component of total soluble sugar was 2.16–2.78%. Other sugars detected in measurable amounts were stachyose, glucose, and fructose. Measurements made on several hundred types have shown that the protein ranges from 9% to 21%. Of the different protein types, prolamine constitutes 40% and globulins 20%. The protein efficiency ratio is reported to be 1.43, which is even better than that of wheat and sorghum (Mc. Donough et al. 2000).

The vitamin values of pearl millet grain are generally lower than those of other grains, although the level of vitamin A is quite good. Pearl millet is still deficient in essential amino acids but averages 35% more lysine (Mc. Donough et al. 2000). Grains contain traces of barium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, silver, strontium, tin, titanium, vanadium, zinc, and iodine. Even when grown in highly stressed conditions, the grain is essentially free of aflatoxins and fumonisins but 5–6% oil and a lower proportion of the less digestible cross-linked prolamines.

The nutritional capability of pearl millet is quite good, but no work of technological or scientific significance has been reported in the literature on the method of standardization of pearl millet *weaning food* or its flavor enhancement. It was, therefore, planned to carry out feasibility studies on the development of such a product, with sensory attributes forming the basis of the feasibility study.

2.2 Sources of Literature

A detailed search in PubMed, Scopus, and Google Scholar was made, the search strategy for described below. The search was limited to:

1. Pearl millet
2. Nutritional value of pearl millet and other cereals
3. Fermentation and weaning food
4. Effect of fermentation on nutritional composition of foods

On these keyword, abstracts of the articles were read, and from relevant article, full-length data was collected, reviewed, and analyzed.

2.3 Nutritional Parameter

During the data acquisition of the above keyword-based search, the following nutritional parameters were discussed. These were carbohydrates, protein, fat, vitamins, and minerals. A comparative analysis of nutritive value of cereal grains was done.

2.4 Pearl Millet

Wholesome, insect-free, pearl millet grains with average mass of each kernel 8.9 mg of variety SDMV 89004 were procured from local market of Karnal district of Haryana (Fig. 2.1).

2.5 Skim Milk and Starter Culture

Fresh mixed skim milk was collected from the Experimental Dairy, NDRI, Karnal. The fat and total solid (TS) content of skim milk ranged from 0.1% to 0.3% and from 8.5% to 9.1%, respectively. The titratable acidity of skim milk was between 0.14% and 0.16%. Mesophilic mixed strain curd culture NCDC-167 was supplied by the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, National Dairy Research Institute, Karnal.

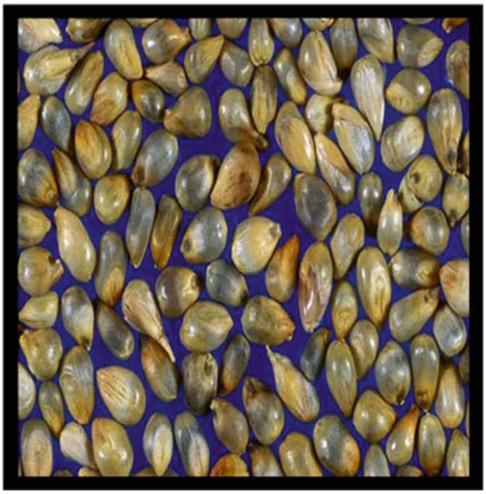

Fig. 2.1 Pearl millet grains (variety SDMV 89004)

2.6 Salt, Cumin, and Chemicals

Commercial grade refined iodized salt was procured from the local market. Commercial grade cumin seeds are procured from the local market roasted and ground into fine powder and sieved through 52 mesh sieves. All the chemicals utilized for the preparation of different reagents were of analytical grade and were procured from standard companies. The reagents required for analysis were freshly prepared adopting standard procedures.

2.7 Maintenance of Cultures

Starter culture obtained from NCDC was maintained in sterilized skim milk. The fresh skim milk (60 ml) was taken into a 250-ml conical flask and plugged with nonabsorbent cotton plugs. The flasks were transferred in autoclave and sterilized at 15 psi for 15 min. The culture was propagated by inoculating sterilized skim milk at the rate of 2% using laminar air flow chamber. The flask were incubated at 37 °C for 10–12 h and stored at 5–7 °C. The propagation of culture was done at regular intervals to maintain culture activity.

2.8 Processing Methods

The processing methods and study designed as per given schematic representation (Fig. 2.2) are as given below.

2.9 Development of Sensory Evaluation Parameter

The fermented and non-fermented pearl millet *weaning food* would be evaluated for its sensory characteristics by a panel of judges using a 9-point hedonic scale (Stone et al. 1974). For sensory assessment, *weaning food* (fermented as well as non-fermented pearl millet) will be served on to coded petri plates. Panelists were asked to assess their degree of liking of samples on paper ballot with a 9-point hedonic rating scale where 9 is like extremely, 8 like very much, 7 like moderately, 6 like slightly, 5 neither like nor dislike, 4 dislike slightly, 3 dislike moderately, 2 dislike very much, and 1 dislike extremely. Ten experienced judges will evaluate randomly coded *weaning food* in terms of color, appearance, aroma, texture, taste, and overall acceptability. Assessors will instruct to cleanse their palate with cold, filtered tap water before testing each sample. Product characterization will carried out under “daylight” illumination.

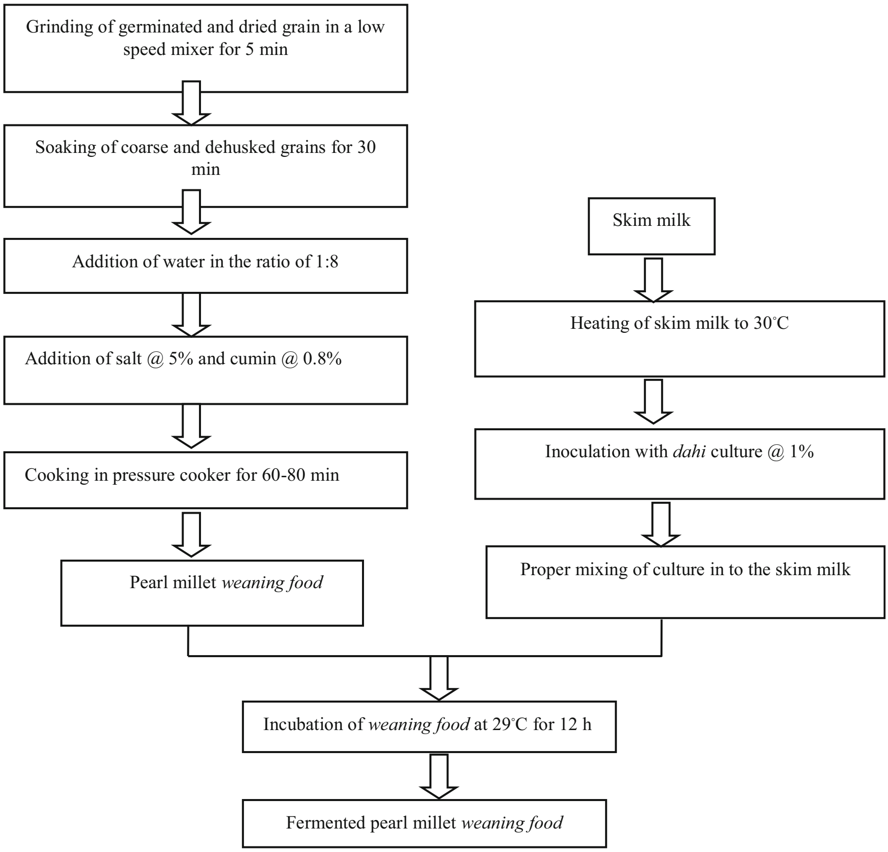

Fig. 2.2 Schematic diagrams for the standardized process for fermented pearl millet *weaning food*

2.10 Outcome

Published studies showed that in India, consumption of pearl millet is very high (42%) compared to other countries (Fig. 2.3).

2.11 Nutritional Value of Pearl Millet

Pearl millet is also termed as "nutracereal" in view of its unique nutritional profile. The grain contains complex carbohydrates, proteins, high proportion of dietary fibers, and myriad of phytochemicals such as fat, vitamins, and minerals with nutraceutical qualities (Fig. 2.4) (Sharma and Kapoor 1996).

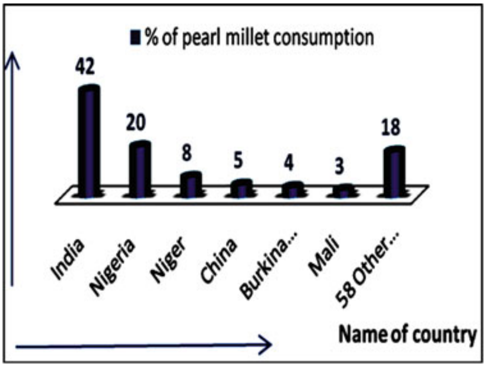

Fig. 2.3 Average millet consumption in the world

2.12 Effect of Fermentation on Nutritional Composition of Foods

Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids, and proteins, by improving protein and fiber digestibility, by enhancing micronutrient bioavailability, and by degrading antinutritional factors. The processes also enhance food safety by reducing toxic compounds such as aflatoxins and cyanogens and producing antimicrobial factors such as lactic acid, bacteriocins, carbon dioxide, hydrogen peroxide, and ethanol which facilitates inhibition or elimination of food-borne pathogens. Therapeutic properties of fermented foods have also been reported with the following changes:

(a) Changes in proximate composition and soluble component (Zamora and Fields 1979)
(b) Changes in composition of amino acids and vitamins (Soni and Sandhu 1989)
(c) Changes in undesirable components (Lopez et al. 1983; Boralkar and Reddy 1985)
(d) Changes in biological value (Khetarpaul and Chauhan 1990a, b, 1991)

2.13 Sensory Evaluation

Based on the preliminary trials, the process for manufacturing of fermented *pearl millet weaning food* was developed. Preliminary trials were conducted to choose the pearl millet grain size for which the pearl millet grains were taken as whole grain and ground for 5 min and 10 min using low-speed mixer. The sample ground for 5 min was found to be the best.

For the selection of water quantity to be added, trials were conducted using different pearl millet to water ratios, i.e., 1:6, 1:7, and 1:8. The ratio of 1:8 was

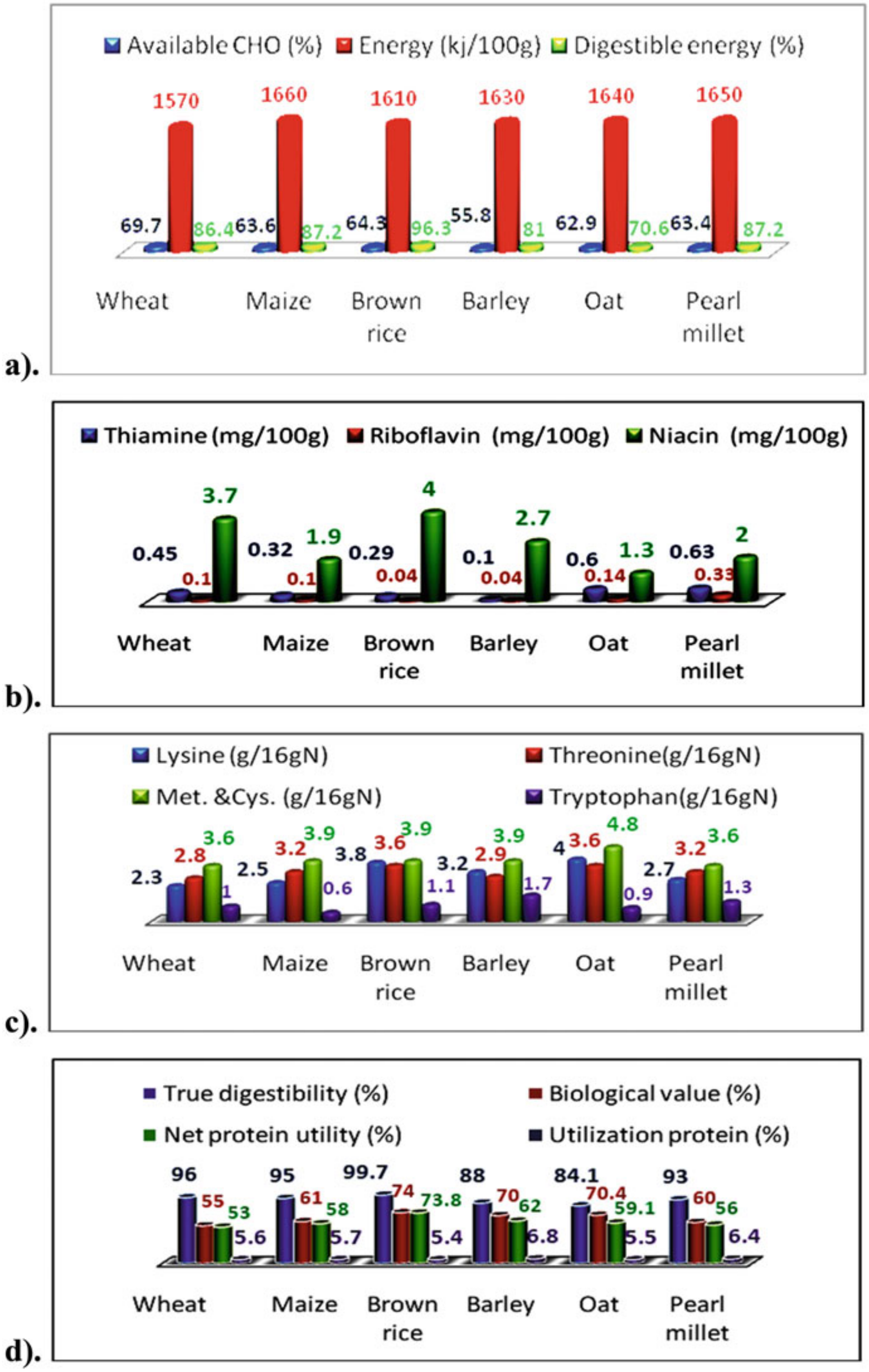

Fig. 2.4 Comparative nutritive value of cereal grains and pearl millet: (**a**) bioenergetics, (**b**) vitamin, and (**c**) amino acids and (**d**) protein quality (Haard 1999)

found to be significantly better than the other two samples with average overall acceptability score of 7.15 on 9-point hedonic scale. For further experiments, this sample was used. Trials were conducted using different time intervals of incubation of *pearl millet weaning food* incorporated with *dahi* culture for 8 h, 10 h, 12 h, and 14 h at 29 °C. Out of the four combinations tried, incubation time for 12 h was found to be significantly better than the other two samples with average overall acceptability score of 7.15 on 9-point hedonic scale. For further experiments, this sample was used.

In the present study, ANOVA was used to explain the sensory attributes such as appearance, flavor, consistency, mouthfeel, acidity, and overall acceptability of non-fermented and fermented *pearl millet weaning food.*

Conclusion of sensory attributes of final product is given below.

1. The standardized product consisted of 1 part of pearl millet cooked with 8 parts of water, 5% salt, and 0.8% of cumin powder. The standardized product consisting of this formulation scored 6.73 for appearance, 7.03 for flavor, 7.24 for consistency, 7.24 for mouthfeel, 7.21 for acidity, and 7.33 for overall acceptability scores on 9-point Hedonic scale.
2. The fermented *pearl millet weaning food* contained 9.55% total solid, 4.45% fat, 3.30% protein, 0.5% ash, and 82.2% carbohydrate.

2.14 Discussion

Fermentation has been found to increase pepsin digestibility of millet protein and decrease the concentration of phytic acid and polyphenols (Mahajan and Chauhan 1987) with improvement in the availability of minerals (Khetarpaul and Chauhan 1989). Fermentation of the processed grains for 12 h slightly affected the protein content of the pearl millet. The effect of fermentation on protein content varied between the cultivars and the processing treatment applied. However, the protein digestibility was significantly improved when the processed grains were fermented for 12 h. Fermentation of the germinated grains improved the in vitro protein digestibility (IVPD) of Gadarif cultivars to 91.1% while that of Gazeera improved to 92.0%. Fermentation of dry heated, debranned, soaked, and coarse ground grains for 12 h also improved the IVPD (El Hag et al. 2002; Hassen et al. 2006). Further increase in the fermentation time to 24 h was observed to cause a further improvement in IVPD of the cultivars. The improvement in IVPD caused by fermentation could be attributed to the partial degradation of complex storage protein to more simple and soluble products (Chavan et al. 1988) and to the degradation of tannins, polyphenols, and phytic acid by microbial enzymes. Enhanced proteolytic activity during fermentation is generally associated with improved protein digestibility, which increases amino nitrogen by partial breakdown of proteins, peptides, and amino acid (El Hag et al. 2002). Lactic acid fermentation of different cereals, such as maize, sorghum, and finger millet, is reported to effectively reduce the amount of phytic acid and tannins and improve protein availability (Chavan et al. 1988).

Table 2.1 Comparative nutritive value of cereal grains

Factor	Wheat	Maize	Brown rice	Barley	Oat	Pearl millet
Available CHO (%)	69.7	63.6	64.3	55.8	62.9	63.4
Energy (kj/100 g)	1570	1660	1610	1630	1640	1650
Digestible energy (%)	86.4	87.2	96.3	81	70.6	87.2
Vitamins (mg/100 g)						
Thiamine	0.45	0.32	0.29	0.1	0.6	0.63
Riboflavin	0.1	0.1	0.04	0.04	0.14	0.33
Niacin	3.7	1.9	4	2.7	1.3	2
Amino acids(g/16gN)						
Lysine	2.3	2.5	3.8	3.2	4	2.7
Threonine	2.8	3.2	3.6	2.9	3.6	3.2
Met. and Cys.	3.6	3.9	3.9	3.9	4.8	3.6
Tryptophan	1	0.6	1.1	1.7	0.9	1.3
Protein quality (%)						
True digestibility	96	95	99.7	88	84.1	93
Biological value	55	61	74	70	70.4	60
Net protein utility	53	58	73.8	62	59.1	56
Utilization protein	5.6	5.7	5.4	6.8	5.5	6.4

Increased amounts of riboflavin, thiamine, niacin, and lysine due to the action of lactic acid bacteria (LAB) in fermented blends of cereals were also reported (Hamad and Fields 1979). Khetarpaul and Chauhan (1990a, b) reported improved mineral availability of pearl millet fermented with pure cultures of lactobacilli and yeasts (Khetarpaul and Chauhan 1990a, b). Fermentation of processed pearl millet grains caused significant reduction in antinutritional factors of the grains, which was accompanied by significant improvement in the protein digestibility.

Digestibility values for the pearl millet were higher than that of sorghum and maize. In contrast to sorghum, digestibility of pearl millet and maize did not decrease significantly upon cooking (Table 2.1). Protein distribution patterns of uncooked pearl millet and shifts in the different fractions as a result of cooking also resembled that of maize and not sorghum. The amino acid profile of pearl millet is more favorable than that of normal sorghum and normal maize and is comparable to the small grains, wheat, barley, and rice. On the basis of these findings, it appears that pearl millet is a nutritious and well-digested source of calories and proteins for humans. Fermentation of processed pearl millet grains leads to significant reduction in antinutritional factors of the grains, accompanied by significant improvement in the protein digestibility.

2.15 Conclusion

Some fermented products, such as rabadi, buttermilk maize, flour preparation, barley rabadi, nutritious medida, bircher muesli, crowdies, Egyptian kishk, and trahanas, are of use in daily life. As we know, pearl millet is an economical food of rural India. The technology of producing these traditional foods from pearl millet remains a household art. Great prospects exist in India for value addition and improving health benefits of pearl millet by combining milk and milk by-products and applying advanced technologies for their processing and preservation. Therefore, in order to enhance the flavor and digestibility of traditional pearl millet *weaning food*, an attempt was made to conduct feasibility studies on the acceptance of fermented pearl millet *weaning food* (made on the basis of milk-cereal weaning foods employing similar technology).

Dedication

This article is dedicated to Senior Police Officer of State Government, Uttar Pradesh, India, late Sri Satish Chandra Shukla (1959–2019), father of Author Pragya Mishra, who lost his life in an accident.

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3

Processing Methodologies for Few Plantation Crops in India (Arecanut, Betelvine, Cashew, Cocoa and Oil Palm)

C. S. Karthik, A. Pariari, Karma Chewang Bhutia, and Sonam Ongmu Bhutia

Abstract

Plantation crops include a variety of high-value commercial crops, which play a vital role in the agricultural economy and trade of many developing and developed countries. These crops are perennial in nature, and the cultivation is labour-intensive, generating considerable employment opportunity during on farm operations and compulsorily off-farm processing activities. The plantation crops are also known for its various medicinal properties, whereas in case of arecanut other than its traditional huge masticatory purpose, it is also reported in the preparation of Ayurvedic and veterinary formulations. Betel leaves are used in Indian System of Medicine against indigestion, stomach ache and diarrhoea. Cashew is also a well-accepted ingredient in dietary preparation, cocoa and oil palm are widely known for its anti-carcinogenic effects and rich source of Vitamin A and E content. India's share in combined export and import of these plantation commodities were about US $ 0.8 billion and US $ 0.4 billion, respectively (FOSTAT). Global competence can come only along with world-class quality, competitive pricing and a well-known brand. Efforts towards improvement in quality and making Indian brands popular abroad will be the key for plantation sector in the international market. Encouragement should be given to form strategic alliances or joint ventures in this sector with the objective of bringing better processing technologies and value addition, apart from branding. These agencies should constantly be updating to the latest technologies for its economic benefits with due consideration for changing consumer preferences and market structure. The Commodity Boards should activate an in-house processing

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control mechanism with legal status to enforce adherence of market-related quality parameters.

Keywords

Processing · Arecanut · Betelvine · Cashew · Cocoa · Oil palm

3.1 Arecanut

Arecanut (*Areca catechu* L.) is one of the commercial crops in India. It is used extensively in the country by all section of people as masticatory nut, popularly known as arecanut, betelnut or supari and is an essential requisite for several religions and social ceremonies. Arecanut industry forms the economic backbone of nearly six million people in India. The nuts also have medicinal and pharmacological properties. It is estimated that one tenth of the world population has the habit of betel chewing. India ranks first in both area (58%) and production (53%). However, a small quantity of arecanut is exported in its processed form or other products like panmasala, scented supari and gutka.

3.1.1 Processing of Arecanut

Arecanut is processed by two methods in different states 'chali' or 'kottapak' fully ripe dehusked graded nuts account for about 80% of production, and 'saraku' or 'kalipak' semi-ripened, dehusked, boiled, coloured and dried nuts account for about 15% (Prakash et al. 1989). The most popular type of arecanut is dried whole nuts. After harvesting, dry nuts are sun dried for 40–45 days. It is essential to spread the nuts in single layer for drying. Proper drying is essential to avoid fungal infections. Turning of nuts once in a week helps in uniform drying. The dry nuts are dehusked manually or mechanically and marketed. Good-quality chali is free from immature nuts, surface crackling, husk sticking, fungal and insect infection. Depending on the sizes, it is further classified into moti, srivardan, Jamnagar and jinni. The main producing centres of chali are Karnataka, Kerala, Assam and Maharashtra.

If the requirement for the market is tender processed nuts, then the nuts are harvested at 6–7 months maturity when they are green and soft. Karnataka and Kerala are main processing areas (Sivashankar et al. 1969). The processing consists of dehusking, cutting nut into halves and boiling with water or dilute extract from previous boiling. After boiling arecanut pieces are coated with 'kali' which is a concentrated extract after boiling 3–4 batches of arecanut, to get good-quality processed nuts. These nuts are generally sun-dried though occasionally oven drying is followed. The well-dried product should be crisp and dark brown in colour. Glossy in appearance and well-toned astringency.

Arecanut is also used for making scented 'supari'. Dried arecanuts are broken into bits, blended with flavour and packed for marketing. The flavouring of supari varies with region and market preferences. The products of tender nut processing are widely used in making scented supari. Spices and synthetic flavours are added. Rose essence and menthol are common additives. The scented supari is packed in aluminium or butter paper pouches for marketing. Recently a product blended with cashew nut bits called 'kaju-supari' and also areca tea has been developed and marketed.

3.2 Betelvine

Betelvine (*Piper betel* Linn), commonly known as pan is a perennial, dioecious, evergreen creeper, cultivated in moist, tropical and sub-tropical regions of India. It is an important cash crop of Andhra Pradesh, Assam, Bihar, Karnataka, Kerala, Madhya Pradesh, Orissa, Tamil Nadu, Tripura, Uttar Pradesh and West Bengal. In India it has been cultivated for its leaf since time immemorial but has assumed significant commercial importance in the last 20–25 years. The crop is highly labour-intensive and particularly suited to small holding. Once stablished, it becomes a perennial source of employment and cash flow for day-to-day requirements of the farmers. In India it has an annual turnover of about Rs. 7000 million providing livelihood to millions of people. This crop has great market value both inside and outside India, and leaves are also exported to countries like Bangladesh, Indonesia, Malaysia and Thailand.

3.2.1 Processing of Betelvine

Usually, betel leaves are used for chewing as fresh unprocessed. But, in certain areas leaves are subjected to processing known as bleaching or curing. It is a specialized job and only a certain class of the community is engaged in it. For bleaching only well-matured leaves are selected. Oxidation is vital for bleaching. During bleaching, there is an increase in the essential oil content and reducing sugar and a decrease in non-reducing sugars, starch and dextrose. A bleached leaf has a better taste and aroma and has more medicinal value than a fresh leaf. Betel leaves are bleached by two methods, furnace method and daban method, and the method of bleaching depends on weather conditions particularly temperature and humidity.

For bleaching, the petioles of leaves are removed, and they are arranged in concentric layers within a basket. In some cases, a vertical empty space is left in the centre for aeration. Leaves are covered with a piece of gunny cloth and sprinkled with water. Gunny cloth covering the leaves should not be too wet nor inadequately moist. If it is too wet, the leaves may spoil; on the other hand, inadequate moisture may also affect the ripening, and leaves may get dry and bleaching would be uneven. Baskets are placed in a dark room away from direct sunlight. For proper bleaching, there should be optimum moisture and temperature. Sometimes a heater or burner is kept inside the room to provide heat. As soon as the room attains the required temperature, the burner is removed. While applying heat, care is taken to see that there is no smoke. It is said that the presence of smoke affects the quality of the leaves. Usually charcoal is used as burning material.

During the process of bleaching, the leaves are often inspected and sorted. The process is repeated a number of times at an interval of 2–7 days depending on weather and season. Each leaf is examined individually, and this is continued till the leaves attain a desired level of ripening and colour. It is very important that affected leaves are removed immediately, failing which the entire lot may rot or spoil. Some chemicals like boric acid, citric acid, sucrose and potassium metabisulphite and even green coconut water have a positive effect in improving the storage life of betel leaves. Such chemicals have been used effectively for better preservation of leaves at normal room temperature (Pal et al. 1986; Iyengar 1956). Sometimes leaves are processed by applying pressure. In Kerala betel leaves are subjected to sun drying and ironing to get the desired yellow colour preferred by consumers.

3.3 Cashew

Cashew (*Anacardium occidentale* L.) is popularly known as “gold mine” of waste land. It was introduced into India (Goa) by Portuguese during the sixteenth century for soil conservation, afforestation and waste land development. It is a precious gift of nature to mankind often referred as wonder nut. It is zero-cholesterol nut with a right combination of amino acids, vitamins and minerals. Cashew contains 47% fat, but 82% of this is unsaturated fatty acids. It also contains 21% of protein and 22% carbohydrates. Nutritionally cashew stands at par with milk, egg and meat. Kernel extracted from nuts are often consumed as such or used in confectionary items and various kinds of dishes. Kernel oil pale yellow colour is used in pharmaceutical industries. Cashew nut shell liquid (CNSL) extracted from its shell has many industrial applications for making paints, resins, flame retardants, etc. Testa covering the kernel is rich in tannin. India is the largest importer (raw nuts), processor and consumer and second largest producer and exporter of cashew kernel. In India cashew is mainly grown in Maharashtra, Goa, Karnataka and Kerala along the West Coast of Tamil Nadu, Andhra Pradesh, Orissa and West Bengal. At present 3796 processing units are functioning in India under organized and unorganized sector with a processing capacity of 16.23 MT. The state Kerala have maximum processing capacity (6 lakh MT) followed by Tamil Nadu (4.0 lakh MT) and Karnataka (3.0 lakh MT).

3.3.1 Processing of Cashew

Cashew nut processing can be defined as the recovery of edible kernel from raw nut by manual or mechanical means. In India, the processing is mostly done manually and it consists of moisture conditioning, roasting, shelling, drying, peeling, grading and packing (Bhaskara Rao et al. 1993).

3.3.1.1 Moisture Conditioning

It involves sprinkling of water on dried nuts to bring to an optimum moisture level of 15–25%. Roasting of raw nuts is done to separate adhering shells from the kernels. There are three types of roasting, viz. drum roasting, oil bath roasting and steam roasting (Balasubramanian 2000). Steam roasting is the commonly used method by most of the processing units.

3.3.1.2 Drum Roasting

In this method, the nuts are fed into an inclined rotating drum which is heated to such an extent that the excluding oil ignites and burns, thus charring the shell. The drum maintains its temperature due to burning of cashew nut shell liquid (CNSL) oozing out the nuts. Roasting generally takes about 3–5 min, and the drum is rotated by hand during this period. The roasted nuts which are still burning are covered with ash to absorb the oil on the surface. The shell becomes brittle, and the rate of shelling and the outturn of whole kernels are reported to be highest in this method.

3.3.1.3 Oil Bath Roasting

This is traditional method being followed only in few processing industries of Kerala and Karnataka. In this method, raw nuts are passed for 1–3 min through a bath of heated CNSL, maintained at a temperature ranging from 190 °C to 200 °C by means of screw belt conveyor. The shell gets heated, and cell wall gets separated releasing oil into the bath. The oil is recovered by continuous overflow arrangement.

3.3.1.4 Steam Boiling

This method is adopted in the factories where hand and leg shelling machines are used. After conditioning of nuts, mild roasting is done for 20–25 min at 100–120 PSI. The nuts are allowed to cool for 24 h and taken for shelling.

3.3.1.5 Shelling (Manual)

Cashew nuts, after roasting and cooling are to be shelled (decortication) to remove kernels. In manual shelling, nuts are carefully broken using wooden mallet or light hammer in a way that whole kernel is released without damage or breakage. The expected outturn of the kernels by this method is estimated to be 90%.

3.3.1.6 Shelling (Mechanical)

Commercial processing units use foot-operated shell cutters (mechanical device) for shelling. The device consists of a pair of blades (knives) shaped in the counter half of a nut which could be operated by foot. The blades cut through the shell all around the nut, leaving the kernel untouched. After shelling, the kernels and shell pieces are separated manually. The output per worker (8 h) is estimated to be 14–22 kg of kernel as compared to 15–20 kg in case of manual shelling. The main disadvantage of this method is, while handling the mild roasted kernels, the CNSL oil may contaminate it, and varying size of the nuts require careful manipulation during cutting to avoid injury to the hands.

3.3.1.7 Drying

After shelling either manually or mechanically, the kernels are dried to reduce the moisture and to loosen the adhering testa. Most commonly used driers are the Broma driers. The kernels are to be dried to a moisture content of about 4–5%. This is done by drying the kernels in hot chambers at 70–80°C in perforated trays for about 6–8 h. After drying, the kernels are kept in the moist chamber for 24 h which facilitates easy removal of testa (peeling) and minimizes broken kernels.

3.3.1.8 Peeling

The process involves the removal of testa (seed coat) from the kernels. Peeling is done using a sharp knife or bamboo piece. Care has to be taken while removing the testa. If kernels are scratched more, it results in poor-quality kernels.

Grading and Conditioning: kernels are graded on the basis of specification prescribed by the Govt. of India under the export (quality control and inspection) act 1963, which recognizes 26 different export grades of kernels. The largest kernels come in the grade of W-210 (440–460/kg), and the smallest of the seven grades is W-500 (1000–1100/kg) (Gopinathan Nair 2002).

3.3.1.9 Packing of Kernels

Cashew nuts are subjected to rancidity and they quickly go stale. Therefore, packing should have low permeability of oxygen and moisture. Methods of packing should involve either vacuum or inert gas inside the packing materials. At present, the bulk cashew nuts are packed in containers weighing 25 lbs. Tins kept on vibrating platforms are filled by a chute. After filling and weighing, the tins are evacuated filled with CO_2 with the help of “VITAPACK” machine and sealed.

3.4 Cocoa

Cocoa (*Theobroma cacao* L.) is one of the important commercial crops of the world. It is a native of the Amazon region of South America and was known as the beverage crop before tea and coffee. The main cocoa producing countries are Ghana, Nigeria, Ivory Coast, Brazil, Malaysia and Cameroon. The cultivation of cocoa on a large scale started in India in the early 1970s. It is mainly grown as a mixed crop in coconut and arecanut plantations and as understorey crop in partially cleared forests. At present, cocoa is cultivated in an area of 17,800 hectares in India with a production of 10,000 MT. Kerala accounts for 71% of the area and 80% of the production. Though Andhra Pradesh has the second highest area, Karnataka ranks second in production.

3.4.1 Processing of Cocoa

Cocoa like other plantation crops such as tea and coffee needs processing before it may be used as food and beverage. This is because raw cocoa beans are very rich in polyphenols, which are astringent and bitter. Also, the flavour of cocoa is developed only as the beans undergo fermentation. The harvested pods should be kept for a minimum period of 2 days before opening for fermentation; however, the pods should not be kept beyond 4 days. For breaking the pods, wooden billet may be used. After breaking the pods crosswise, the placenta should be removed together with husk, and the beans are collected for fermentation. Fermentation, drying and roasting are the three stages in processing which result in production of wholesome cocoa flavour (Rajagopal and Ananda 2006).

3.4.1.1 Fermentation

Fermentation of cocoa beans is essential to remove the adhering mucilaginous pulp to develop flavour and aroma precursors, reduce bitterness and kill the germ of the seed and to loosen the testa. Different methods of fermentation normally followed are (i) box, (ii) heap, (iii) basket and (iv) tray. However, box and basket methods are recommended depending on the quantity of beans to be fermented.

3.4.1.2 Methods of Fermentation

1. The *box method* of fermentation is used in the West Indies and in many countries in Latin America which is more applicable in large estates or central fermenters. Fermentation in boxes involves the use of strong wooden boxes holding up to 1.5 tonnes of wet beans. The boxes of 60 cm × 60 cm × 45 cm made of wood and having reapers at the bottom to allow the sweating from the pulp to drain out and provide aeration are used. The boxes could be arranged in tiers for transferring beans from one to the next in line below. Two detachable wooden planks are provided on one side of the box for transferring (mixing) the beans by removing the planks.

   The beans are loaded on fermentation box and covered with banana leaves or gunny bags. The mixing of beans is effected while transferring to the next box after 24 h. The mixing is done to facilitate uniform fermentation and to maintain proper temperature, moisture and aeration during fermentation. The temperature of the fermentation mass will rise to 42–48 °C after about 48 h of fermentation. Again transferring of beans is done at 72 h of fermentation, and the final transferring is done after another day's fermentation. A total of 6 days (144 h) are required to complete the fermentation.
2. *Heap method* of fermentation is widely used in West Africa. In the hep method, beans are laid on banana leaves and covered with more leaves when the beans are heaped. The leaves are held in place by pieces of wood. Fermentation lasts foe 4–6 days, during which time the heap may be turned once or twice.
3. *Basket method* Cocoa is also fermented in baskets lined and covered with banana leaves; a method is used in parts of Nigeria and Ghana. Holes are shallow depressions in the ground that have been used in West Africa. A small weight is placed over the banana leaves. The basket is placed over a raised surface to facilitate drainage of the sweating for 1 day. Later the basket is covered with thick gunny bags. The beans are mixed thoroughly on the third and fifth days and again

covered with gunny bags. The fermentation will be completed at the end of the sixth day, and the beans are withdrawn for drying.

4. *Tray method* It is a relatively new method and offers many advantages over the traditional methods. Beans are loaded in trays (3″ × 2″ × 5″), with battens and some matting at the bottom and stacked up to 12 or 14 trays high. The bottom most trays are slightly raised from the ground and are kept empty to improve aeration and sweating. After 24 h, the stack of trays is covered with sacking and allowed to ferment. The fermentation period is shorter. The beans are not turned during fermentation, and with a stack of 12 trays, there is an effective depth of 4 feet of cocoa, and thus less space is required.

3.4.1.3 Drying

After fermentation, the beans can be dried by sun-drying or artificial drying using electric oven. The fermented cocoa beans have considerable moisture (55–69%), and the drying rate is dependent upon temperature and the airflow.

3.4.1.4 Sun-Drying

Sun-drying should be adopted as far as possible, as it gives superior quality produce when compared to that by artificial drying. The fermented beans are spread in thin layer over a bamboo mat or cement floor and dried for 5–6 days. The beans are to be stirred from time to time for uniform drying. The moisture content of well-dried beans is around 6–7%.

3.4.1.5 Artificial Drying (Electric Oven)

During the monsoon period, artificial drying is done using electric oven. The duration of artificial drying varies from 48–96 h. The drying of beans at high temperature should be avoided as it results in low-quality end product. Slow drying in the initial stage gives better-quality beans. Mould growth is to be prevented during drying as it affects the quality of the beans.

In the electric oven, beans are to be dried for 8–10 h at 50–55 °C for the first 2 days followed by continuous drying at 60 °C. The total drying period will be 72–96 h. The beans are to be stirred at regular intervals for uniform drying and to prevent clump formation.

3.4.1.6 Dry Beans Grading and Storage

The dried beans after cooling to room temperature should be cleaned before storage. The flat, slate, shrivelled, broken and other extraneous materials are removed. Where grading is needed, it can be done with a grading machine with reciprocating sieves or a rotating drum; the former type of machine often incorporates a fan that blows away any dust and small pieces of shell. The cleaned beans are packed in fresh polythene-lined (150–200 gauge) gunny bags. In many countries a cocoa sack will hold 63.5 kg, but in countries using metric weights, the bag will hold 67 kg. The bags are stored on a raised platform of wooden planks, and also high temperatures favour the development of stored product pests. Also humidity may be high to allow development of moulds. The beans should not be stored in rooms where spices, pesticides and fertilizers are stored as they may absorb the odour from these materials (Amma et al. 2004). A cocoa store must be built with these requirements in mind. The floor and walls must be of brick and cement with no cracks and crevices. The relative humidity of cocoa stores should not exceed 80% for any length of time.

3.5 Oil Palm

The oil palm (*Elaeis guineensis* Jacq.) has spread throughout the tropics from its centre of origin in Africa and being a native to West Africa. It was introduced to Malaysia during the twentieth century and commercially produced since 1917. Today Malaysia's oil palm plantations cover 40% of its cultivated land, and it has become the world's largest producer and exporter of palm oil. Oil palm is the highest oil-yielding crop unit^{-1} area and, hence, is the best source to overcome the edible oil shortage in India. The commercial cultivation of this crop in India started during 1970, and now it is grown in 16 or more countries in the world. It was introduced into India as an ornamental palm in the Botanical Garden of Calcutta. Among the ten important cultivated annual oilseed crops, i.e. groundnut, rapeseed, mustard, soybean, linseed, sesamum, safflower, sunflower, niger and castor contribute to 80% of India's vegetable oil production. Although there has been a continuous increase in the production of edible and non-edible oil, the country has been facing deficit in edible oil requirement. Our per capita annual consumption of edible oil is only 5 kg as against 20 kg or more in the developed countries.

3.5.1 Processing of Palm Oil

Processing is an integral part of oil palm plantation development. Modern palm oil mills (POM) therefore are digested to match with plantation size for their captive use. In palm oil processing, there exists two contrasting situations: (a) modern large-scale POM designed to process 20–80 tonnes fresh fruit bunches (FFB)/hour and (b) traditional processing (Arumughan et al. 1989). While a high degree of process efficiency and good product quality have been achieved by large-scale mills, the traditional processing suffers from lack of efficiency and quality problems.

3.5.1.1 Sterilization

This serves the dual objectives of inactivation of the enzyme lipase and loosening of fruits from the bunch. The loose fruits are stripped off with the aid of a mechanical bunch stripper. This device is a rotary drum with baffles and perforations. When the sterilized bunches are thrown into a rotating drum (20 rpm), the fruits are separated from the bunches, loose fruits fall through the perforators and the empty bunches pass through the other end of drum.

3.5.1.2 Digestion

Purpose of digestion is to convert the loose fruit into pulp, and in the process the cell walls are broken facilitating release of oil with the help of thermal and mechanical energy. The digester is a vertical jacketed cylindrical vessel fitted with a centrally mounted agitator having specially designed blades rotating at slow speed (25 rpm). The loose fruits are charged into the digester from the top with the aid of an elevator. Live stream is injected into the jacket and into the vessel to maintain the temperature at 95 °C (Hartley 1988). The digested mash with semisolid consistency is discharged through a discharge at the bottom.

3.5.1.3 Pressing

The digested mash consisting of oil, wafer, seed, fibre and other suspended matter is charged into a perforated cage and subjected to pressing. The hydraulic press is fitted with a plunger matching with the cage diameter. The pressing is done while the mash is hot (80–90 °C) at 750 psi. The hot oil-water mixture with suspended solids is expelled through the perforations leaving the solid press cake in the cage.

3.5.1.4 Clarification

The oil -ater mixture is filtered to remove the fibrous debris and is collected in a clarification tank. Clarifier is a vertical cylindrical vessel filled with steam coil. The oil-water mixture is diluted with hot water (1:2) and heated to 95 °C. The oil being lighter rises to the top and is decanted continuously into a collection tank. The watery sludge at the bottom is discharged as waste.

3.5.1.5 Purification and Storage

The crude palm oil from the clarifier is passed through a high speed centrifuge at 80 °C to remove the traces of solid impurities and water. The pure raw palm oil thus obtained is stored in tanks provided with steam coils.

3.5.1.6 Refining

Refining of crude palm oil (CPO) is carried out for removing impurities, free fatty acids, colour and odour. This is achieved through two routes, chemical and physical refining.

3.5.1.7 Chemical Refining

Major unit operations involved are degumming, neutralization and soap stock separation, bleaching and filtration, deodorization and polishing.

3.5.1.8 Degumming

It is the process of precipitating impurities like phosphatides, protein fragments and mucilaginous matter. The degummed oil is treated with calculated amount of caustic soda to remove free fatty acids in the form of soap. The soap stock is separated by centrifugation, and neutralized oil is washed free of alkali and soap using hot water at 90 °C followed by vacuum drying. It is adopted in the process to remove excess moisture in the oil leads to FFA release and quality deterioration storage.

3.5.1.9 Bleaching

It is done to obtain palm oil with lighter colour to remove traces of soap. Depending upon quality of CPO, 1–3% bleaching earth at temperature ranging from 100 °C to 150 °C under vacuum for 15–30 min is employed. Oil is pumped through a filter press to separate the spent earth.

3.5.1.10 Deodorization and Polishing

This is achieved by passing superheated steam at 230–240 °C under vacuum for 2 h. When fully deodorized, the oil is cooled to 55°C and pumped through a polishing filtrate to give the oil its final sparkle.

3.5.1.11 Physical Refining

Crude palm oil is first mixed with 0.1% phosphoric acid, followed by bleaching with 1–2% earth under vacuum at 100–150 °C. After suitable contact time, the spent earth is removed by filtration. This degummed and bleached oil is deaerated and steam-stripped under vacuum at 240–270 °C. Finally, oil is cooled and passed through a polishing filter. Physical method yields better-quality oil and avoids pollution. The end product of both refining methods is the same refined, bleached and deodorized palm oil (RBDPO).

3.5.1.12 Fractionation

The refined palm oil is separated into liquid fraction or palmolein and solid fraction by different processes such as crystallization, detergent fractionation and solvent fractionation.

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4 Omega-3 Fatty Acid from Plant Sources and Its Application in Food Technology

Poonam Yadav, Anil Kumar Chauhan, and Mohammed A. Al-Sebaeai

Abstract

Lipids are considered as one of the most elemental nutrients for humans. Lipid metabolism generates many bioactive lipid molecules, which are fundamental mediators of multiple signaling pathways, and they are also indispensable compounds of cell membranes. Omega-3 fatty acids are also found in smaller quantities in nuts, seeds, and soy products, as well as beans, vegetables, and whole grains. They are also involved in inflammatory processes in the body. The three types of omega-3 fatty acids involved in human physiology are α-linolenic acid (ALA) (found in plant oils) and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (both commonly found in marine oils). It is important for vegetarians to include foods that are rich in omega-3 fatty acids on a daily basis. Alpha-linolenic acid is found in many vegetables, beans, nuts, seeds, and fruits. The best source of alpha-linolenic acid is flaxseeds or flaxseed oil. For those seeking to increase their intake of omega-3 fatty acids, more concentrated sources can be found in oils such as canola (also known as rapeseed), soybean, walnut, and wheat germ. Omega-3 fatty acids can be found in smaller quantities in nuts, seeds, and soy products, as well as beans, vegetables, and whole grains. Fortification and encapsulation are the most common methods used for addition of omega-3 fatty acids to food products such as yogurt, juices, grains, nuts, fresh produce, oil, and baby food.

Keywords

Omega-3 · Fatty acid · Plant · Food

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4.1 Introduction

Omega-3 polyunsaturated fatty acids (FA) include α-linolenic acid (ALA, 18:3ω3), eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3). The potential health benefits of regularly consuming omega-3 FA have been extensively studied. EPA and DHA are recognized as the principle omega-3 FA associated with increased health benefits. Omega-3 FA have been associated with reduced risk and alleviation of many health conditions, such as coronary heart disease and arthritis, and are important for the development and functions of the brain and nervous systems (Simopoulos 1999; Ruxton et al. 2004).

Fatty acids are the basic structural components of fats and oils present in foods. Based on the presence of double bonds in their structure, they are classified into saturated fatty acids (no double bond), monounsaturated fatty acids (MUFA) (single double bond), and polyunsaturated fatty acids (PUFAs) (more than one double bond). PUFAs are grouped into omega-3 (n-3) and omega-6 (n-6) based on the presence of the first double bond from the terminal methyl carbon. Linoleic acid (LA; 18:2 n-6) and α- linolenic acid (ALA; 18:3 n-3) are the precursors of other omega-6 and omega-3 PUFAs, respectively, and are considered essential fatty acids (EFA) (Gogus and Smith 2010). In humans, ALA is converted to eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) which are considered long-chain n-3 PUFAs (Williams et al. 1983). ALA is present in high amounts in various vegetable sources such as flaxseed, canola, and soy oils, whereas fish oil and other marine food products such as algae are a good source of EPA and DHA (Moghadasian 2008).

4.2 Definition of Omega-3 Fatty Acids

Omega-3 fatty acids (n-3) are a group of polyunsaturated fatty acids (PUFAs) which include α-linolenic acid (ALA, C18:3 n-3), its long-chain metabolites eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C 22:6 n-3) (Fig. 4.1). Humans can synthesize EPA and DHA through desaturation and elongation of ALA (Gogus and Smith 2010). However, this conversion has been found to be limited. There is increasing evidence on the importance of these essential fatty acids in relation to human health and disease prevention such as cardiovascular diseases, hypertension, diabetes, arthritis, other inflammatory diseases, and autoimmune disorders (Gogus and Smith 2010; Calder 2006). Dietary recommendations for omega-3 fatty acids (2.2 g of ALA/day and 0.22 g/day of EPA and DHA; International Society for the Study of Fatty acids and Lipids, 2004) can be obtained from the diet by the consumption of foods rich in these fatty acids (Gebauer et al. 2006).

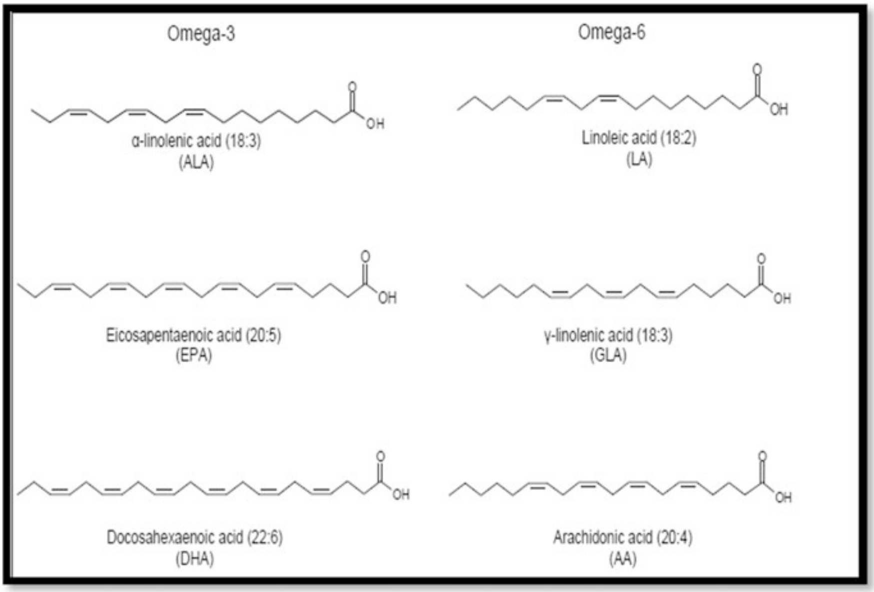

Fig. 4.1 Structures of major omega-3 and omega-6 fatty acids

4.3 Sources of Omega-3 Fatty Acid

Major sources of ALA include the seeds and oils of flaxseed, soybean, and canola, with flaxseed containing 50–60% ALA (Moghadasian 2008). EPA and DHA are obtained in the diet from aquatic and marine products only such as fish, shellfish, algae, and their oils. However, fish consumption is low in the current North American diet and consequently low in EPA and DHA levels (Moghadasian 2008). Most North Americans consume foods high in omega-6 PUFA which has resulted in 10–15 times more omega-6 than omega-3 fatty acids in the diet (Simopoulos 2008). Increased knowledge of the health benefits of omega-3 fatty acids especially EPA and DHA has led to a growing demand for products rich in omega-3 fatty acids (Table 4.1). These products represent one of the fastest growing trends in the food industry which has opened up new era for functional foods. A wide variety of omega-3 fatty acid-enriched foods are available to consumers today (Jacobsen et al. 2008).

4.4 Omega-3 Fatty Acids as Nutraceuticals

Ongoing debate about appropriate levels of long-chain fatty acid consumption has led to some confusion among consumers. The food standards code for Australia does not prescribe specific quantities of ω-6 and ω-3 fatty acid content in food products

Table 4.1 Plant sources of omega-3 fatty acids (United States Department of Agriculture 1986)

	Sources	18:3 (g/100 g)
Fruits	Avocados, raw, California	0.1
	Raspberries, raw	0.1
	Strawberries	0.1
Grains	Barley, bran	0.3
	Corn, germ	0.3
	Oats, germ	1.4
	Rice, bran	0.2
	Wheat, bran	0.2
	Wheat, germ	0.7
	Wheat, hard red winter	0.1
Legumes	Beans, common, dry	0.6
	Chickpeas, dry	0.1
	Cowpeas, dry	0.3
	Lentils, dry	0.1
	Lima beans, dry	0.2
Vegetables	Broccoli, raw	0.1
	Cauliflower, raw	0.1
	Kale, raw	0.2
	Radish seeds, sprouted, raw	0.7
	Soybeans, green, raw	3.2
	Spinach, raw	0.1
Nuts and seeds	Butternuts, dried	8.7
	Walnuts, English/Persian	6.8
	Chia seeds, dried	3.9
Oils	Linseed oil	53.3
	Rapeseed oil (Canola)	11.1
	Walnut oil	10.4
	Walnut oil	6.9

(S. Food n.d.). The food standards code specifies only the amount of PUFAs and total saturated fat content, which is inadequate for consumers wishing to make informed decisions about their dietary intake. PUFAs derived from plant sources are consumed in higher abundance than those from fish. In particular plant ω-6 PUFAs are consumed in higher quantities than ω-3 PUFAs (Newton 2008). This overconsumption of ω-6 relative to ω-3 oil has been linked to increased risk of cancer, diabetes, and cardiovascular and neurodegenerative diseases (Simopoulos 2006). To restore a more balanced consumption ratio, the ratio of ω-3 fatty acids to ω-6 fatty acids should be increased. Ratios of ω-6 to ω-3 between 5:1 and 3:1 have been suggested as optimum for human consumption (Simopoulos 2008). A recent report by the National Health and Medical Research Council included references to the amount and types of fat consumed. The consumption of PUFAs at an average of 6% of total energy and not exceeding 10% was recommended, with a reduction in the consumption of saturated fatty acids to maintain levels at 8–10% of total energy intake. In the revised versions of the same report, the recommended intake of long-chain omega-3 fatty acids (DHA/EPA) in order to lower the risk of chronic diseases was 610 mg/day for men and 430 mg/day for women (NHMRC 2003, 2005) Adequate to high dietary intake of EPA and DHA may help in the prevention of inflammatory diseases, including cardiovascular diseases, bowel diseases, cancer, and arthritis (Wall et al. 2010).

4.5 Biosynthesis of Omega-3 Fatty Acids

Animals, including humans, lack the Δ-12 and Δ-15 desaturase enzymes, which are essential for the synthesis of ω-3 and ω-6 fatty acids. As such, they are not capable of synthesizing omega-3 fatty acids de novo and must acquire them from their diets. Humans and other animals can synthesize some DHA and intermediate products such as EPA through the bioconversion of α-linolenic acid (18:3ω-3); the rest is obtained from direct consumption of DHA itself (Innis 2008). α-Linolenic acid is primarily acquired in our diet from plant sources. Further metabolism of α-linolenic acid is characterized by the action of the Δ-6 desaturase enzyme for the unsaturation of the fatty acid, followed by the action of the elongase enzyme for the addition of two carbon atoms to the molecular chain, leading to action by Δ-5 desaturase to form EPA. Earlier studies revealed that the occurrence of the desaturation process for long-chain PUFAs takes place in the endoplasmic reticulum (Innis 2008). Interestingly, the contribution of Δ-4 desaturase in the final steps of DHA synthesis (22:5ω-3 to 22:6ω-3) was established in the microbial pathway. This part of the synthesis was later interpreted in a different manner after the discovery of 24:5ω-3 with the help of the elongation of 22 carbon atoms in the mammalian pathway (Sprecher et al. 1999). The 24:5ω-3 is then desaturated to yield 24:6ω-3 in the peroxisomes where DHA is formed by the partial oxidation process (Ferdinandusse et al. 2001). Discovery of the mechanism for DHA synthesis may aid in the treatment of peroxisomal disorders such as Zellweger syndrome in infants, using DHA-supplemented diets. The unsaturation (addition of a double bond) and elongation (addition of a 2-C unit) mechanisms in the conventional fatty acid synthesis of these fatty acids are shown in Fig. 4.2. Besides the conventional fatty acid synthase (FAS) system in thraustochytrids for fatty acid synthesis, one distinct pathway named polyketide synthase (PKS) pathway (Fig. 4.3) has been observed in *Schizochytrium* sp. In contrast to the FAS system, this pathway does not require molecular oxygen.

Two distinct pathways have recently been shown to be involved in the synthesis of PUFAs in *Thraustochytrium aureum* ATCC 34204, the PKS pathway reported in lower bacteria and the conventional FAS pathway with the active role of Δ-12 fatty acid desaturase (Tau Δ-12 des) (Matsuda et al. 2012). The Δ-4, -5, and -9 desaturases and elongase enzymes have been isolated from *Thraustochytrium aureum* and heterologously expressed in yeasts such as *Pichia pastoris* and *Saccharomyces cerevisiae* (Lee Chang et al. 2013; Qiu et al. 2001). These desaturases are

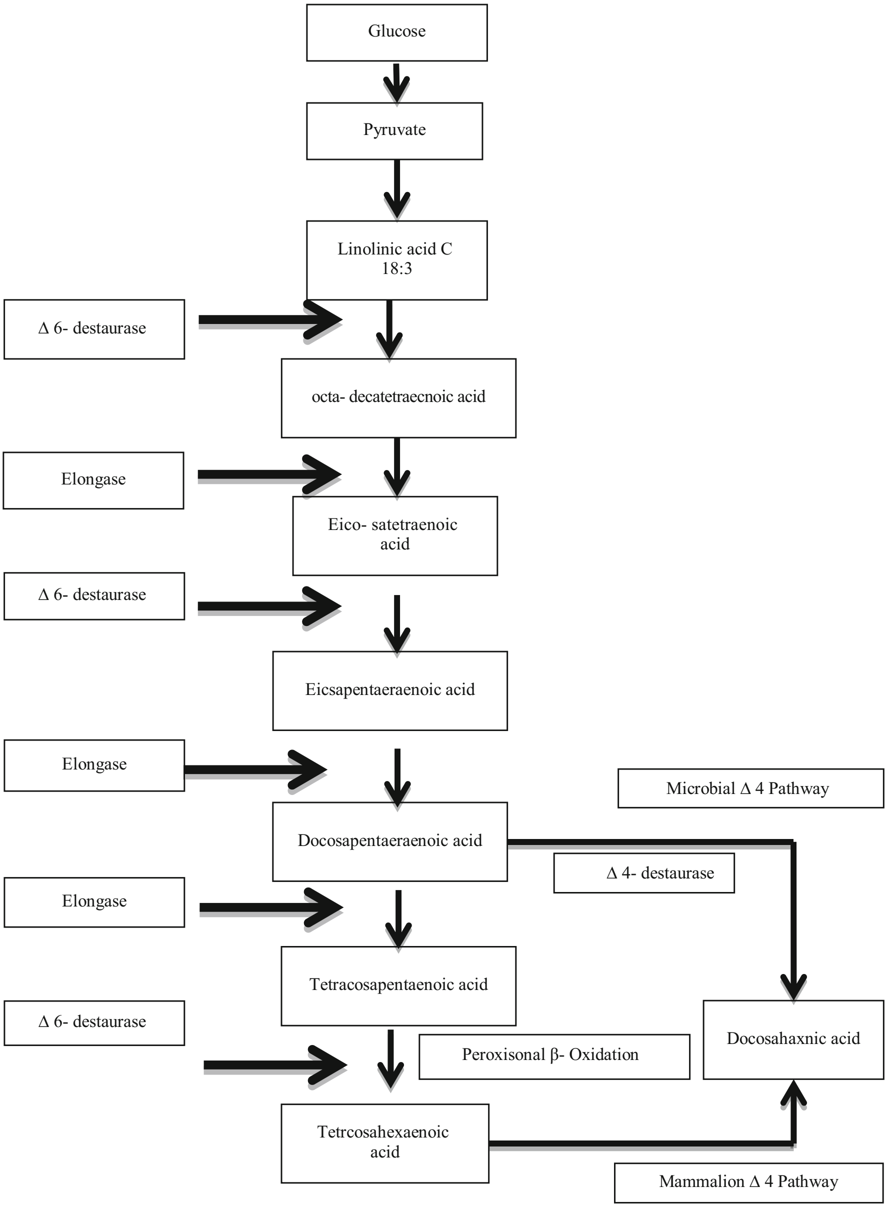

Fig. 4.2 Biosynthesis of ω-3 fatty acids (Innis 2003)

purported to be involved in the production of EPA and DHA and are the subject of a patent application (Burja et al. 2007). However, the absence of the Δ-12 desaturase enzyme in *Schizochytrium* sp. makes it incapable of converting the C16:0 and C18:0 synthesized by the FAS pathway to long-chain unsaturated fatty acids such as DHA

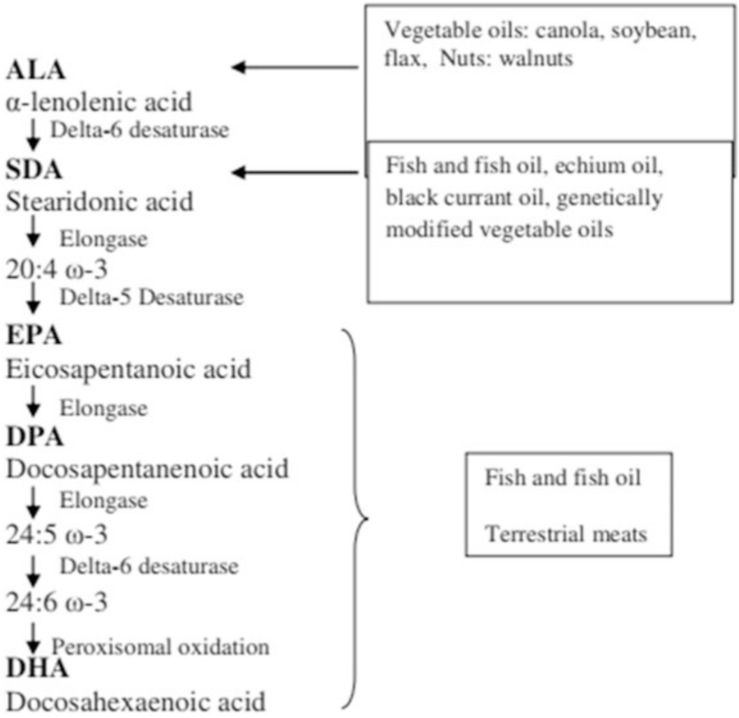

Fig. 4.3 Metabolic pathway of ω-3 polyunsaturated fatty acids (PUFAs) and traditional food sources of these fatty acids in the diet (Jay and Cheryl 2006)

(Lippmeier et al. 2009). Hence, the long-chain PUFAs are synthesized through a distinct PUFA synthesis pathway involving a multi-subunit PUFA synthase system (Hauvermale et al. 2006).

4.6 Health Benefits of Omega-3 Fatty Acids

Omega-3 fatty acid ALA, found in vegetable oils, is the precursor of EPA and DHA, which occur in marine sources. The health benefits of DHA and EPA have been extensively evaluated through a wide range of clinical studies (Innis 2003; Barrow and Shahidi 2007; Kang and Weylandt 2008) Omega-3 fatty acids have been shown to help prevent heart attack as well as decrease the overall risk of cardiovascular disease in general (Takahata et al. 1998; Masson et al. 2007). DHA is “physiologically essential” for brain and eye development, especially in infants, which has led to the addition of DHA to most infant formulas and many other infant-related food products (Russo 2009). The ability of EPA and DHA to decrease blood triglyceride levels and lower cardiovascular disease risk led to the FDA approval of the drug Lovaza™, which is an 85% EPA and DHA concentrate derived from anchovy oil.

This drug had worldwide sales of more than $1 billion and is primarily prescribed for hypertriglyceridemia 33.

EPA and DHA have been specifically recommended for secondary prevention of cardiovascular disease (Biopharma 2012) and are the focus for prevention and treatment of disorders with an inflammatory component, including type 2 diabetes, irritable bowel syndrome, rheumatoid arthritis, asthma, and several cancers. Omega-3 PUFAs have also been suggested to have an improving effect on psychiatric disorders. Ingestion of EPA and DHA partially replaces omega-6 fatty acids (especially AA) in cell membranes, particularly those of platelets, erythrocytes, neutrophils, monocytes, and liver cells. This leads to (Simopoulos 1999):

- Decreased production of prostaglandin E2 metabolites 8
- Decreased concentrations of thromboxane A2 (a potent platelet aggregator and vasoconstrictor)
- Decreased formation of leukotriene B4 (an inducer of inflammation)

Rodent studies have demonstrated that diets enriched with n-3 polyunsaturated fatty acids (PUFAs) reduce the development of diet-induced obesity (Kris-Etherton 2002). Both omega-3 PUFAs and omega-6 PUFAs are substrates for cyclo- and lipoxygenases, and omega-3 PUFAs are assumed to act as anti-inflammatory agents by competitive inhibition of the biosynthesis of arachidonic acid-derived proinflammatory prostaglandins of the 2-series. Omega-3 PUFA-derived prostaglandins of the 3-series are believed to be less inflammatory (Arai et al. 2009). Metabolic effects of long-chain (LC) omega-3 PUFAs largely depend on modulation of genes involved in fatty acid oxidation through expression of peroxisome proliferator-activated receptor (PPARα) and inhibition of lipogenesis through modulation of Srebf1 (Calder 2009).

4.7 Foods Fortified with Omega-3 Fatty Acids

A wide range of foods can be fortified with omega-3 fatty acids. Examples are given below.

A. Eggs

Commercial table eggs are a poor source of ω-3 fatty acids although they contain a high proportion of n-6 PUFA (mainly 18:2 ω-6). The simplest way is to produce an egg enriched in linolenic acid (Madsen et al. 2005), which is a precursor of DHA (Van Elswyk 1997). The hen is fed with a diet rich in linseeds, flaxseeds, or their corresponding oils; as a result the egg's yolk is enriched with alpha-linolenic acid (ALA), and the level of DHA is also enhanced (Lorgeril et al. 2013). Eggs that are derived from ALA-fed hens have higher ALA contents, while hens fed EPA and/or DHA produce eggs with higher DHA levels. In either case, arachidonic acid levels are significantly reduced. This is important because eggs are particularly rich in arachidonic acid, and attenuating arachidonic acid and its subsequent metabolism to eicosanoids is believed to be a targeted effect underlying some of the benefits of n-3 PUFA (Ferrier et al. 1995). However, most of the health-promoting properties of ω-3 fatty acids are associated with DHA; the health benefits of ALA-enriched eggs could be limited (since the conversion of linolenic acid into DHA in the human body is not always effective). This is especially so in the elderly and children when their diets are rich in ω-6 PUFAs. The second group or route to enhancing levels of ω-3 in the egg, by including preformed DHA in the hen's diet, usually in the form of fish (menhaden, herring, or tuna) oil, is a more promising one (Larsson et al. 2004).

B. Milk and Milk Products

UFA content in milk fat varies from 25% to 35% depending on different factors like breed, period of lactation, feeding regimen, and season. More than 95% of UFA in milk fat is in the form of oleic acid, linoleic acid, and α-linolenic acid (21–30%, 2–2.5%, and 1–1.3% of total fat, respectively) (Leskanich and Noble 1997). Unsaturated fatty acids (UFA) are claimed to have beneficial health effects; therefore recent studies have focused on increasing the extent of unsaturated fatty acids (UFA), particularly of conjugated linoleic acids (CLA) in milk and milk products (Collomb et al. 2000). Collomb et al. (Jones et al. 2005) showed that the concentrations of oleic (C18:1), linoleic (C18:2), and α-linolenic (C18:3) acid and CLA isomers in milk depend upon the fat source fed to the cows. A dietary supplementation with sunflower seeds led to the highest content of the cis-9, trans-11 CLA (c9t11 CLA) isomer, which is considered a very health-promoting fatty acid (FA). It represents 75–90% of the total CLA concentration in milk fat and was reported to show anticarcinogenic (Parodi 1994), body fat-reducing, and growth-promoting properties.

C. Pasta, Breads, and Cereal (Granola) Bars

Bread products may also be fortified with ALA from flaxseed (up to 300 mg per 25 g slice) and some with a combination of fish oil and a product containing DHA and EPA in a ratio of 3.5:1. Some ω-3-fortified pasta products are being made with eggs from hens fed linseed oil (>50% ALA), a novel approach for the use of these eggs. Cereals and cereal bars high in ω-3 PUFA are typically fortified with ALA from flaxseed, with levels ranging from 2000 to 5343 mg per 55 g serving (one cup) for cereals and 1500 to 2200 mg ALA per bar. No cereals that contained EPA/DHA could be identified (Jay and Chery 2006).

D. Infant Formulas and Baby Foods

Without radical changes of eating habits, an alternative way to increase the intake of omega-3 PUFA is a fortification of various food products with fish oil (Kolanowski et al. 2006). Despite a desirable elevation of omega-3 PUFA intake, fortification of foods with fish oil might negatively impact sensory quality of foods, depending on the amount of added fish oil. These are the main limitations of fish oil use for food fortification with omega-3 PUFA (Lovegrove et al. 1997). However, microencapsulation is believed to stabilize fish oil against oxidation. It makes possible to transform the oil into a powder, where the small droplets of oil are surrounded by a dry matrix of proteins and/or carbohydrate coating materials (Keogh et al. 2001).

4.8 Conclusion

Omega-3 fatty acids are considered essential fatty acids, meaning they are essential to human health. Changes in agricultural practices have decreased the content of omega-3 fatty acids (18:3ω3, 20:5ω3, 22:6ω3) in the food supply, while there has been an increase in the intake of 18:2ω6 from vegetable oils and 20:4ω6 from meat and dairy products. Leafy wild plants contain more 18:3ω3 and less 18:2ω6, whereas cultivated plants and seeds are higher in 18:2ω6 with the exception of flax.

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5 Taizzy Smoked Cheese: Updated Information

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Abstract

Taizzy smoked cheese (TSC) is one of the traditional cheeses of Yemen, specifically in Taiz City. It is delicious and more favorable by the local consumers of Yemen. Taizzy smoked cheese is produced through traditional process using clot (rennin) animal sources or microbial sources. Clot from animal sources are communally used. Taizzy smoked cheese is a semihard cheese in small wheels with thin brown smoked rind. The shelf life varies depending on the salt content. The shelf life of low-salt cheese (Sweet) is 7–10 days, where it is extended to 2–4 weeks for salted cheese. The disadvantage of cheeses is that they are exposed to different types of contamination during manufacturing or processing with different kinds of microbial contamination and heavy metals.

Keywords

Taizzy · Smoked · Cheese · Traditional cheeses · Yemen

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5.1 Introduction

Taiz is the main area for farmstead cheese production in Yemen. It is a milk cheese-oriented system including farmers and shepherds. Milk production, cheese processing, and marketing are the main economic activities and the sources of income for farmers and shepherds in rural areas of Taiz, Yemen (Aita and AL Ramesy 2013).

Smoked cheese is a cheese that has been specially treated by smoke curing. It typically has a yellowish-brown outer pellicle which is a result of this curing process. Smoke curing is typically done in one of two ways: cold smoking and hot smoking. The cold smoking method (which can take up to a month, depending on the food) smokes the food between 20° and 30 °C (68° and 86 °F). Hot smoking partially or completely cooks the food by treating it at temperatures ranging from 40 °C to 90 °C (104–194 °F). Another method of "curing" used in less expensive cheeses is to use artificial smoke flavoring to give the cheese a smoky flavoring and food coloring to give the outside the appearance of having been smoked in the more traditional manner (James n.d.). The most common smoked varieties of cheese are Seretpanir (Iran), Caramakase (Germany), Bandal (India), and Provolone (Italy) (Amran and Abbas 2011).

Taizzy smoked cheese is the traditional cheese and the most commonly consumed cheese in Yemen. The product is considered as a semihard cheese with about 40% moisture content and characterized as a salted cheese with an attractive light brown color imposed by smoking (Thabet et al. 2013).

5.2 Cheese Production in Yemen

According to FAO Statistics in 2010, total cheese supply in Yemen was 33,737 tons, of which 11,000 tons were imports, while the balance (22,737 tons) was manufactured locally (Table 5.1). Table 5.2 refers to the breakdown of cheese exports and imports in 2010. Consumption of cheese in Yemen is relatively low. Cheese may be eaten solely or with other dishes (sahawek). Cheese production in Yemen constitutes the primary activity at the small dairy sector. Typically, the amount of milk processed to cheese by farmers is seasonal as the supply of milk.

Table 5.1 Annual cheese productions (tons, Yemen, 2005–2011)

Cheese product	2008	2009	2010	2011	2012	2013	2014
Cheese of goat milk	4932	5228	5345	5345	6037	6763	7511
Cheese of sheep milk	3900	4212	4300	4300	5664	6137	6637
Cheese of skim cow milk	6238	6675	6890	6890	5069	5261	5487
Cheese of whole cow milk	5678	5987	6202	6202	4444	4563	4789
Cheese (all kinds) + (total)	20,748	22,102	22,737	22,737	21,214	22,724	24,424

Table 5.2 Cheese import and export (tons, 1000$, Yemen, 2005–2010)

	Cheese, processed		Cheese, whole cow milk		Total	
	Export	Import	Export	Import	Export	Import
2008	0	4773	323	11,588	323	16,361
2009	0	6448	180	13,617	180	20,065
2010	0	1928	0	11,000	0	12,928
2011	0	5618	146	15,676	146	21,294
2012	0	8205	19	17,042	19	25,247
2013	0	7223	0	19,743	0	26,966

The amounts of milk processed to cheese are 6–12 kg of milk in summer and 2–7 kg in winter. In general, the quantity of smoked cheese produced per day would be at a range of 1–4 kg and that of soft cheese produced per day would be around 1–2 kg (Aita and AL Ramesy 2013).

5.3 Smoking of Food

Smoking of food is one of the oldest methods used from 10,000 years. Sensory active components such as phenol derivatives, carbonyls, organic acids and their esters, lactones, pyrazines, pyrroles, and furan derivatives are responsible for many of the aromatic properties of meat products (Šimko 2002). Khamis (2011) reported that smoking of food and food products is a common and old process and one of the best ways to keep food from spoiling since wood smoke contains substances that inhibit the growth of spoilage organisms, thus keeping the quality of the product, and also imparts an agreeable taste and appearance to the food. The smoking process of cheese is well known and widespread in some European countries and the United States long ago. On the other hand, the smoked cheese is recently known in Egypt as a product that covers the needs of foreigners and tourists.

Traditional methods of smoking foods involve fairly simple equipment based upon easily obtainable sensory properties. The traditional equipment used for smoking foods usually consisted of a smoker where pyrolysis of wood was induced. The smoker was loaded with wood that was burned, and the resulting smoke was channeled in a direction so that direct contact with the food could be obtained. These traditional goals of smoking foods were to impart and develop desirable sensory (flavor, aroma, and appearance) properties as well as rendering the food product safe to eat. The same author illustrated that additional application technology allows for alternative means for smoking such as glimmer smoke, liquid smoke, friction smoke, wet smoke, and smoke chambers which can accommodate both batch and (semi-) continuous flow systems in conjunction with computerized controls. Also, new smoke generation systems are mandated to employ equipment or scrubbers to aid in the cleaning of smoke, which strengthens its case for an environmentally friendly processing technology (Fessmann 1995).

There are different smoking methods that can be used like:

Smoking with freshly generated smoke from wood
Smoking with smoke regenerated from smoke condensates
Flavoring with smoke flavor preparations derived from smoke condensates
Flavoring with smoke flavors prepared by mixing chemically defined substances

The smoke flavorings can be divided into two main groups (Šimko 2010): smoke flavorings made from smoke from burning wood, i.e., condensed smoke possibly with adjuncts added and where some components of health concern may have been removed, and synthetic smoke flavorings.

There are many methods in using synthetic smoke flavorings like liquid smoking flavorings (LSF). LSF is used in the following forms (Ismail 2016):

Liquids for spraying, nebulization, immersion, or showering
Emulsions incorporated into foods by injection or mixing
Water-mixable emulsions for showering or curing brine
Powders such as maltodextrins, salt, saccharides, starch, proteins, and seasoning solutions in vegetable oils

5.4 Taizzy Smoked Cheese

Smoked cheese, also called Taizzy cheese, is the traditional cheese and the most commonly consumed cheese in Yemen. Taiz is located in the southern Yemen. The smoked cheese is produced from mostly raw goats and powder milk in farms or mostly private houses. The product is considered as a semihard cheese with about 40% moisture content and characterized as a salted cheese with an attractive light brown color imposed by smoking (Al-Zoreky 1998). The smoked cheese is popular among consumers, and, while large quantities of cheeses are sold immediately after production, its texture is semihard or hard, and it is sold in disks, at room temperature. The fresh cheese product has a characteristic with a nice brown color all over the surface of the cheese and has a characteristic aroma (Thabet et al. 2013).

5.4.1 Taizzy Smoked Cheeses Processing

There is no standardized technique for the manufacture of smoked cheese, only using traditional methods in the different geographical locations in Yemen without species starters. It is true that it is potentially unsafe and could cause problems in the future if its production conditions are not improved. These types of cheese were marketed 7 days after production.

5.4.2 Clot Milk Preparation

Clot milk is extracted from the stomach of young goats, which are no older than 2 weeks, to be used as the milk curdles. (Fig. 5.1).

5.4.3 Smoking Wood

A certain type of wood known as Mazeez/Sarab is used as a smoking agent in the final stage of cheese processing. Mazeez trees are naturally grown in rural areas. It is important to use dry wood to give cheese the typical smoked flavor and brown color (Fig. 5.2).

Fig. 5.1 Clot milk uses in producing of Taizzy smoked cheese

Fig. 5.2 Almazeez wood used in cheese smoking

5.4.4 Processing Method of Taizzy Smoked Cheeses

The processing methods of Taizzy smoked cheese are shown in Scheme 5.1 (Fig. 5.3).

5.4.5 Chemical Composition of Taizzy Smoked Cheese

Taizzy cheese is a traditional cheese made of raw goat and cow's milk. The average composition for smoked Taiz cheese is 47.17% moisture, 21.22% fat, 13.92% protein, and 5.22% salt. The average composition of Oob is 58.02% moisture, 19.75% fat, 15.27% protein, and 3.21% salt (Aita and AL Ramesy 2013). Chemical compositions of the smoked and nonsmoked cheese samples are presented in Table 5.3.

Scheme 5.1 Processing of methods of Taizzy smoked cheese

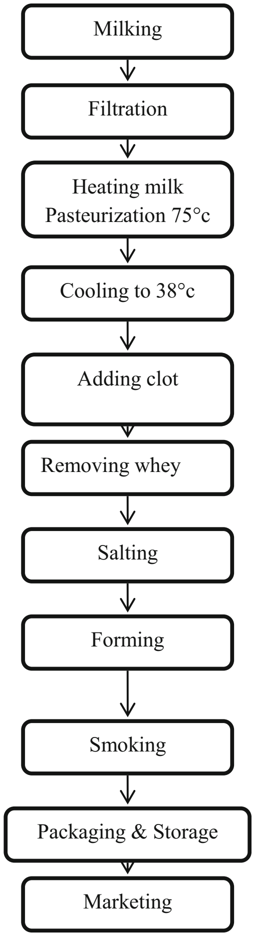

Fig. 5.3 Marketing of Taizzy smoked cheese

Table 5.3 Chemical compositions of the smoked and nonsmoked cheese samples

Sample	Moisture (%)	Fat (%)	Protein (%)	Salt (%)	References
Taizzy smoked cheese	44.09	21.6	18.7	4.3	Amran and Abbas (2011)
Nonsmoked cheese	56.9	21.6	18.7	4.3	Amran and Abbas (2011)
Naturally smoked Circassian cheese	62.17	27.56	25.26	3.71	Aydinol and Ozcan (2013)
Liquid smoked Circassian cheese	62.27	30.05	25.15	2.97	Aydinol and Ozcan (2013)
Nonsmoked Circassian cheese	60.02	28.10	26.51	2.98	Aydinol and Ozcan (2013)

Table 5.4 Mineral composition in Taizzy smoked cheese

Element	Cd (μg/g)	Fe (μg/g)	Cu (μg/g)	Mn (μg/g)	Pb (μg/g)
Amount	0.73	7.84	1.56	0.47	0.53

5.4.6 Mineral Compositions in Taizzy Smoked Cheese

The traditional smoked cheese, which has been produced at home or in small scale and sold in the market without packing, result to major heavy metal-contaminated smoked cheese, leading to environmental pollution. Many studies showed that cadmium and lead are heavy metals with the highest concentration in Taiz areas compared to Ibb area; that is due to the many industry factories in Taiz and transportation vehicles. Most of the minerals in this study has higher concentration compared with earlier reports, since these minerals are transferred to smoked cheese from water, containers, and utensils during manufacturing processes and the selling of cheese (Thabet et al. 2013) (Table 5.4).

5.4.7 Microbial Content in Taizzy Smoked Cheese

The microbiological accounts of Taizzy smoked cheese depend on many factors, viz, verities of milk, type of cloth, processing, and handling. The microbiological accounts studied by Thabet et al. (2013) and the results are shown in Table 5.5.

5.4.8 Hygienic Conditions of Taizzy Smoked Cheeses

Most traditional cheeses are usually produced under poor hygienic conditions with different manufacturing technologies that are dependent on the geographical location (Freitas and Malcata 1999). It has to be noted that there is no standardized processing method for smoked cheese production. Raw milk contaminated with foodborne pathogens and introduced into dairy processing plant constitute a risk to human health if used unpasteurized for the production of some types of cheeses or in case of cross contamination with pathogens (Kousta et al. 2010). The safety of raw milk cheese has been questioned, however, as several large outbreaks of foodborne disease due to consumption of raw milk cheeses have been reported in the past 10–20 years (Marth and Steele 2001). Humans have been found to serve as contamination source of cheese with pathogenic bacteria like *S. aureus* (Callon et al. 2008).

Cheese are exposed to different types of contamination during manufacturing processes with different kinds of heavy metals such as cadmium, nickel, lead, copper, and mercury (Ayar et al. 2009). It causes many health problems such as weakness, heart failure, and induced cancer diseases and also affects the kidney. In 2008, the National Oncology Centre (NOC), located in the capital, Sana'a, reported that there are 360,000 cancer cases in Yemen at present, with 22,000 new cases each year and 12,000 annual cancer-related deaths. Most of these cases and kidney failures were related to consumption foods and water, which contaminated with minerals. The heavy metal content of cheese is variable due to factors such as differences between species, geographical area, characteristics of the manufacturing practices, and possible contamination from the equipment during the process (Thabet et al. 2013).

Table 5.5 Microbiological accounts of Taizzy smoked cheese

Parameter	log (cfu/g)
Mesophilic	5.08
Proteolytic	2.66
Halotolerant	4.72
Staphylococcus	4.16
Coliform	4.04
E. coli	3.06
Yeast and molds	6.12
Salmonella spp.	Nd

5.5 Conclusion

Taiz cheese is not well known in the world; it is the only kind of cheese known in Yemen and some of the Arab countries. It is a very old cheese as observed from its old method of manufacturing. Taiz cheese is originally made from raw sheep, goat, cow, and camel's milk or a mixture of these milks. Taiz cheese has many kinds (fresh, soft, dry, half-dry, smoked and unsmoked, with and without salt). No enough studies were performed before on this type of cheese.

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Plant-Based Milk Substitutes: A Novel Non-dairy Source

6

Sonika Pandey and Amrita Poonia

Abstract

Plant-based milk substitutes or non-dairy substitutes are among the fastest growing beverage segments worldwide. There are many reasons behind such growth. Lactose intolerance is one of the main reasons. Such milk substitutes are naturally free from lactose and have lower amount of cholesterol and fat than milk from animals. These substitutes are often more easily digested than dairy products and these also favor those people who do not suffer from lactose intolerance. Other than soy, which is the main driver of this growth instead, innovative beverages are made from nuts, grains, and seeds. These substitutes are manufactured by extracting the plant material in water, separating the liquid, and formulating the final product. Homogenization and thermal treatments are necessary to improve the suspension and microbial stabilities of commercial products that can be consumed as such or be further processed into fermented dairy-type products. The nutritional content depends on the plant source and further fortification.

Keywords

Non-dairy substitutes · Lactose intolerance · Homogenization · Thermal treatments · Fortification

6.1 Introduction

India follows a tradition of consuming milk that goes back to 2000 BC. Milk is mainly used in daily cooking and during festivals and is also offered as prasadam to deities in temples. Going dairy free through whole food and plant-based diet, though

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a relatively new concept in India, is steadily increasing in scope in the recent 4–5 years. It is very difficult to remove dairy from the diets of vegetarians, chiefly Indians. Nevertheless, over the years, dairy quality is deteriorating, as well as the overall health of people.

Dairy milk that comes from animals is a very good source of many vitamins and minerals including calcium. It is also sometimes fortified with vitamins A and D. Plant-based milk substitutes are the non-dairy beverages and may or may not be nutritionally equal to dairy milk. But they can be fortified with nutrients to make them nutritionally equal to dairy milk. Still, non-dairy beverages are good for those suffering with milk allergies or lactose intolerance. They are also very easily digested. Plant-based milk substitutes are boon for the countries where the supply of milk is not enough. The most broadly consumed plant-based milk substitute is soy milk. The first commercially booming product was launched in Hong Kong in 1940. The market grew rapidly during the 1970s and early 1980s in Asia after the technology for large-scale production of soy milk was developed (Chen 1989). The two major raw materials used for preparation of milk-like products are groundnut and soybean (Sangita et al. 2016). Overall, the dairy substitute market is still growing.

6.2 Types of Plant-Based Milk Substitutes/Non-dairy Alternatives

6.2.1 Almond Milk

Probably the most widely used milk substitute, almond milk, is a creamy, sweet, healthy, and nutritional powerhouse among foods. It is rich in omega-6 fatty acids. It is made from ground almonds and filtered water. It usually contains sweeteners and other ingredients. The shelf life can also be increased by the added ingredients. It may also be fortified with vitamins and minerals (Fleming 2008). This milk has alpha-tocopherol which is a powerful antioxidant and protects against harmful free radicals (Burton and Ingold 1989).

6.2.2 Soy Milk

Soy milk has the most of protein and in that view is comparable to cow's milk**.** It is made from ground soybeans and filtered water (Fleming 2008). It may contain sweeteners and flavors. It is very rich and creamy. Soy milk can be taken directly, with cereal or in coffee. The taste is nutty and slightly sweet. For the cooking purpose, soy milk is one of the best milks to choose. Its stability at high temperatures makes it a good choice for savory dishes and sauces. Soy milk because of its high protein content is best choice in baking. Soy milk has goodness of isoflavones and phytosterols which are effective against cancer, cardiovascular disease, and osteoporosis (Omoni and Aluko 2005).

6.2.3 Peanut Milk

It is produced by soaking and grinding full-fat raw peanuts with water to get a slurry subject to filtration. The milk-like product produced is further homogenized and pasteurized in the same manner as fresh milk and also fortified with vitamins and minerals and is at times flavored (Chan and Beuchat 1992). It has been well-known that peanut milk is rich in protein, minerals, and essential fatty acids such as linoleic and oleic acids, which are considered to be highly valuable in human nutrition. It is widely used in India and other emerging countries by vegetarian mass and in recent times by children allergic to cow's milk (Kouane et al. 2005). It is good in phenolic compounds and protective against diseases like coronary heart disease, stroke, and cancers (Wien et al. 2014).

6.2.4 Rice Milk

This is typically made from rice and filtered water. It may have some oil added. It also may be fortified with vitamins and minerals. It may be sweeter than cow's milk (Fleming 2008). It is commonly used milk which is a healthy and nutritious option for those looking for an alternative to dairy products. As compared to products like cashewnut milk and coconut milk that are more expensive, rice milk, made from one of the world's most common and frequently cultivated grains, is typically found at a cheaper price point than other non-dairy milks. It is a healthy blend of carbohydrates and protein, showing a very minimal amount of fat per serving. It is best in taste when mixed with the powerhouses of kale, berries, bananas, flax seeds, and avocados. Rice milk is rich in β-sitosterol and γ-oryzanol that lower cholesterol, hypertension, anti-diabetic, anti-inflammatory, and antioxidative effects (Biswas et al. 2011).

6.2.5 Coconut Milk

It is prepared by steeping of finely grated coconut meat in hot water and then filtering to get milk. It is rich in fat but low in proteins. Coconut milk is best for drinking straight from the glass, added to coffee or smoothies or poured over cereal. It works well in cooking and baking (Fleming 2008). It is found to be rich in lauric acid which stimulates brain development, lifts immune system, and sustains the elasticity of the blood vessels (Seow and Gwee 1997).

6.2.6 Hemp Milk

It is made from soaked hemp seeds and filtered water. It may also contain sweetener according to the requirement, followed by fortification with vitamins and minerals (Fleming 2008). Hemp milk has more protein than other non-dairy milks other than soy milk and is also rich in omega-3 fatty acids. It is thick and creamy. It has a strong taste that may be appropriate for making savory dishes, and its protein content is useful in baking.

6.2.7 Oat Milk

This is formulated with presoaked oat groats that are hulled grains broken into fragments. Oat milk is light with little sweet texture. It is compatible to low-fat or fat-free cow's milk. It can be taken directly from the glass, over cereal or in smoothies. It can be applied for both sweet and savory dishes. Since it is light in texture, it is good for light cream soups and curries, while its sweet taste makes it work for baked goods. It has goodness of β-glucan that reduces the blood glucose level and hypocholesterolemic effect by lowering total and LDL cholesterol (Deswal et al. 2014).

6.2.8 Cashew Milk

Cashew milk is similar to almond milk in that it does not contain much protein. It is not only made from nuts, but also it is less known than rice, coconut, and oat milks. But due to its high nutritional content, it must be considered important. It is used in baking or making of sweets, directly consumed, or taken with cereal. Cashew milk is nutrient dense as compared to soy and dairy milks with less calories. It promotes cardiovascular health and reduces the deficiencies of trace mineral such as zinc and iron (Manzoor et al. 2017).

6.3 Process

Plant-based milk substitutes are colloidal suspensions or emulsions mainly containing dissolved and disintegrated plant substance. These are prepared firstly by grinding the raw material into slurry and then straining it to eliminate coarse particles. There are many kinds of methods of processing, but the general outline of a modern industrial-scale process is essentially the same. The plant material is both soaked and wet-milled to extract the milk constituents, or alternatively the raw material is dry milled, and the flour is extracted in water. The waste generated by grinding is separated by filtering or decanting. After standardization, the kind of additives and other ingredients, such as stabilizers, coloring agents, and sweetening agents, may be added, followed by homogenization and pasteurization/ultra-high-temperature treatment to improve suspension and microbial stabilities. Spray drying process is also useful for production of powder (Diarra et al. 2005).

6.4 Pre-treatment of Raw Material

Methods involved in pre-treatment include dehulling, soaking, and blanching (Debruyne 2006). To inactivate trypsin inhibitors and lipoxygenase, blanching is done; otherwise that would result in off-flavors in soy milk (Giri and Mangaraj 2012). On roasting of the raw material, it increases the aroma and flavor of the final product, but heating reduces the protein solubility and extraction yield (Rustom et al. 1991; Hinds et al. 1997a).

6.4.1 Extraction

As the temperature of extraction is raised, it increases the extractability of fat, but the denaturation of proteins decreases their solubility and yield (Rustom et al. 1991).

When the hot water extraction of cowpea milk was done, it decreased the yield and protein content as compared to the cold water extraction, but it slightly improves protein digestibility due to trypsin inhibitor inactivation and leads to a decreased extraction of phytic acid (Akinyele 1991). During extraction, alkaline pH increased the protein extractability, but a neutralization step may be required in the process (Rustom et al. 1991; Aidoo et al. 2012).

6.4.2 Separation

Once the extraction step is completed, coarse particles are removed from the slurry by filtration, decanting, or centrifugation (Lindahl et al. 2001; Diarra et al. 2005). Peanuts, as raw materials, has high amount of fat; the excess fat can be removed using a separator as in dairy processing (Diarra et al. 2005).

6.4.3 Formulation of Product

After separation of coarse plant material, other ingredient may also be added like vitamins and minerals for fortification, with sweeteners, flavorings, stabilizers, and emulsifiers. Since the suspension constancy is a problem in plant milk substitutes, hydrocolloids are often used to increase the viscosity of the continuous phase, and also emulsifiers have been proved successful in some beverages. Sodium stearoyl-2-lactylate (SSL), a lipid surfactant, has been found to bind particularly to partially hydrolyzed oat proteins and thus enhance the stability of oat protein suspensions (Chronakis et al. 2004). Rice-based beverage stability was increased by using pine nuts as these beverages contain proteins with good emulsifying properties (Lee and Rhee 2003).

By using a stabilizer mix for dairy products containing mono- and diglycerides, glyceryl monostearate, guar gum, and carrageenan, it gave the most stable peanut milk (Rustom et al. 1991), whereas good results were also found with 0.02–0.04% carrageenan and 0.2–0.4% mono- and diglycerides (Hinds et al. 1997c).

6.4.4 Homogenization

Homogenization decreases the particle size distribution and improves the stability of plant milk substitutes by disturbing aggregates and lipid droplets (Malaki Nik et al. 2008). Due to high quantity of lipids present, an emulsion is formed resulting in a creamier, more homogenous product. Homogenization as in the conventional dairy-processing pressure range (ca. 20 MPa) is reported to increase the suspension stability of at least soy, peanut, and rice milk substitutes (Rustom et al. 1991; Hinds et al. 1997b; Lee and Rhee 2003). Higher homogenization temperature has been reported to increase the stability of peanut milk (Rustom et al. 1991; Hinds et al. 1997a). When ultra-high-pressure homogenization (UHPH) of soy milk at 200–300 MPa was done, it reduced the particle sizes intensely and improved the stability compared with conventionally processed products. The treatment also reduced microbial counts and can be used for preservation (Rustom et al. 1996). A greater homogenization temperature has been reported to increase the stability of peanut milk (Rustom et al. 1991; Hinds et al. 1997a).

6.4.5 Storage and Shelf Life

A peanut beverage was treated for 4 and 20 s at 137 °C. The longer treatment time increased the taste and quality but decreased the suspension stability. Both the treatments increased the microbial shelf life, and no growth of spores and mold was seen (Rustom et al. 1996). The commercial plant-based milk substitutes are pasteurized or UHT treated to improve the shelf life. But the heat changes that occur in protein properties not only influence the stability but change the flavor, aroma, and color (Kwok and Niranjan 1995; Rustom et al. 1996).

To avoid UHT-related changes in flavor, Sikhye, a Korean rice beverage, is commonly sold frozen. However, *Bacillus cereus* spores are a risk, and their number has successfully been reduced by a procedure consisting of heating to 80 °C to activate spore germination, followed by heating to 95 °C known as tyndallization (with CO_2 injection) (Kim et al. 2012).

6.5 Acceptability

Now people around the world are accepting plant-based milk substitutes. The major problems in developing countries are due to the deficiency in protein intake by poor people. The quick solution for this problem is there should be the utilization of vegetable protein with low cost and good quality. Many a time the milk substitutes are blended together to improve taste and nutritional quality of the product. Based on

Table 6.1 Some plant-based milk fermented products

S. No.	Fermented products	Benefits	References
1.	Fermented soy milk beverage	Reduced the amount of flatulence-inducing oligosaccharides depending on the a-galactosidase activity of the strain and increased the angiotensin- converting enzyme (ACE) inhibitory activity	Donkor et al. (2007)
2.	Fermented oat milk beverage	Strains of *Leuconostoc mesenteroides*, *Leuc. dextranicum*, *Pediococcus damnosus*, and *Lactobacillus kefiri* produced the highest levels of lactic acid, resulting in a pleasant flavor	Martensson et al. (2000)
3.	Peanut milk with kefir	High minerals and essential amino acids content	Bensmira and Jiang (2012)
4.	Probiotic milk with added quinoa milk	Improved nutrition	Casarotti et al. (2014)
5.	Lupin milk-based cheese	Enhance taste, texture, flavor, and overall acceptability of both fresh and mature cheese	Elsamani et al. (2014)
6.	Fermented tiger nut milk	Enhanced the growth of the incorporated probiotic bacteria strains	El-Shenawy et al. (2016)

roasted peanut milk, a probiotic fermented beverage was developed. It was a blend of yellow millet and roasted peanuts (Kabeir et al. 2015). Two peanut-based beverages were formulated due to their good source of protein, wide offer, and low cost. The peanut milk was enriched with umbu and guava pulp (de Albuquerque et al. 2015). Flavored lactose-free milk, non-dairy powders, low-calorie cheese, and lactose-free ice cream are some non-dairy milk products (Dekker et al. 2019). Few plant-based milk fermented products are listed in Table 6.1.

6.6 Conclusion and Future Research

Plant-based milk substitutes represent a huge expansion for the healthy food market. In the future, we anticipate many different new product launches in this expanding section of the dairy industry. It needs a proper research through the development of advanced processing, technological interventions, and fortification techniques, for developing a complete nutritional beverage with high overall acceptability. To keep up with the competitive demand of the market, research is a true challenge: further studies on functional properties, stability, and sensory acceptance are needed while keeping up with the cost and environmental impact in mind.

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Makhana: Dry Food and a Potential Aquatic Cash Crop 7

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Abstract

In traditional makhana markets, four quality types of pop are distinguished: lava, murha, turi, and mix. The differences in quality are almost exclusively linked with the size of the pop. Makhana transactions in these traditional markets are done in gunny bags. These gunny bags are standardized in size, and the weight of such a gunny bag is indicative of the quality of makhana. If makhana is processed well, makhana pops are larger and weigh less, and a low-weight bag is thus an indication of good quality.

Keywords

Makhana · Dry food · Cash crop · Quality · Processing

7.1 Introduction

Makhana also known as Gorgon not or Fox nut or Prickly water lily is an important a kind of dry fruit produced from an aquatic plant Euryle ferox Salisb. It is grown in stagnant perennial water bodies like ponds, land depressions, oxbow lakes, swamps, and ditches. Vernacular names are as follows: in Sanskrit, makhana or padma; Hindi and Bengali, Makhana or makhna; Telugu, Mellunipadmamu; Uriya, Kuntapadamu; Punjab, jewar or jaibar; Gujarati, makhana; "thangjing" in Meeteilon, i.e., Manipuri, makhana, nikori (in Assamese), Marati makane, kamalabeeja, or makhana; Indochina, kedru or khiem; China, chien shish or chi-t'ou; Malaya, siewsat; Persian, mukareh or mukhareh; Urdu, akhana or kautapadma; and Onibas (Onibasu) in Japanese. Synonyms of Makhana are as follows: Makhonna, Makhana, Padmabijabha, Padma, Padmabija, Padmaksham, Padmakam, Padmakarkati,

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Paneeya phala, Bheda, Bhedaa, Krouncha, Krounchadhana, Krounchadhini, Kandhalee, Naapaka, Gaalodya, Galodya, Gilodya, Kalodya, Ankalodya, Hraswotpalakanda, Ciccodaka, Kalankataka, Kantapadma, Andika, and Andira. Makhana is a species of Nymphaeaceae family. It is also known as Kenjitsu, Komsil, Hin sat, Qian shi, Ji tou, Ji tou shi, Tao Quin shi, Su quin, ji tou mi, and prickly lily seed in different parts of the world. Evidence from archaeobotany indicates that *Euryale ferox* was a frequently collected wild food source during the Neolithic period in the Yangtze region, with large numbers of finds coming from the sites of Kuahuqiao, Hemudu, and Tianluoshan (Goren et al., 2014.) The earliest recorded use of *Euryale ferox* to date was found in Gesher Benot Ya'aqov, Israel, among artifacts of the Acheulean culture 790–750,000 years ago.

The texts of Ayurveda describe properties of foxnut or makhana as follows:

makhannam snigdhavrishyam cha garbhasamsthapakam param I *Vatapitta haram balyam sheetam pittasradaahanut* II.

Makhana increases stickiness of secretions by increasing moisture level in the body. Hence it increases quality and quantity of semen and is useful in impotence. It helps to increase the fertility in women and reduces vata and pitta. It strengthens the body and reduces burning sensation and quenches thirst.

"Makhana" is derived from the Sanskrit word for grain Mak. Mak is the meaning of sacrifice – grain that is widely used in sacrifice. Makhana was the food of the gods they worship, and it comes in a gift. Makhana is aphrodisiacs food.

Euryale ferox is the only extant species in the genus *Euryale*. It is a flowering plant classified in the water lily family, Nymphaeaceae, although it is occasionally regarded as a distinct family Euryalaceae. Unlike other water lilies, the pollen grains of Euryale have three nuclei (Cronquist 1981).

7.1.1 Characteristics

7.1.1.1 Nature: Neutral; Sweet and Sour; Attributive to Kidneys and Spleen

Morphology

Root

The germination of makhana seed is of hypogeal type. Upon the germination, the cotyledons and hypocotyls of seeds remain in the soil. It has thick fibrous roots comprising 3–5 clusters each consisting of about 15 rootlets. The roots are thick, long (40–50 cm), fleshy, and fibrous in nature and also have a number of air pockets. The plant has rhizomatous stem. The rhizome is characterized as short, thick, and erect. Stem is less prickly, aquatic herb , rootstock short, and rhizome thick.

Leaf

The leaves (Figs. 7.1, 7.2, 7.3, and 7.4) are submerged; oblong, peltate, elliptic, or orbicular in shape; corrugated about 6–175 cm in diameter; reddish green above and deep bluish purple beneath with strong spiny rib downy; and densely spinous. Spines are sharp and curved on the under and upper surface. Ribs are dichotomously branched over the whole leaf. The petiole is long, wavy, and spiny in the leaf while in the bud is curiously folded up and enclosed in an involucre which bursts as the leaf expands. Leaf stalk is attached in the center of the lower surface. The leaves have a quilted texture, although the stems, flowers, and leaves which float on the surface are covered in sharp prickles. Other leaves are submerged.

Flower

The flowers (Figs. 7.5, 7.6, 7.7, and 7.8), however, are very small related to the size of the plant, and they open during the day. You have to be vigilant to see them because they only open for a brief time in the morning, and this often occurs under the water. It is rare but rewarding for the flowers to open above the surface. Flowers

Fig. 7.1 One-day-old leaf

Fig. 7.2 Two-day-old leaf

are bright purple color, long pedicel, and fleshy and goblet-shaped thalamus (04 nos.) and covered with dense and sharp prickles. As a result of the flowers opening under water, *Euryale* is almost exclusively self-pollinated. It produces pollen the day *before* it opens. In the wild, flowers open above the water at the end of the season, allowing some opportunity for cross-pollination. Size of flower is 1–2 inches long (2.5–5 cm) and bright red or violet inside and green and shiny outside. The flowers are solitary, submerged, and epigynous with four persistent, thorny sepals inserted on the torus above the level of the ovary, together with many-seriate petals. Most flowers are cleistogamous, but chasmogamous flowers may also be produced. Sepals are four in number, erect in shape, and inserted on the edge of the torus above the carpels. Petals are numerous (about 20) in number, violet in color, 3–5-seriate, and shorter than the sepals. Stamens are many in number and many-seriate. It is fascicled in eight filaments and linear, and pollen is spherical in shape and three nucleated. Ovary is eight celled sunk in the dilated top of the torus. Stigma is sessile, discoid, and concave in shape. The inferior, multicarpellary ovary develops into a spongy berry-like fruit. Berry is spongy and 5–10 cm in diameter, and it is crowned with persistent sepals (Jha et al. 1991a, b).

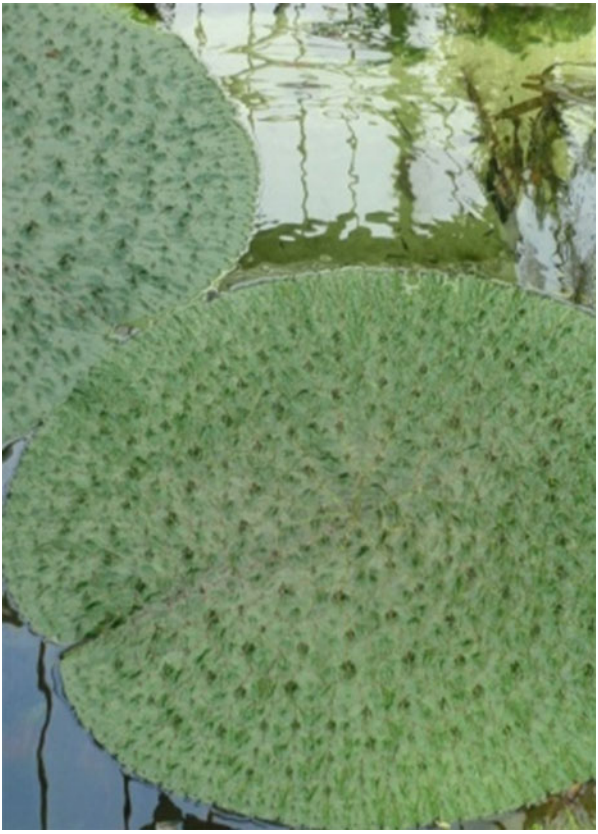


Fig. 7.3 Fully open leaf

Fruit

Fruits (Figs. 7.9, 7.10, and 7.11) are big and spheroidal in shape, protuberant in appearance, and densely covered with sharp prickles and have whitish brown outer skin. The fruit is described as berry, large (5–8 cm in diameter), spongy, spiny, and crowned with persistent sepals. Each fruit has 20–200 seeds with hard black seed coat and a pink-colored mucilaginous aril. The fresh pulpy aril keeps the seeds (Fig. 7.11) floating for a few days (about 3–4 days) after they dehisce, before they finally settle down after decomposition of fresh pulpy aril to the bottom of the water (Jha et al. 1991a, b).

Seeds

The fresh seeds (Figs. 7.12 and 7.13) are lumpy and surrounded by streaked bright red and pinkish arils and keep on floating on water surface until the red aril gets partially decomposed and sticks with seed coat. Thereafter, the seeds get settled in

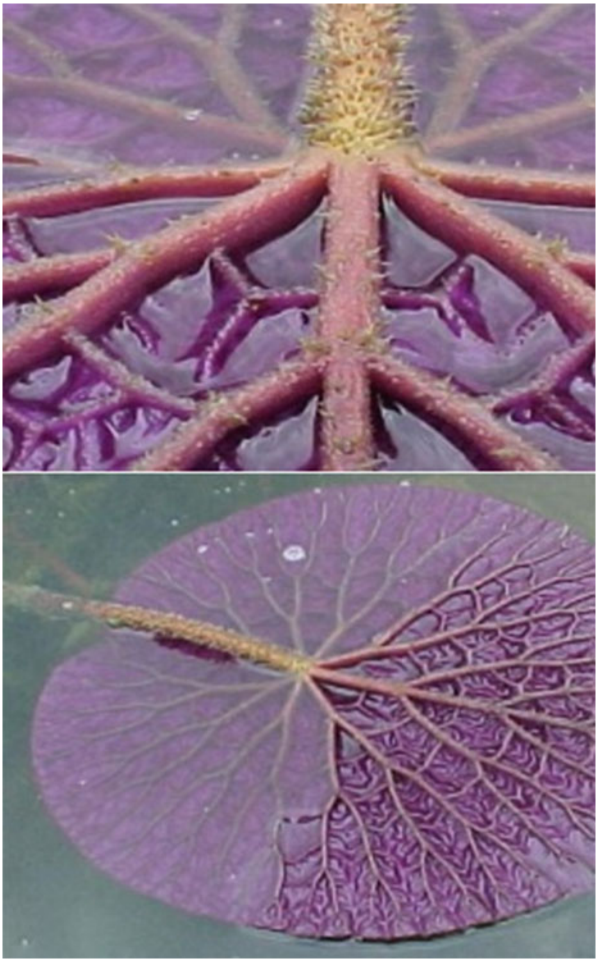


Fig. 7.4 Lower side of leaves

the bottom surface of the pond and finally acquired black color. Seeds (diameter: 0.5–1.5 cm) are enough bold and have a hard outer covering. Seedcoat is thick, albumen merely with small embryo. The seeds are oval with a black-colored hard seed coat and contain about 10–15% protein and lots of starch. It is a superior herb highly regarded for restoring sexual vigor and youthful energy in older men and for retarding aging. It is commonly used as food. The different dietary components of the seeds were investigated to assess its nutritional significance.

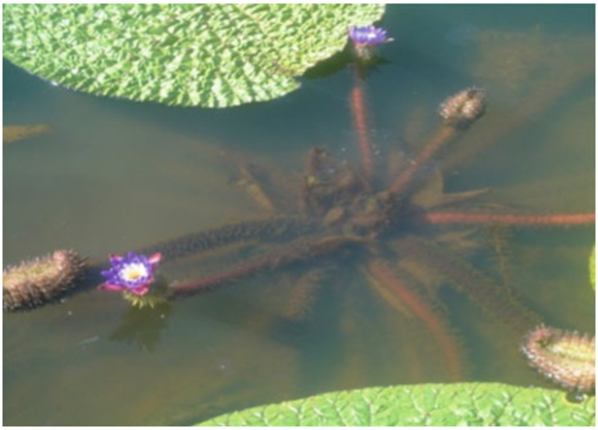


Fig. 7.5 Flowers bearing plant of makhana

Fig. 7.6 Fully open flowers of makhana

7.2 Composition and Uses

A lot of medicinal uses are recommended in the Indian and Chinese system of medicine. Makhana is one of the most common dry fruits utilized by the people due to its low fat content and high contents of carbohydrates, protein, and minerals. Both raw and fried Makhana are fairly rich in essential amino acids. Edible perisperm constitutes 80% starch. *Euryale ferox* is a storehouse of macro- and micronutrients.

Fig. 7.7 Size of makhana flowers

The seeds are eaten raw or roasted. The seeds are sold in market and used as a farinaceous food. Based on the recent studies revealing antioxidant activities of *Euryale ferox* and its glucosides composition, we sought to determine if *Euryale ferox* seeds (Makhana) could reduce myocardial ischemic reperfusion injury. Two different models were used: acute model, where isolated rat hearts were preperfused for 15 min with Krebs-Henseleit bicarbonate (KHB) buffer containing three different doses of makhana (25, 125, or 250 μg/ml) followed by 30 min of ischemia and 2 h of reperfusion, and chronic model, where rats were given two different doses of makhana (250 and 500 mg/kg/day) for 21 days, after which isolated hearts were subjected to 30 min of ischemia followed by 2 h of reperfusion. In both cases, the hearts of the Makhana-treated rats were resistant to ischemic reperfusion injury as evidenced by their improved post-ischemic ventricular function and reduced myocardial infarct size. Antibody array technique was used to identify the cardioprotective proteins. The Makhana-treated hearts had increased amounts of thioredoxin-1 (Trx-1) and thioredoxin-related protein 32 (TRP32) compared to the control hearts. Western blot analysis confirmed increased expression of TRP32 and thioredoxin proteins. In vitro studies revealed that Makhana extracts had potent reactive oxygen species scavenging activities. Taken together, the results of this study demonstrate cardioprotective properties of Makhana and suggest that such cardioprotective properties may be linked with the ability of makhana to induce TRP32 and Trx-1 proteins and to scavenge ROS (Das et al. 2006).

Fig. 7.8 L.S. of makhana flowers

Fig. 7.9 Mature fresh fruit of makhana

Fig. 7.10 T.S. of fresh fruit of makhana

Fig. 7.11 Newly burst fruit of makhana

7.2.1 Uses

In India, in the northern (Punjab) and western parts of the country, *Euryale ferox* seeds are often roasted or fried, which causes them to pop like popcorn. These are then eaten, often with a sprinkling of oil and spices. In Mithila culture of Mithilanchal, makhana known as Makhana in Maithili is an auspicious ingredient

Fig. 7.12 Fresh makhana seeds

Fig. 7.13 Germinated makhana seeds

in offerings to the goddesses during festivals and is used to show reverence and in cooking, specially to make a porridge/pudding called kheer of makhana or "makhaanak kheer" or "makhaanak payasam" and Makhane Ka Rayta (Makhana yogurt blend). Makhhaan along with Paan (betel leaf) and Maachch (fish) is symbolic to Maithil culture.

Eaten raw or roasted. Sometimes seeds are boiled in salt water. On roasting in hot sand, the seed coat swells and bursts and can be easily peeled off. The seeds are sold in market and used as a farinaceous food. The seed flour is used as a substitute for arrowroot. It is nutritious and easily digested. In Kashmir fruits are edible. Seeds eaten raw or roasted forms, and dried seeds are crushed and used in production of nutritious bread. *E. ferox* is used as delicious vegetable by the people of Manipur during autumn and summer. The leaf petiole and seeds are taken raw in salad and chutney forms. Vegetable dishes and curry is also prepared with it. However, in some other areas, people also use tender leaves, seed aril, and fruit skin in the preparation of chutney after removing the prickles by means of fire or some other means. In North India and Bihar, makhana serves as a dessert delicacy. After frying the seeds are used as snacks as well as in the preparation of vegetable dishes.

Makhana pop has several uses. It is a highly relished food consumed as *namkeen*, *kheer*, curry, and so forth (Mishra et al. 2003). *Makhana* pop is traditionally consumed as a snack; high-protein, low-fat food; or sweet component; and it has been used in traditional medicine. *Makhana* further holds special importance in the cultural and social life of Bihar. It is considered obligatory for brides' parents to send *makhana* to the house of the groom to serve the latter's family before the marriage. It is usually eaten with betel and betel nut. It is also used as a part of final rituals in the case of death.

7.2.1.1 Snacks

Besides from having herbal and medicinal properties, Makhana is used in various purposes. Makhana is consumed as dry fruit snacks by roasting. Homemade roasted makhana can get rid of the junk chips and other snacks from the children's ill habits. In 100 gms Makhana has 360 k calorie.

7.2.1.2 Organic Herb

Makhana is harvested from the stagnant wetlands. In the whole cultivation and even post-harvest process, neither fertilizers nor pesticides are used. After harvesting the leaves and stems remained in the same water bodies which serve as fertilizer for the next crop; hence it is called organic naturally. Being herb, Makhana is one of the main ingredients of herbal/Ayurvedic medicine and TCM (traditional Chinese medicines).

7.2.1.3 Food Preparation

Makhana is one of the most essential ingredients for delicious food preparation. Makhana Kheer and Sewai made of makhana only are lazeej itself. It is used in pudding, milk-based sweets. Dal makhani and vegetable curries prove delicious when makhana is spread for taste and thickening object. Makhana raita is very tastier and it is digestive in nature. The month of "saawan" is here. If you are into Monday fasting, you can also make kheer of makhana.

- It is digestible and it is used as food. Kids can dip it in melted butter or oil to make snacks like Civde. They like it. It can be found in the pudding. In this panjeeri, pies can also be mixed.
- Because of its medicinal properties, the Associated American Herbal Products has rated it as class 1 food. It is benefitial for chronic diarrhea, leukorrhea, decreased sperm count, etc.
- This has the most antioxidant properties. Therefore, it is advantageous in diseases of the bladder.
- Per day consumption of Makhana reduces blood pressure, and waist and knee pain is controlled.
- Women had to overcome the weakness of prenatal. It has been cooked in milk.
- It is a good sources of protein, carbohydrates, fat, calcium, phosphorus, iron, acid, and B vitamins.

7.2.1.4 Puja and Havan

Being the *god*'s *food*, Makhana is used in all the worshipping ceremonies, in puja as offering, and in havan due to its heavenly nature and can also be eaten during fasting (vrat). In big temples and religious ceremony, Makhana is offered as Prasad and distributed among devotees. Hence we can say:

> ZINDGI SE PAHLE, ZINDGI KE SATH BHI AUR ZINDGI KE BAAD BHI.

7.2.1.5 Industrial Use

Makhana is used as starch for coating in the quality fabrics like Banarasi sarees, etc.

In spite of the unique properties of makhana, the consumers have lack of information about the product and its various uses, and hence it has not been able to make enough strides into the consumers' mind space. Benefits of the wonder crop have not been disseminated properly, and the sector needs urgent attention on this matter.

7.3 Origin and Distribution

The main distribution of *Euryale* is in Pakistan (Kashmir) eastward to India (Qaiser 1993), Korea, Japan and China, Oudh, Kashmir, East Bengal, and China. Makhana plant is considered as a native of Southeast Asia and China but distributed to almost every parts of the world. In general, its distribution is extremely limited to tropical and subtropical regions of Southeast and East Asia and known to exist in China, Japan, Korea, Russia, North America, Nepal, Bangladesh, Marala Headworks wetlands, and northwest side of the Punjab, as an aquatic plant species commonly growing along the water inlets on the left bank of River Chenab, facing Village Gondal, District Gujrat, Pakistan (Muhammad et al. 2010), and some parts of India. In India, it is distributed in Bihar, West Bengal, Assam, Manipur, Tripura, Eastern Odisha, Madhya Pradesh, Rajasthan, and Eastern Uttar Pradesh. However, its commercial cultivation is limited to North Bihar, Manipur, parts of West Bengal, and Madhya Pradesh. In the state of Bihar, major makhana-producing districts include Darbhanga, Sitamarhi, Madhubani, Saharsa, Supaul, Araria, Kishanganj, Purnea, and Katihar. Approximately, 80% of total production of processed makhana comes from Darbhanga, Madhubani, Purnea, and Katihar districts alone. Area under makhana cultivation in Bihar is about 13,000 ha. *Makhana* is an aquatic crop that is largely grown in Northern India. Though *makhana* is also found in wild form in China, Japan, and Russia, India is the only country where *makhana* is cultivated as a crop, mainly in the states of Bihar and some parts of Assam (Mishra et al. 2003). *Makhana* as a crop can be cultivated in any shallow and stagnant pond. *Makhana* has shown important production increases in the last decades, and *makhana* cultivation has endogenously (without public research or extension intervention) spread to off-season rice fields in the districts of Bihar. It is estimated that *makhana* cultivation done in ponds accounted for 90% of total production 10 years ago, while 65% and 35% of current production comes from ponds and rice fields, respectively.

The distribution of this species is now limited to the tropical and subtropical regions of Southeast and East Asia. It is found growing wild in India in the south to as far north as Manchuria (Regel 1862; Komarov 1927). Further it has been reported to grow as a native plant in the lakes of Manchuria (Sukatscheff 1906). It has completely vanished from Tegelen clay in Holland and at Lachvin in Russia (Sculthrope 1967). In China it has been cultivated in the Hainan and Taiwan islands for 3–4 millennia (Jha et al. 1991a, b). Its distribution includes the islands of Taiwan (Formosa) and Kyushu, Shikoku, and Honshu in Japan (Okada 1935). The northern limit in Japan corresponds to about 38° 30′ N on the Pacific coast and 37° 55′ N on the Japan sea coast. In India also Makhana has been growing in the temperate lakes of Kashmir as an ancient natural crop. The deteriorating conditions of the lakes in Kashmir had diminished the number of plants to few in Dal and Manasbal lakes and a single plant in Nagin Lake (Kak 1985). The Chinese have cultivated the plant for over 3000 years (Mabberley 1987). Archaeobotany indicates that *Euryale ferox* was a frequently collected wild food source during the Neolithic period in the Yangtze region, with large numbers of finds coming from the sites of Kuahuqiao, Hemudu, and Tianluoshan – used for medicinal purposes in China.

According to a popular adage in Mithila, betel leaves and makhana are not found in heaven. So, one should relish them on Earth so as not to regret later. The plant is also found in the fresh water tank ponds and jhils of northern central and western parts of India.

7.3.1 Nutritional Value

It is one of the most common dry fruits utilized by the people due to low fat content and high contents of carbohydrates, protein, and minerals. The calorific value of raw seeds (362 k cal/100 g) and puffed seeds (328 k cal/100 g) lies close to staple foods like wheat, rice, other cereals, and some aquatic plants like *Nelumbo* and *Trapa* (Jha UN 1968). Biochemical analysis of *Euryale ferox* seeds is revealed: 61% carbohydrate, 15.6% protein, 12.1% moisture, 7.6% fiber, 1.8% ash, and 1.35% fat. The seeds were found to contain 12 amino acids, which are histidine, leucine, isoleucine, glutamic acid, lysine, tyrosine, valine, aspartic, threonine, alanine, methionine, and arginine (Alfasane et al. 2008). Roasting and popping cause a loss in the calorific value. Makhana is a good source of carbohydrate, protein, and minerals. The chemical constituents of the popped kernels (g/100 g) are 12.8 moisture, 76.9 carbohydrate, 9.7 proteins, 0.1 fat, 0.5 total minerals, 0.02 calcium, 0.9 phosphorus, and 0.0014 iron (Jha et al. 1991b). Bilgrami et al. (1983) found makhana superior to dry fruits such as almond, walnut, cashew nut, and coconut in contents of sugar, proteins, ascorbic acid, and phenol. According to Nath and Chakraborty (1985), nitrogen content of defatted seed powder is 1.36% by Kjeldhal's method which means 8.5% protein. Sixteen types of amino acid are present in the kernel. Both raw and fried Makhana are fairly rich in essential amino acids. The values relating to essential amino acid index (EAAI) and chemical score (CS) of makhana are close to that of fish (Jha et al. 1991a, b). The values of EAAI in raw Makhana and popped Makhana are 93% and 89% which are higher than the values for rice (83%), wheat (65%), Bengal gram (81.5%), cow's milk (88.8%), fish (89.2%), and mutton (87.24%). Makhana protein (10–12%) is a bit lower when compared to cereals. Still it is nutritionally superior to many plant- and animal-based diets due to high EAAI and CS (Sikka et al. 1979; Jha 1987). The biological value (BV) of puffed seeds was found to be 55 which is lower than in other plant- and animal-based diets. It may be due to the high ratio of leucine to isoleucine present in it. The lower BV recommends its use as a complementary food item. The ratio of arginine + lysine to proline shows better utilization of protein in rat growth. It is 6.3 in raw and 4.74 in puffed makhana seed. A+L/P was found to be higher (7.6) in a wild population from Tripura (Nath and Chakraborty 1985). Protein and amino acid composition (g/16gN) of makhana when compared with egg and FAO/WHO pattern showed higher content of arginine, alanine, and tyrosine. A remarkable loss was seen in the values of tyrosine, while the values were higher for lysine, arginine, threonine, serine, glutamic acid, glycine, alanine, valine, cystine, isoleucine, leucine, and phenylalanine on popping (Jha et al. 1991a, b). Jha (1987) reported net protein utilization (NPU 49.3), true digestibility (TD 89.6), apparent digestibility (AD 69.1) of makhana were comparable to the values of most cereals. The above values were lower when compared to soya bean, egg, and human and cow's milk (Jha et al. 1991a, b).

7.3.2 Phytochemistry

Makhana is a good source of carbohydrate (Tables 7.1, 7.2 and 7.3). Edible perisperm constitutes 80% starch. Nath and Chakraborty (1985) reported 77% starch in the perisperm. Protein-free starch was fractionated into 25.3% amylose and 74.7% amylopectin. The iodine-binding capacity of amylopectin indicated that 0.47% amylose was present in this fraction. The chemical composition and properties of starch are given in Table 7.4. Trace metals like Cu, Na, Ca, Fe, and Mg reported a declining trend on purifying starch (Table 7.5). The loss was more pronounced for Ca and Mg illustrated in Table 7.6 (Nath and Chakraborty 1985).

Euryale ferox is a storehouse of macro- and micronutrients (Dutta 1984). Vegetative parts also contain good amount of N (0.167% and 0.197%) along with the edible seed (1.56% N equivalent to nearly 10% crude protein). The values are considerably higher than that present in some of the most common fruits

Table 7.1 Protein and amino acid composition (g/16gN) of makhana when compared with egg and FAO/WHO pattern

Amino acid	Makhana		Egg	FAO/WHO (1973)
	Raw	Fried		
Lysine	3.79	4.69	6.7	5.4
Histidine	3.15	3.12	3.5	2.5
Arginine	15.19	16.07	6.7	5.2
Aspartic acid	5.76	5.05	10.4	7.7
Threonine	3.34	3.51	5.1	4
Serine	5.05	5.64	6	7.7
Glutamic acid	16.64	17.06	25.02	14.7
Proline	4	3.24	–	10.7
Glycine	3.01	3.28	3.6	2.2
Alanine	5.5	5.84	3.5	6.1
Valine	5.18	5.49	7.5	5
Cystine	0.75	1.21	3.0	–
Methionine	3.06	2.95	2.3	3.5
Isoleucine	4.18	4.8	5.8	4
Leucine	8.34	8.85	8.9	7
Tyrosine	6.38	2.91	3.6	3.05
Phenylalanine	5.78	6.12	6.7	3.05
Tryptophan	n.d	n.d.	1.5	1
Ammonia	0.9	1.16	–	–
Protein (%)	11.1	11.5	–	–

Source: Jha et al. (1991a, b). n.d. = not determined

Table 7.2 Comparative value of essential amino acid index (EAAI) and biological value (BV) of foods

Feeds	EAAI	BV	CS (% EGG)
Edible items			
Rice	82.88	68	54.93
Wheat	65.18	62.6	39.7
Bengal gram	81.55	68	53.33
Soya bean	85.59	50.7	52.6
Amaranth	57.72	–	40.93
Human milk	81.55	–	59.7
Cow's milk	88.8	84.5	52.54
Fish	89.2	59.7	65.7
Mutton	87.24	74	71.46
Makhana			
Fried	89.97	55	56.57
Raw	93.63	–	70

Source: Jha et al. (1991a, b). n.d. = not determined

Table 7.3 Comparative value of leucine to isoleucine and arginine + lysine proline ratios in foods

Feeds	Leucine/isoleucine	(Arginine + lysine)/proline
FAO/WHO pattern	1.75	0.99
Rice	1.66	4.00
Wheat	1.66	0.71
Soya bean	1.45	2.86
Amaranth	1.27	3.41
Human milk	–	1.58
Cow's milk	1.76	–
Fish	1.71	5.18
Mutton makhana	1.56	–
Raw (Tripura sample)	1.9	7.6
Raw (Bihar sample)	1.84	6.3
Fried (Bihar sample)	1.99	4.74

Source: Jha et al. (1991a, b). n.d. = not determined

Table 7.4 Chemical composition and properties of starch of *Euryale ferox*

Determination	Whole starch	Protein-free starch
Yield (%) from seed meal	52.5	n.d.
Moisture (%)	14.4	13.5
Ash (%)	15	0.15
N (%) by Kjeldahl method	1.36	Nil
Protein (%) by amino acid analysis	7.32	Trace
Total carbohydrate (%)	77.33	86.85
Amylose (%), potentiometrically	n.d.	25.3
Amylopectin (%) with respect to amylose	n.d.	74.7

Source: Nath and Chakraborty (1985); n.d., not determined

Table 7.5 Properties of starch, amylose, and amylopectin from the seeds of *Euryale ferox*

Determination	Starch	Amylose	Amylopectin
Iodine-binding capacity	5.36	21.2	0.1
Blue value	0.31	1.28	0.05
Specific rotation	142.5	135	132.5
Average chain length			
By periodate oxidation	29	380	23(22)
Average chain length			
By methylation	–	395	20
Intrinsic viscosity [h](dl/g)	1.15	0.78	1.2

Source: Nath and Chakraborty (1985); n.d., not determined

Table 7.6 Detection of trace metals present in the starch of *Euryale ferox*

Sample	Cu ppm	Na ppm	Ca ppm	Fe ppm	Mg ppm
Seed meal	<1	1000	>1000	100	>2000
Whole starch	1	1000	>1000	100	>2000
Purified starch	<1	800	<200	80	100

Source: Nath and Chakraborty (1985); n.d., not determined

(0.6–4.4%). P content in makhana (2397 mg/kg) was higher than common fruits like guava, litchi, and mango (300–800 mg/kg). K is accumulated more in rhizome (2170 mg/kg) than in the seeds (159–240 mg/kg). *E. ferox* is a sodium-loving crop. It accumulates sodium even in low-sodium soils. Na content in the seeds ranged between 180 and 200 mg/kg. Various common fruits like mango, litchi, and banana had Fe content in the range of 105–678 mg/kg, while Fe in various parts of makhana plant was found to be 1994–2236 mg/kg (Belavady and Subramanian 1959). Zn content in makhana fruit (42.9–66 mg/kg) was also reported to be higher than in common fruits like mango and banana and vegetables like cucumber and *Colocasia* (23.3, 7.3, 5.3, and 15.5 mg/kg, respectively) (Dutta et al. 1986). Both the plant and the seed contain Cu in the uniform range of 8.3 mg/kg, but it ranged from 12.5 to 16.7 mg/kg in fruit sheath and petiole. Mn content also showed the same trend. The value was higher for makhana fruits (25–35 mg/kg) than for mango, cucumber, and banana (7.3 mg/kg, 14.4 mg/kg, and 29.6 mg/kg, respectively) (Dutta 1984). The above data revealed that fruit sheath was a good source of minerals.

A lot of medicinal uses of Makhana in the Indian and Chinese system of medicine. Makhana is recommended for treatment of diseases regarding respiratory, circulatory, digestive, excretory, and reproductive systems (Qudrat et al. 2000). The edible seed is known for its tonic, astringent, deobstruent, anti-rheumatic, anti-diuretic, and roborant properties. It is also utilized to overcome postnatal weaknesses in women, and in case of men, its aphrodisiac and spermatogenic potential is utilized (Jha et al. 1991a, b). Ayurveda, the Indian system of medicine recommends makhana to be beneficial in Tridosas (the seminal Ayurvedic theory of diagnosing diseases on the basis of three principal defects of the body), especially in vata (rheumatic disorders) and pitta (bile disorders). In Unani system of medicine, makhana is used against dysmenorrhea. According to the principles of Chinese medicine, its main functions are to notify the spleen and stop diarrhea, to strengthen the kidneys and control the essence, and to dispel dampness (Hsu and Cho 1951). Makhana is used as a tonic and for the treatment of leucorrhoea and good immunostimulant (Puri et al. 2000).

Besides the above it has iron, zinc, thiamine (vitamin B1), nicotinic acid, carotene, and minerals. It is low in saturated fats, sodium, and cholesterol and high in magnesium potassium and phosphorus. So it is very useful for people with high blood pressure. It contains flavonoids which are antioxidants; thus it is an anti-aging food too. It is high in fiber; thus it helps to deal with constipation. Foxnut is helpful in treatment of arthritis and rheumatic pains. It also helps in treating insomnia and irritability. It also helps increasing appetite. Makhana helps strengthen the heart and kidney. It is a good food for diabetic too. It acts as an aphrodisiac; thus it increases quality and quantity of semen, prevents premature ejaculation, increases libido, and helps in female infertility. Makhana increases stickiness of secretions by increasing moisture level in the body. Hence it increases quality and quantity of semen and useful in impotence. It helps to increase the fertility in women and reduces vata and pitta. It strengthens the body and reduces burning sensation and quenches thirst. So it would be really stupid to not include this pious and heavenly food in our diet. It can be eaten raw (just roast it with little ghee and add salt and pepper) or can be made into various preparations.

7.3.3 Medicinal and Therapeutic Value

It gives strengthen to the kidney to preserve essence; deprives dampness to relieve leucorrhea and emission; reduces frequent urination; strengthens the spleen to relieve diarrhea; regulates blood pressure; and relieves numbness and aching near the waist and knees. It is suitable for arthritis; impotence; and premature aging. It has been widely used in traditional oriental medicine to cure a variety of diseases including kidney problems, chronic diarrhea, excessive leucorrhea, and hypofunction of the spleen. A lot of medicinal uses are recommended in the Indian and Chinese system of medicine. Makhana is recommended for treatment of diseases regarding respiratory, circulatory, digestive, excretory, and reproductive systems. Dragendroff (1898) found the whole plant to bear tonic, astringent, and non-obstructing properties. Diseases of the spleen, polyuria, spermatorrhea, gonorrhea, articular pains, micturition, and seminal loss are also treated with it. Stuart (1911) and Kariyone and Kimura (1949) reported the seeds to be effective in increasing the secretion of hormones. It acts as an expectorant and emetic (Nadkarni 1976). Sharma (2005) noted its medicinal impacts in treating circulatory disorders and also as a cardiac stimulant. The farinaceous seeds have binding action in dysentery. But in overdoses, it causes constipation and flatulence. The edible seeds are known for its tonic, astringent, deobstruent, anti-rheumatic, anti-diuretic, and roborant properties. It is also utilized to overcome postnatal weaknesses in women.

In case of men its aphrodisiac and spermatogenic potential is utilized (Jha et al. 1991a, b).

Ayurveda, the Indian system of medicine, recommends makhana to be beneficial in Tridosas (the seminal Ayurvedic theory of diagnosing diseases on the basis of three principal defects of the body), especially in vata (rheumatic disorders) and pitta (bile disorders). In the Unani system of medicine, seeds are used against dysmenorrhea. According to the principles of Chinese medicine, its main functions are to tonify the spleen and stop diarrhea, to strengthen the kidneys and control the essence or jing, and to dispel dampness. To treat diarrhea, *Euryale* seed is typically incorporated into a larger formula containing white atractylodes and dioscorea. It is included as "Chien-Shih" in the Chinese medicinal formula "Su-Shin" (a tonic especially required for the growth of the children) (Hsu and Cho 1951). Makhana seeds contain sufficient amount of vitamins, so it is suitable to be used to treat beriberi, a disease caused by deficiency of vitamin B1 (Ho H, Cheu Y, Luo I 1953). Quadrat-I-Khuda et al. (2000) found its starch granules to be very small (1–3 um as compared with 2.2–7.5 um of *Nymphaea stellata* Willd. and 15.91–39.0 um *Trapa bispinosa* Roxb.) making it effective against digestive disorders. *E. ferox* is used as a tonic and for the treatment of leucorrhoea. Puri et al. (2000) reported *E. ferox* to be a good immunostimulant. Feeding of *E. ferox* stimulated humoral immunity and suggested its applications in mothers after delivery and invalids. Gordon *Euryale* seed is analgesic (insensitizes pain) and aphrodisiac. It is taken internally in the treatment of chronic diarrhea, vaginal discharge, kidney weakness associated with frequent urination, impotence, premature and involuntary ejaculation, and nocturnal emissions. It also regulates blood pressure; and it relieves numbness and aching near waist and knees. It is suitable for arthritis; impotence; and premature aging (McGuffin et al. 1997). The American Herbal Products Association has given *Euryale* seed a class 1 rating, meaning that it can be safely consumed when used appropriately.

7.4 Species and Cultivars

7.4.1 Species

Euryale ferox is the only species in the genus *Euryale*. The name *Euryale* comes from the mythical Greek Gorgon by the same name (Regel 1862). It is a flowering plant classified in the water lily family, Nymphaeaceae, although it is occasionally regarded as a distinct family Euryalaceae. Unlike other water lilies, the pollen grains of Euryale have three nuclei. The genus *Euryale* was first described by Salisb in 1806 as a monotypic genus of the family Nymphaeaceae but sometimes treated under separate family Eurylaceae (Qaiser 1993). *Euryale* (ur-ee-al-ee), the "Gorgon plant," is first cousin to *Victoria*. There is just one species in the genus, *ferox*, and the plant is indeed ferocious! Every part of it has thorns, even the tops of the pads. It can get quite large but lacks the upturned rims of *Victoria* (Blatter 1927). It is diploid having chromosome number 2n = 2x = 58. It is an exclusively self-pollinated plant in which fertilization takes place at an early stage of their development.

7.4.2 Cultivars

7.4.2.1 Swarna Vaidehi

The first ever variety of Makhana was released in India. The variety of makhanas has been developed and released by ICAR Research Complex for Eastern Region, Patna, for the first time in India. The Institute Variety Release Committee approved the release of Makhana variety for Bihar, Assam, Chhattisgarh, and Odisha on 15 November 2013. This variety developed through pure line selection has the production potential of 2.8–3.0 t/ha. in farmers' field which is almost twofold higher than the productivity of traditional cultivars. The Sel-6 strain of makhana has been released as a first ever variety of makhana under the name of "Swarna Vaidehi" by the Institute Variety Release Committee. The seeds of Swarna Vaidehi (Fig. 7.14) are bold with average productivity of 2.8–3.0 t/ha. This variety sowed has 60% higher seed yield compared to local check.

7.4.2.2 Sabour Makhana-1

The Makhana variety Sabour Makhana-1 (Fig. 7.15) gave seed (Guri) yield of 32–35 q/ha and pop (Fig. 7.16) recovery of 55–60% as compared to previously released variety 'Swarna Vaidehi' (seed yield 28–30 q/ha and pop recovery 35–40% only) and other genotypes under test. The Sabour Makhana-1 variety has large spherical leaf, dark purple flowers, medium-size fruit, and small, oval, and smooth seeds with very thin seed coat (0.29 mm) in comparison with released variety 'Swarna Vaidehi' with large spherical leaf, light purple flowers, medium-size fruit, and large, round, and rough seed with slightly thick seed coat (0.99 mm). The variety Sabour Makhana-1 is moderately field resistant to important insect pests, viz., aphid (*Rhopalosiphum nymphaeae*), case worm (*Elophila depunctalis* and *E. crisonalis*), and leaf midge (*Chironomus* spp.), and also leaf blight disease (*Alternaria* spp.). Maturity (range in number of days) seed to seed is 240–250 days.

7.5 Soil and Climate

7.5.1 Soil

Makhana is best grown in age-old perennial water bodies with a rich mucky bottom providing nutrients to the plants. The plant is cultivated for its seeds in lowland ponds in India. Initially, makhana is grown in stagnant perennial water bodies like ponds, land depressions, oxbow lakes, swamps, and ditches, but realizing the importance, it has been commercialized as a major aquatic horticultural crop. Now, Makhana cultivation is started in lowland area in shallow water under field

Fig. 7.14 Seeds and field view of Swarna Vaidehi

condition. Clay to loam soil is suitable for growing successful makhana crop. The plant is cultivated for its seeds (Mabberley 1987) in lowland.

7.5.2 Climate

For geographical and climatic reason, Bihar is the heaven for Makhana production. Climatically the area is characterized by hot, dry summers and mild winters. The maximum daily temperature in summer is 41–46 °C and minimum 15–20 °C (Jalali and Jamzad 1990; Nasir and Rafiq 1995). The plant does best in locations with hot, dry summers and cold winters. It is plant of tropical and subtropical climate. For its proper growth and development, the conducive range of air temperature is 20–35 °C, relative humidity 50–90%, and annual rainfall 100–250 cm (Mandal et al. 2010); being a popular aquatic fruit crop has a heavy water requirement. Thus, assured availability of irrigation water is the prime need for its cultivation especially in field

Fig. 7.15 Seeds of Sabour Makhana-1

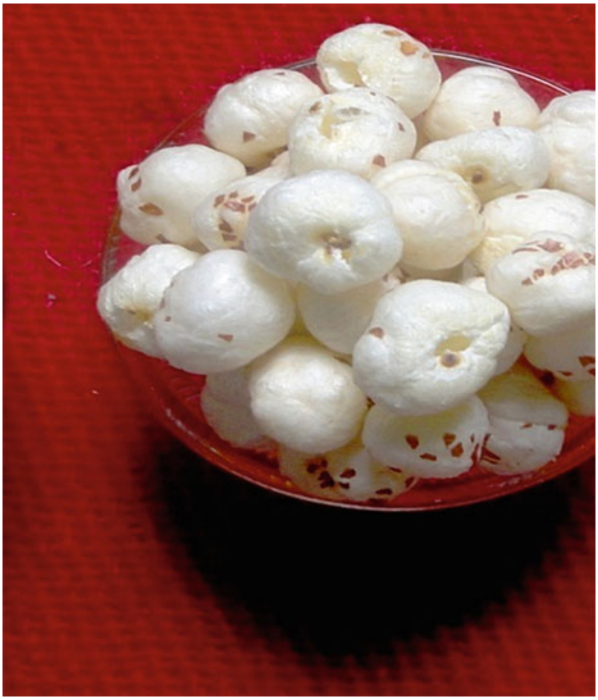


Fig. 7.16 Pop of Sabour Makhana-1

condition. Moreover, it also needs permanent standing water throughout its growth period. Seeds are collected in the late summer and early autumn and may be eaten raw or cooked.

7.6 Area Production and Productivity

It is estimated that Bihar accounts for more than 80% of total makhana production in the country. Production takes place in 20 out of its 38 districts, mostly situated in the north of the state. Darbhanga, the district where the survey was fielded, is one of the most important makhana-producing districts in Bihar. The total makhana area cultivated in Darbhanga amounted to about 1215 ha in 2009, compared with a total of 15,182 ha. in Bihar (Minten et al. 2014). The average production and productivity of makhana crop in Bihar are 3,18,750 q and 21.25 q/ha, respectively (Minten et al. 2014). Also, increased commercialization has been noted over the years. While, before the 2000s, only a tiny share of makhana was exported outside the district, it was estimated in 2009 that almost 60% was sent outside. *Makhana* is an aquatic crop that is largely grown in Northern India. Makhana is best grown in age-old perennial water bodies with a rich mucky bottom providing nutrients to the plants. Growth of plants is not proper in freshly excavated ponds or water area because they lack the highly nutritive mucky bottom (Thakur 1978). Annual production of Makhana pop is estimated at around 96,000 tonnes, with major producing districts being Darbhanga, Madhubani, Purnea, and Katihar.

7.7 Propagation

Makhana is commercially propagated by seeds. Makhana cultivation requires minimum expenditure as new plants germinate from the seeds left over from the last harvest. Transplanting into sparse areas, adding insecticides, and collecting dispersed seeds from the pond bed during harvest (Jha and Prasad 1996).

7.7.1 Seed

The seeds of the plant are starchy, white, small, and round, with a black to deep brown outer covering. In India, particularly in the northern and western parts of the country, Makhana seeds are often roasted or fried, which causes them to pop like popcorn. They can be consumed either raw or after being stir-baked, often with a sprinkling of oil and spices. Seed germination is started in December, water temperature maintained at 20 ~ 25 °C, the night is over 15 °C (best in the greenhouse or in soaking, germination bed).

7.7.2 Seed Viability and Storage

Seeds need to be stored cold (45 °F or 7 °C) to prevent sprouting when germination is desired; seeds will sprout at room temperature and higher. "Nicking" (removing the operculum) isn't necessary.

7.7.3 Seed Germination and Seedling Vigor

It is an absolutely seed-propagated plant. The germination of makhana seed is of hypogeal type. Upon the germination, the cotyledons and hypocotyls of seeds remain in the soil. It has thick fibrous roots comprising 3–5 clusters each consisting of about 15 rootlets. The roots are thick, long (40–50 cm), fleshy, and fibrous in nature and also have a number of air pockets. The plant has rhizomatous stem. The rhizome is characterized as short, thick, and erect.

7.8 Cultivation

Makhana production technology provides the way for better utilization and to get maximum net return from underutilized and neglected waterlogged areas without eroding natural resources.

7.8.1 Preparation of Nursery and Transplanting

7.8.1.1 Preparation of Nursery

A nursery (Fig. 7.17) area 500–600 m^2 is sufficient to transplant 1 hectare of main field. About 22–25 kg seed is for raising seedlings and 40–45 kg seed for direct sowing enough for 1 hectare area. Seeds are treated with imidacloprid 70 WS or

Fig. 7.17 Healthy makhana nursery plot

thiamethoxam 25 WG at 5 g/kg seed. Soaked seeds are kept on the floor in thin layer under shade for 2–3 h. Pre-germinated seeds are broadcast (depth of sowing at the bottom surface of field/pond) uniformly over well-prepared nursery field/pond. 25–30 kg well-decomposed farmyard manure and 20–25 kg lime dust a day prior to sowing of treated makhana seeds are essential for raising healthy seedlings. Gradually, water level is raised and maintained to a depth of as per need and water level of main field. Level of water in nursery in field and pond system is minimum 6 inch and maximum 5 to 6 ft., respectively. Proper time of sowing of seed in nursery field/pond is December in this agro-climatic zone.

7.8.2 Field Preparation and Transplanting

Field should be plowed 2–3 times and puddled 3–4 days before transplanting of makhana seedlings.

7.8.2.1 Transplanting

Approximately 55–60-day-old seedlings (4–5 leaf stage), when spines are soft with 7–9 cm in diameter and small sized and leaf color of seedling turns bronze to light green color, are suitable for transplanting during summer season (February to March). A spacing of 1.25 m × 1.25 m is adopted, and planting of single seedling per hill is recommended, which reduce the seed rate even with ensuring optimum flower stalk number per unit area. Transplanting (Fig. 7.18) should be completed within February to March to achieve higher yield.

Fig. 7.18 Transplantation of makhana seedlings in field condition

7.9 Irrigation

Makhana, being a popular aquatic crop, has a heavy water requirement. Thus, assured availability of irrigation water is the prime need for its cultivation. Moreover, it also needs permanent standing water throughout its growth period. Standing water (minimum 6 inch) should be maintained in main field from transplanting to flower initiation, i.e., up to first fortnight of April. Thereafter, the depth is to be increased to 1 foot, and it is continued to maturity as well as harvesting (buharai) of Makhana seed (Gurri), i.e., up to first fortnight of August in field condition. But, in pond system, standing water (minimum 3 feet and maximum 7 feet) should be maintained. This practice helps in good plant establishment and weed control. Thus, whenever precipitation does not occur, farmers become helpless to meet out the required quantity of water to the crop. In addition to this, farmers also face difficulty in doing any cultural practices which are essential for increasing the yield potential of the crop. The crop growth period in pond system generally varies between 9 and 10 months; thus, farmers are unable to get more than one crop in a year.

7.9.1 Manuring and Fertilization

Fertilizer is the key input for realizing the potential yields of makhana. At the time of field preparation, about 20–22 tonnes of farmyard manure with dose of NPK fertilizers at 75:45:30 + 40–50 kg lime per ha is recommended, of which entire phosphate and potash are to be given as basal at least 5 days before transplanting. Half dose of nitrogen should be applied after making solution in 4–5 splits and sprayed on leaves after 20–25 days interval.

7.9.2 Weed Management

The weeds need to be kept under control especially during early stages after transplanting, since these compete with the main crop for nutrients, water light, and space growth. Depending on weed infestation, at least 2–3 hand weeding are necessary at 2–3 weeks interval starting from early stage (25–30 days after transplanting) till the water surface is covered by large spherical makhana leaves. Once, water surface is completely covered by large spherical makhana leaves, the growth of weeds is suppressed (Fig. 7.19).

7.9.3 Cropping System

Normally grown in lowland ponds, it will now be possible to grow the nutritional plant in low farmland and cropping system with rice, wheat, and green fodder.

Fig. 7.19 Field view of makhana crop in field condition

Cultivation of Makhana at shallow water depth in cropping system mode would help to increase its area from the present 20,000 hectare.

Sl. No	Problem/issues observed	Suggested Makhana-based cropping system
(i)	**Up lowland area:**	**Field system:** Makhana production technology
	Water stagnation in month of July up	Makhana-rapeseed
	to 1–2′ and dry in the month of	Makhana-berseem
	October. In these area farmers are	Makhana-paddy-field pea
	growing garama (boro) paddy, but	Makhana-paddy-lentil
	income from this crop is very low	Makhana-paddy-linseed
		Makhana-paddy-Bankala
		Makhana-paddy-Kerao
(ii)	**Mid lowland area:**	**Pond system:** Makhana production technology
	Water stagnation in month of July up to 3–4′ and dry in the month of January–February. Farmers are unable to grow any crop, but they harvest less quantity wildly grown fish	Makhana-water chestnut
(iii)	**Deep lowland area:**	**Pond system:** Makhana production technology
	Water stagnation in month of July up to 7–10′ and inundated throughout the year. It is heavily infested by aquatic weeds specially water hyacinth. Farmers are unable to grow any crop, but they also harvest less quantity wildly grown fish	Makhana-water chestnut
		Makhana-cum-fish production

With the help of new research, Makhana can be harvested in 5–6 months. Makhana plantation done in February–March can be harvested in July–August. Then the farmers can grow paddy in this field, after which they can take rabi crop from the same piece of land. Kumar et al. (2011a, b) showed that the cultivation of makhana could be done in general agricultural fields having clayey soils which are being used for rice cultivation. High-yielding and makhana varieties *Swarna Vaidehi* and Sabour Makhana-1 cultivars of other crops hold enormous potential to produce more in irrigated areas than rice-wheat cropping system or rice, wheat, and berseem alone, thus improving the total productivity of the cropping system. Therefore, crop diversification with higher biomass-producing and fertilizer-responsive cereals is of paramount importance in mitigating the problem arising due to low biomass-producing rice-wheat or rice alone crops in the system. In addition to this, growing these cereals in combination with makhana would certainly have the potential to achieve a high grain yield per unit area and consequently greater land use efficiency than sole cropping, through efficient use of resources such as light, water, and nutrients. This crop combination also helps to minimize risk of complete crop failure and bring stability of crop production under wetland condition. In the field condition, the productivity of improved strain of makhana varied between 26 and 30 q/ha (Kumar et al. 2013). They further reported that in field condition the crop growth period was of only 4 months. Hence, some other crops can also be taken in the same field after taking makhana crop. In this regard they had further developed some makhana-based cropping systems such as makhana-makhana, makhana-rice, makhana-wheat, makhana-berseem, and makhana-chestnut. As a result of this, farmers nowadays are adopting the makhana-based cropping systems in their own fields in addition to natural ponds. Makhana is also known to incorporate huge amount of organic matter to the soil on account of having large leaf size and extensive root system. Soil organic matter serves as an important storehouse of nutrients, drives nutrient cycle, improves soil productivity, promotes water retention, and reduces erosion (Bulluck et al. 2002). Under crop cultivation changes in soil organic matter status would determine the dynamics of alluvial soil quality of wetlands. In addition to makhana, the other component crops too would certainly add an appreciable amount of organic matter to soil. Jha and Dutta (2003) had reported about the chemical changes in soil under makhana plants growing in naturally existing ponds. However, the impact of various makhana-based cropping systems on soil properties, yield attributes, and yields of makhana and other associated component crops as related to seasons, particularly in silty clay loam soils of this region, has not been studied to date. Cropping systems are developed in response to the demand for particular crops, soil nutrient status, soil physical structure, and biotic completion and are constrained by available resources (including climate) and knowledge. Pulses can assist in soil improvement, particularly when used as green manure crops when the whole plant is plowed down into the soil. Fixed nitrogen and in some cases phosphorus are made available to subsequent crops, and the added organic matter contributes to improved soil structure. The cycling pattern of nutrients with different cropping system has not yet been studied. Thus, judicious application of nutrients to the associated crops needs to be studied well so that the makhana-based cropping systems such as makhana-makhana, makhana-rice, makhana-wheat, makhana-berseem, and makhana-chestnut may be a substitute over the dominant rice-wheat cropping systems and fulfill the future demand of human and animal feed.

7.10 Flowering, Floral Biology, and Pollination

The flowers, however, are very small related to the size of the plant, and they open during the day. You have to be vigilant to see them because they only open for a brief time in the morning, and this often occurs under the water. It is rare but rewarding for the flowers to open above the surface. Flowers are characterized by its bright purple color, long pedicel, and fleshy and goblet-shaped thalamus (04 nos.) and covered with dense and sharp prickles. As a result of the flowers opening under water, *Euryale* is almost exclusively self-pollinated. It produces pollen the day *before* it opens. In the wild, flowers open above the water at the end of the season, allowing some opportunity for cross-pollination. The flower is 1–2 inches long (2.5–5 cm) and bright red or violet inside and green and shiny outside. The flowers are solitary, submerged, and epigynous with four persistent, thorny sepals inserted on the torus above the level of the ovary, together with many-seriate petals. Most flowers are cleistogamous, but chasmogamous flowers may also be produced. Sepals are four in number, erect in shape, and inserted on the edge of the torus above the carpels. Petals are numerous (about 20) in number, violet in color, 3–5-seriate, and shorter than the sepals. Stamens are many in number and many-seriate. It is fascicled in eight filaments and is linear; its pollen is spherical in shape and three nucleated. Ovary is eight celled sunk in the dilated top of the torus. Stigma is sessile, discoid, and concave in shape. The inferior, multicarpellary ovary develops into a spongy berry-like fruit. Berry is spongy and 5–10 cm in diameter, and it is crowned with persistent sepals (Jha et al. 1991a, b).

7.11 Fruit Growth and Development

Fruits are big and spheroidal in shape, with whitish brown outer skin, protuberant in appearance, and densely covered with sharp prickles. The fruit is described as berry, large (5–8 cm in diameter), spongy, spiny, and crowned with persistent sepals. Each fruit has 20–200 seeds with hard black seed coat and a pink-colored mucilaginous aril. The fresh pulpy aril keeps the seeds floating for a few days (about 3–4 days) after they dehisce, before they finally settle down after decomposition of fresh pulpy aril to the bottom of the water (Jha et al. 1991a, b).

7.12 Pest and Diseases

7.12.1 Insect and Pest Management

Need-based plant protection measures have to be given for protecting the crop against insect pest and diseases. Generally, it is observed that makhana is less infested by serious pests and diseases, but few insect pests are known to cause damage to the crop, especially when environmental conditions are very conducive to pest attack. Moderately field resistance to important insect pests, viz., aphid (*Rhopalosiphum nymphaeae*), case worm (*Elophila depunctalis* and *E. crisonalis*), and leaf midge, *Chironomus* spp. It can be managed by in-field system through seed treatment with imidacloprid 70 WS or thiamethoxam 25 WG at 5 g/kg seed and root dip with imidacloprid 70 WS or thiamethoxam 25 WG at 5 g/liter of water for half hour + foliar spray of NSKE at 5% at 25 days interval from 40 DAT.

In pond system applied neem cake 2 q/ha at the time of last plowing/planting + foliar sprays of NSKE at 5% at 25 days interval from 40 DAT.

7.12.2 Diseases

7.12.2.1 Leaf Blight Disease

The causal organism of the disease has been confirmed as *Alternaria tenuis* Nees ex Pers. Severe leaf spot disease is commonly found in makhana crop. The disease is characterized by the presence of dark brown or black, irregular, more or less circular dead areas upon the leaves which usually show a concentric series of rings and ridges giving the lesions a “target board” effect. Several such adjacent spots coalesce to each other forming large spot. Severely affected leaves are completely blighted. The long stalks of seriously affected leaves are yellow and some of them completely dried.

Management

Foliar spray of NSKE at 5% at 25 days interval from 40 DAT is found to be very effective against leaf blight disease (*Alternaria* spp.).

7.13 Harvesting (Buharai)

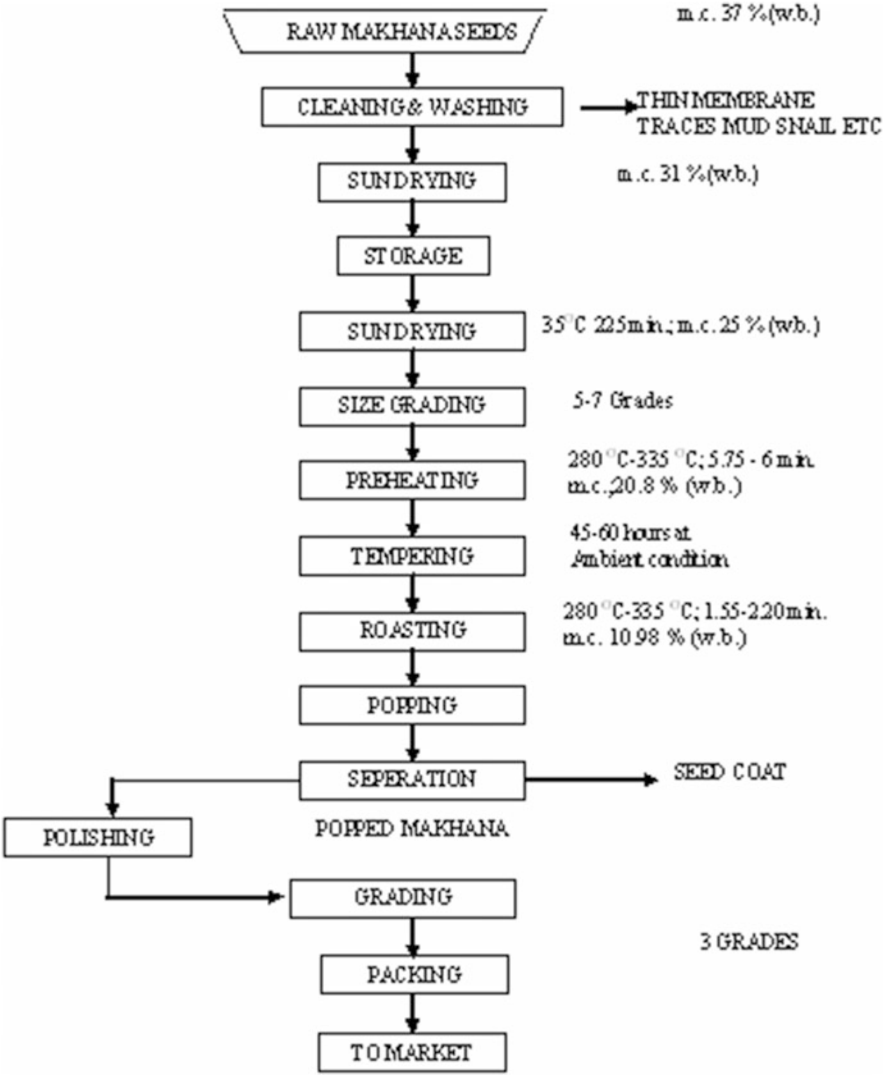
Harvesting and processing of seed is still carried out by traditional methods. The entire floor of the pond is swept by experienced fishermen to form heaps of the sunken seeds that are scooped out with the help of a horn-shaped split bamboo contrivance. Smaller and lighter seeds which float on the water are collected with the help of small nets. Collected seeds are thoroughly thrashed by feet to remove the membranous cover. Time of harvesting is very important for maximum yield. The optimum harvesting time is first fortnight of August (Thakur 1978; Jha and Prasad 1996).

7.14 Yield

The average yield of seeds varies from 2.5 to 3.0 tonnes per hectare of pond.

7.14.1 Processing of Makhana Seeds

Processing of makhana seeds are very tedious and still carried out by traditional methods due to lack of new processing technology. Seeds are sun-dried in the morning, so that the moisture content reaches around 31%. Water is sprinkled to keep the seeds fresh and moisture content optimum. The other steps involved are drying, size grading, preheating and tempering, roasting, and popping. Seeds are now further dried to facilitate removal of kernel from the seed coat. Seeds are passed through different size of sieves to differentiate them into 5–7 grades. Uniform heat transfer occurs when seeds of same size are heated during preheating and roasting. Graded seeds are heated in cast iron pan with continuous stirring over fire at 230 °C–335 °C for approximately 6 min. Tempering of seeds is followed by storing them in open baskets for 40–50 h. This loosens the kernel within the seed coat and increases the yield of popped seeds. Tempered seeds are roasted in 300 gm lots in an open pan over fire at approximately 230 °C–335 °C. When a crackling sound is heard, 5–7 seeds are taken out, kept on a hard surface, and hit with a wooden hammer. Seed coat breaks, and due to sudden release of pressure, the kernel pops out in expanded form, and seed coats are then removed manually. The edible part of makhana seed is perisperm, and the popped kernels, known as makhana, are polished by rubbing it against baskets made of bamboo splits without any delay to avoid absorption of moisture. Grading is done on the basis of size and whiteness. Polished and graded product is finally packed in polyethylene-lined gunny bags (Thakur 1978; Jha and Prasad 1996).



The general rule of thumb is that a bag of 8 kilograms (kgs) is an indication of high-quality *lava makhana*, and a bag that weighs more than 10 kg. contains mostly lower-quality *makhana* (*murha* and *turi*).

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Cereal β Glucan as a Functional Ingredient

8

Neha Mishra

Abstract

Cereal β glucans are linear polymer of β-D-glucose unit linked via (1–3)(1–4) glycosidic bonds. These water-soluble polysaccharides are natural component of the endosperm and aleurone cells of Poaceae family. Numerous studies documented the significant health benefits of β glucan beyond basic nutrition. Regular consumption of β glucan is effective in lowering glycemic index of food and serum cholesterol levels, controlling blood glucose and insulin levels, preventing constipation, decreasing the risk of colorectal cancer, and enhancing the immune system by protecting the body from bacterial and parasitic infections. β glucan also possesses various functional properties such as stabilizing, thickening, gelation, and emulsification. These functional and nutritional benefits of β glucan mainly depend on its concentration, molecular structure, and molecular weight. Over the last few years, β glucan has gained renewed interest in the food industry due to their diverse physicochemical properties that aid in development of new cereal products. To meet the rising demand of the consumer for functional food, modern food technology aimed at utilizing β glucan for the development of new products and delivering numerous health benefits to the community. This chapter presents the sources, structure, positive physiological effects of β glucans, and its utilization as functional ingredient in food industries.

Keywords

β glucan · Physiochemical properties · Biological activity · Functional food · Therapeutic benefits

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8.1 Introduction

The global markets for functional foods have been growing promptly day by day to meet the rising demand of the consumer. Functional foods are the food products that provide additional health benefits beyond basic nutrition. Food industries are in search of functional ingredients that have great potential for development of novel food products along with physiological benefits. Recently β glucan has been recognized as potential functional ingredient that has wide range of pharmaceutical activities with promising physiochemical and rheological properties for application in various food systems. β glucan is the polymer of glucose units linked by β(1,3) and/or β(1,4) linkage with or without side branches via β(1,6)/β(1,2) glycosidic linkage (Barsanti et al. 2011). They are derived commonly from cereals, yeast, algae, bacteria, and mushroom. Over thousands of years, β glucans have been consumed (Chen and Seviour 2007). However their potential as functional ingredients in development of nutraceutical food products has been recognized recently. Several studies documented the biological activities of β glucans such as anti-microbial, anti-diabetes, anti-tumor, anti-cancer, and hypocholesterolemic (Rahar et al. 2011; Suchecka et al. 2017). Besides health benefits, it possesses diverse physicochemical properties such as thickening, stabilizing, emulsification, and gelation (Ahmad et al. 2012) that aid to perform different functions in food processing industry. These health-promoting and physiochemical activities were attributed to its viscosity; this in turn depends on their structure, molecular weight, and concentration. Modern food technologist introduced plenty of β glucan-enriched functional food products in the market, and their number will steadily increase in the future. This chapter has an overview of β glucan sources, structure, physiochemical properties, multiple health benefits, and utilization in food industry.

8.2 Structure and Sources of Cereal β Glucan

Cereal glucans are concentrated in aleurone layer and endosperm cell walls of grain. Structurally cereal β glucan is long linear polymers of glucopyranose unit linked by *β*(1,3)/*β*(1,4) glycosidic bonds as depicted in Fig. 8.1 (Demirbas 2005; Herrera et al. 2016). The blocks of (1→4)-linked D-glucopyranosyl units are separated by single (1→3)-β-linked units. Generally (1→4)-linked blocks are mainly 3 or 4 glucose units long, but longer units of up to 14 glucosyl units are also found occasionally (Skendi et al. 2003; Lazaridou and Biliaderis 2007). This forms the structure consisting of 30% (1→3)- and 70% (1→4)-linked β-D-glucopyranosyl units which determines its functional and therapeutic activities. After hydrolysis β glucan releases two major oligomer units, as 3-*O*- *β*-D-cellobiosyl-D-glucose (DP3) and 3-*O*-*β*-D-cellotriosyl-D-glucose (DP4) (Yoo et al. 2007; Schierbaum 2008). The ratio DP3:DP4 determines its solubility and functional properties which vary with different sources. Generally, the ratio DP3:DP4 of oats, barley, and rye is 1.5–2.3, 1.8–3.5, and 3.0–4.5, respectively (Lazaridou and Biliaderis 2007). Solubility decreases as the degree of polymerization increases. The content of glucan varies

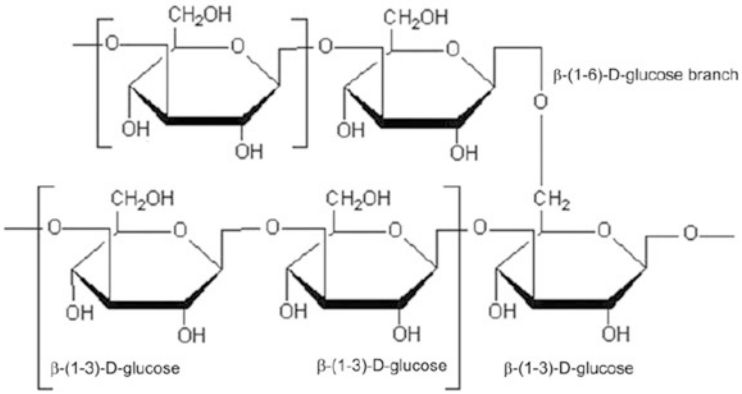

Fig. 8.1 Structure of β glucan (Rahar et al. 2011)

Table 8.1 β glucan content from different cereals

Sources	β glucan content (%)	References
Rice	0.4–0.9	Demirbas (2005)
Rye	0.7–2.4	Genç (2001) and Demirbas (2005)
Spring wheat	0.6–1.1	Demirbas (2005) and Cui and Wang (2009)
Winter wheat	0.4–1.4	HozoVá et al. (2007)
Hulless barley	3.7–9	Bhatty (1992)
Oats	3–7	Skendi et al. (2003); Demirbas (2005)
Corn/Maize	0.1–2.3	Demirbas (2005) and Blakeney and Flinn (2005)

with sources, cultivators, and environmental conditions. Among cereals, highest amount of glucans are in barley (5–11%) and oat grains (3–7%), while in wheat and rye, it constitutes only 0.5–1% and 1.4–2%, respectively (Saastamoinen et al. 2004; Tiwari and Cummins 2009) as shown in Table 8.1. The environmental conditions have little effect on glucan content although it varies with cultivators such as waxy genotypes documented higher amounts of *β* glucan as compared to normal genotypes (Anker-Nilssen et al. 2006).

8.3 Physiochemical and Functional Properties

For the last few years, the physicochemical properties and rheological characteristics have been studied extensively (Lazaridou and Biliaderis 2007). The functional properties particularly viscosity were positively correlated to the health benefits such as prevention of diabetes mellitus, coronary heart disease, cancer, and colon dysfunction (Meyer et al. 2000; Sudha et al. 2007; Pins and Kaur 2006; Aleixandre and Miguel 2008; Khoury et al. 2012; Daou and Zhang 2012; Du et al. 2014). However, the viscosity of *β* glucan depends on the molecular weight (MW), molecular structure, solubility, and concentration (Wood 2004). β glucan with high molecular weight has potential to form more viscous solution than low molecular weight at the same concentration (Colleoni-Sirghie et al. 2003). It was found that molecular mass varies with source and extractability with highest content in oats (about 30 × 105 Da) followed by barley (about 21 × 105 Da) (Wang et al. 2003). Generally, high molecular weight (MW > 100 kDa) β glucans are biologically more active as compared to low molecular weight (MW 5–10 kDa). In addition to molecular weight, the degree of branching also influences the biological activities of β glucans. It was found branching frequency of 0.2–0.33 exhibits high biological activity. Unbranched β glucans are biologically less active than the branched ones. Another factor contributing to the differences in the biological activities of β glucans is their side chain and conformation. For example, sulphated and carboxymethylated derivatives of insoluble glucan showed more activity against sarcoma 180 tumor and gastric carcinoma cells compared to its unmodified, methylated, hydroxymethylated, or hydroxypropylated derivatives (Wang et al. 2005, 2012). β glucans can exist in triple helix, single helix, or random coil conformation (Zeković et al. 2005). Several studies reported that the triple helix conformation is the most biologically active (Sletmoen and Stokke 2008), while others suggest that the single helix is the most active conformation (Aketagawa et al. 1993).

Molecular weight (MW) is the prime determinant of glucans along with physiochemical properties (Lazaridou et al. 2004) and biological activities (Lazaridou and Biliaderis 2007). It determines the physical properties such as water solubility, thickening, gelation, stabilizing, and emulsion properties that are important in formulating different food products and other industrial applications (Siurek et al. 2012; Lazaridou and Biliaderis 2007). In aqueous solution, β glucan is cross-linked at several points to form three-dimensional continuous network that traps liquid and retards its flow. Low molecular weight glucan has high gelation ability and forms soft elastic gel. As short chains of β glucan molecules have higher mobility and is greater possibility of forming junctions with neighboring chains and easily rearranging themselves (Li et al. 2006). This feature has been widely applied to make pseudoplastic solution in food process industry. Low molecular weight glucans have slower gelation time and higher gelation rate. For a specific MW, gelation rate also depended on concentration and had reported gelling capacity and strength can be increased by increasing concentration as more glucans are available for bonding (Skendi et al. 2003). It has been found that percent of β-(1→3)-linked cellotriosyl units has significant effect on gelling properties and elasticity of gels. Cui (2000) and Lazaridou et al. (2004) reported β glucan showed shear-thinning behavior at the low concentration of 0.2–3% while at a higher concentration it forms gel. Similarly gelling characteristics of three sources of β glucans of 10% oat, barley, and lichenan with similar molecular weight were investigated (Regand et al. 2011). Lichenan has the highest proportion of β-(1→3)-linked cellotriosyl units and gelled very quickly. Conversely, oat β glucan has least proportion of β(1→3)-linked cellotriosyl units and thereby required the most time to gel and the least amount of heat to melt (Brummer et al. 2014).

8.4 Biological Activity of β glucan

Numerous clinical studies documented the several health benefits of β glucan such as lowering serum cholesterol levels, controlling blood glucose and insulin levels, preventing constipation, decreasing the risk of colorectal cancer, and increasing the immunity toward bacterial and parasitic infections (Wasser 2011; Yu et al. 2012; Tosh 2013; Silva et al. 2015).

8.4.1 Hypochloesteromic Activity

The biological activity of glucan has been related to its property to form viscous solution. They have the ability to bind water and swell to form a gel-like network, thereby increasing the viscosity of gastrointestinal chyme which delays gastric emptying and reduces enzymatic activity and intestinal absorption of glucose. In addition, the colonic fermentation of β glucan produces short-chain fatty acid (SCFA) especially butyrate and propionic acid which increases glucose transporter type 4 (GLUT-4) via the peroxisome proliferator-activated receptor (γ PPAR) in the muscle and adipocyte (Topping and Clifton 2001). This facilitates the uptake of blood glucose to the muscle and adipocyte and could attenuate the blood glucose level. Andrade et al. (2015) who conducted a systematic review demonstrated that daily dose of 6 β glucans for at least 4 weeks was sufficient to improve blood glucose levels and also lipid parameters of individuals with diabetes mellitus. Several studies observed addition of β glucan lowers glycemic index of foods and consumption of this low glycemic index food is found to be very effective in controlling glycemic response and lowering serum cholesterol levels (Kabir et al. 2002; Andrade et al. 2015). The consumption of β glucan rich diet could reduce the risk of obesity which was attributed to its association with appetite and satiety. It have been reported that consumption of β glucan reduces gastrointestinal motility and food intake by modulating the release of various appetite-regulating hormone-like peptide YY (PYY), glucagon-like peptide 1(GLP-1), and cholecystokinin (CCK) (Beck et al. 2009). Yet further investigations are needed to confirm these mechanisms. Combining previous results, it was found that dose and carrier food forms also play an important role in satiety. A beverage containing 2.5 g or 5 g of oat β glucan significantly increased the feeling of fullness in comparison to beverage free from fiber (Lyly et al. 2009, 2010). In consistent to these, other studies also reported significant increase of postprandial satiety score for the beverages containing 1.2% w/v barley β glucan or 1.6% w/w oat bran (Lumaga et al. 2012; Pentikäinen et al. 2014). In contrast, no significant effect on satiety rating or gastric emptying was observed after intake of meal replacement bars enriched with 1.2 g barley β glucan (Kim et al. 2006; Peters et al. 2008). This could explain that dose is an important determinant on efficiency of β glucan on satiety and observe the significant linear relationship between the dose of oat β glucan and their hypoglycemic responses. In support to this, several studies observed the postprandial hypoglycemic response after intake of 6.2 g beta-glucan in a bar, 7.3 g in a breakfast cereal, and 9.4 g in flour

(Jenkins et al. 2002; Tapola et al. 2005). However, in healthy individuals, snack bar containing 1.5–3 g β glucan has no significant effect in glycemic response in comparison to control snack bar containing 11.9 g total dietary fiber (Panahi et al. 2014). These illustrate that for healthy individuals higher dose of glucan is required to alter their glycemic response. Willis et al. (2009) reported no significant effect on satiety and gut hormone response after intake of 0, 4, 8, and 12 g of mixed fiber in muffins; this could be solid foods mask the satiety potential of β glucan (Kirkmeyer and Mattes 2000; Khoury et al. 2012).

8.4.2 Hypocholesteromic Activity

Several mechanisms were proposed to explain the hypocholesterolemic effect. The most accepted theories are the following: (1) β glucan reduces blood cholesterol levels via interrupting bile acid metabolism. Consumption of β glucan increases the excretion of bile acid by inhibiting its reabsorbtion (Gunness and Gidley 2010; Gunness et al. 2016; Grundy et al. 2017). This upregulates the hepatic bile acid synthesis by utilizing plasma cholesterol which serves as substrate for newly synthesized bile acids and leads to a reduction of serum cholesterol levels in blood (Drzikova et al. 2005; Cohen 2008). (2) Cholesterol-lowering effect is also attributed to microbial fermentation of β glucans in the colon and produces short-chain fatty acids (SCFAs) primarily acetate, propionate, and butyrate as primary end products. Propionate in turn reduces cholesterol by suppressing cholesterol synthesis in the liver due to the inhibition of HMG-CoA reductase (Gunness and Gidley 2010; Khoury et al. 2012). Kerckhoffs et al. (2003) reported that regular consumption of soluble fiber (3 g) may lower the total cholesterol by 0.41 $mmolL^{-1}$ and 0.13 $mmolL^{-1}$ in hypercholesterolemic and normocholesterolemic persons, respectively. In support studies reported daily consumption of 5 g of β glucan significantly lowers the serum cholesterol levels (Naumann et al. 2006; Theuwissen and Mensink 2007). In 2006, the European Food Safety Authority (EFSA) recommended daily dose of 3 g β glucan significantly lowers serum cholesterol and supports healthy glucose metabolism by maintaining healthy digestive system (Health Canada 2002). Meta-analysis of previous studies confirmed the effect of cereal β glucan on significant reduction of total cholesterol as well as LDL cholesterol levels (Ames et al. 2006; Ho et al. 2016). Wang et al. (2017) indicated that β glucan decreased total cholesterol (TC) levels due to increased bile acid synthesis rather than inhibition of cholesterol absorption or synthesis.

8.4.3 Anti Tumor Activity

It is also documented that β glucans have a potential to prevent colonic diseases, ulcerative colitis, and colon cancer (Kim et al. 2006; Nilsson et al. 2008). It inhibits the growth of already degenerated cells and induces apoptosis by arresting cell division through its ability to regulate histone deacetylase inhibitor (Borowicki et al. 2010, 2011; Schlörmann et al. 2012). β glucans can act as prebiotics and stimulate the growth of *Bifidobacteria* in the colon (Hamaker and Tuncil 2014). Butyrate is the energy source for the healthy colonic epithelial cells and repairs the damaged DNA (Toden et al. 2007; Topping et al. 2008). These chemopreventive actions reduce the risk of colonic cancer.

8.4.4 Other Activity

The effects of cereal β glucan on the immune system and their role in preventing infections are not well documented. Vetvicka (2011) showed the protective effect of β glucans to infections caused by fungi, protozoa, and bacteria such as *Staphylococcus aureus* and *E. coli.* Commonly daily consumption of β glucans isolated from fungi significantly increases the immuno-modulatory and anti-inflammatory activity (Silva et al. 2015). It is documented that they are potent inducers of humoral and cell-mediated immunity and increase the production of interleukin (IL)-1 and tumor-necrosis factor (TNF)-α. Recently the roles of β glucan on improving the skin health were reviewed by Du et al. (2014). They noted the antioxidant activity, anti-wrinkle activity, anti-ultraviolet light, wound healing, and moisturizing effect of β glucan which improves the overall health of skin.

8.5 Application in Food Industry

From the last few decades, the knowledge and consumer awareness for the functional foods have been raised. Recently β glucan is recognized as a functional ingredient that along with several health benefits possesses varied rheological properties such as gel formation, emulsification, and thickening required to develop innovative nutraceutical food products. Health Canada (2002) recommended that 3–5 g of β glucan/day reduces cholesterol and blood glucose level and supports to maintain a healthy digestive system. In order to reach the daily amount, β glucan-rich fraction is successively incorporated into products such as breakfast cereals, baked goods (bread, muffins), and dairy meat products (Cavallero et al. 2002; Lazaridou et al. 2007). Cereals could be a desirable vehicle for food fortification as they are widely consumed by large population. From the last few years, incorporation of glucan in bakery industries has stemmed to enrich wheat flour with soluble fiber (Trogh et al. 2004). There are several studies on β glucan-enriched bread with improved physiochemical, sensory, and rheological properties (Cavallero et al. 2002; Flander et al. 2007; Lee et al. 2009; Škrbić et al. 2009; Skendi et al. 2010). Several studies showed β glucan improved the rheological and viscoelastic properties of batter which might be due to better gas retention (Moriartey et al. 2011). Owing to its high apparent viscosity, water holding capacity, and emulsion stabilizing property, β glucan has potential to be used as a fat replacer in meat, beef products, and dairy products (Tudorica et al. 2004). Low-fat products with reduced calorie and cholesterol and improved texture properties were developed by

Table 8.2 Utilization/incorporation of β glucan into various food products

Type	Product	Physiochemical effects	Reference
Oat β glucan	Bar	No negative effect on sensory properties	Jenkins et al. (2002)
Barley β glucan	Bread	Reduced starch breakdown No negative effect on sensory properties	Cavallero et al. (2002)
Barley β glucan	Low fat sausages	Function as fat replacer No significant effect on product texture or flavor at 0.3% (w/w)	Morin et al. (2002)
Barley β glucan	Orange-flavored beverage	Increases viscosity	Temelli et al. (2004a, b)
Oat β glucan	Probiotic milk-based drinks	Increased stability	Angelov et al. (2006)
Oat β glucan	Low-fat yogurt	Increased the viscosity and decreased the separation of whey	Sahan et al. (2008)
Oat β glucan	Bread	Improved crust color, softness, and taste, increased firmness of the bread crumb	Lazaridou et al. (2007)
Oat β glucan	Beverages	Thicker, more extensible, grainier, and slimy	Lyly et al. (2009)
Oat β glucan	Noodle	Decreased firmness, elasticity, surface smoothness, and flavor	Choo (2007)
Oat β glucan	Yogurt	Increased firmness and stickiness in texture	Nikoofar et al. (2013)

incorporation of β glucan in breakfast sausages, frankfurters, and mayonnaise (Morin et al. 2002; Thammakiti et al. 2004). Additionally the high water holding capacity and thickening property are often used to improve the consistency of food products, e.g., drinks, dressings, fermented dairy products, dairy desserts, or ice cream (Dongowski et al. 2005; Worrasinchai et al. 2006). Food processors have been utilizing glucan for replacement of thickeners such as pectin, gum arabic, and alginates in beverages, ice creams, sauces, and salad dressings (Ahmad et al. 2010).

Sharafbati (2012) prepared a low-calorie dairy product by incorporation of β glucan with similar texture, firmness, and sensory attributes. β glucan has a great potential to be incorporated in dairy products such as in yogurt (Sahan et al. 2008; Ozcan and Kurtuldu 2014), dairy gels (Sharafbafi 2012), cheese (Konuklar et al. 2004; Volikakis et al. 2004), low-fat ice creams (Brennan et al. 2002), and low-fat cheese curds (Tudorica et al. 2004). Symbiotic association of microorganism and β glucan results in the release of several bioactive compounds which modify textural attributes of milk products by controlling phase separation of milk proteins and polysaccharides (Lazaridou et al. 2014). Awareness of symbiotic (probiotic + prebiotic) food products has been rising in consumers and thereby in food industries. Angelov et al. (2006) prepared β glucan-enriched fermented drink by fermenting whole oat grains by lactic acid bacteria. Temelli et al. (2004a, b) formulate β glucan-enriched beverage having 0.5% β glucan by use of orange flavor/whey protein isolate. No significant difference was observed in all attributes of sensory except sourness, and orange flavor decreased with increasing whey protein isolates. Barley *β* glucan is well suited for such applications in beverages, being capable of imparting a smooth mouth feel (Table 8.2).

8.6 Conclusion

The epidemiological studies documented that β glucan intakes have been associated in promoting health and lowering risks of many diseases such as CVD, diabetes, gastrointestinal disorder, obesity, and cancer. Due to their physicochemical properties such as viscosity, water holding capacity, solubility, and gel formation, the carbohydrates present in β glucan not only improve the functional properties but also improve the textural and rheological properties of food products. Numerous studies focused on various possibilities of exploitation of cereal β glucans as important functional ingredients for the development of new cereal products in food industry. Emerging researches have to focus on different technologies to obtain more concentrated sources of β glucan and production of functional food with high β glucan to meet the needs of β glucan set by health agency.

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Part II

Food and Industrial Microbiology

9 Rediscovering Medicinal Activity and Food Significance of Shogaol (4, 6, 8, 10, and 12): Comprehensive Review

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Abstract

Ginger *Zingiber officinale* Roscoe is a natural dietary rhizome. The plant is a herbaceous tropical monocotyledon perennial plant which belongs to the family Zingiberaceae and subfamily Zingiberoideae and possesses various biological properties and/or activities. Ginger has been reported to play several roles in ameliorating several health conditions which might be linked to the presence of numerous biological components including gingerols, gingerdiols, shogaols, paradols, and zingerones. However, shogaol has been found to be a major active component of ginger which exists in various forms such as 4-, 6-, 8-, 10-, and 12-shogaol. Also, 6-shogaol has been discovered to be the most active component which possesses a non-pungent metabolite called 6-paradol. 6-Shogaol has

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various importance in the health, food, and beverage industries. The wide range of the usefulness of shogaol is associated with its taste, biocompatibility, and effect in ameliorating and/or preventing health challenges. Some uncountable medical benefits of shogaol include their application as anticancer drugs, antimicrobials, antioxidants, cardiovascular, anti-ulcer, and neuroprotective among others. Therefore, this review highlights recent advances and findings to the use of shogaol present as a bioactive substance in ginger and its wide application in medical, biological, and food industry.

Keywords

Ginger · Shogaol · Antimicrobials · Antioxidants · Biocompatibility · Medical · Food industry

9.1 Introduction

Ginger, a natural dietary rhizome of the *Zingiber officinale* Roscoe, is a herbaceous tropical monocotyledon perennial plant which belongs to the family Zingiberaceae and subfamily Zingiberoideae (Fatoki et al. 2016; Vasala 2001). The classes of bioactive components of ginger include gingerols, gingerdiols, shogaols, paradols, and zingerones (Baliga et al. 2011; Ali et al. 2008). The pharmacological essentiality of ginger such as antioxidant, anti-inflammatory, anti-neuroinflammatory, anti-emetic, anti-neoplastic, antitumor, and hypotensive effects has been majorly attributed to two of its bioactive components, 6-gingerol and 6-shogaol, and attracted research interests (Seow et al. 2017; Kim et al. 2014; Shim and Kwon 2012; Ha et al. 2012; Dugasani et al. 2010; Weng et al. 2010; Betz et al. 2005; Kim and Kim 2004). Numerable commercial varieties of ginger exist globally, and about 25 species of Zingiberaceae are used as spices and ethnomedicinal cure for multiple diseases such as gastrointestinal inflammations, hypertension, microbial infections, dementia, osteoarthritis, cancer, and others, since ancient years, owing to its rich chemical constituents (Seow et al. 2017; Fatoki et al. 2016; Rahmani et al. 2014). Shogaol is a phenolic compound that is derived from gingerol conversions through thermal processing of ginger, having various components such as 4-, 6-, 8-, 10-, and 12-shogaol, and is the most reactive component of ginger that has the ability to relieve various health challenges. In view of the aforementioned, this chapter provides holistic reports on recent utilization of shogaol in food and medical sector.

9.2 Antimicrobial Activity

Arshad and Shadab (2017) tested the antimicrobial effect of ginger using disk diffusion against *E. coli*, *S. epidermidis*, *S. aureus*, *and P. mirabilis.* The result obtained showed that the methanolic extract exhibited more enhanced antimicrobial activity when compared to the hexane extract. Some of the active components confirmed present in the crude extract include α-curcumene, gingerol, β-sesquiphellandrene, zingiberene, shogaol, β-bisabolene, and α-farnesene. They also execute computational studies which were used for the evaluation of drug-likeness and physicochemical attributes for all the constituents in the crude extract. The result obtained from the computational results indicated that all the components possessed enhanced bioactivity score but with the exception of component-7; when compared to all other components, the component-2, component-3, component-4, and component-7 were discovered against the Lipinski's rule of five with respect to the partition coefficient. Moreover, molecular docking trial was performed to collaborate the experimental results which state that hydrogen bonding with the receptor is discovered only in shogaol and gingerol and both the constituents were discovered mainly in methanol extract.

Riaz et al. (2015) assessed the antimicrobial effectiveness of ginger grown in the local area of Punjab, Pakistan. The crude extract of the ginger was tested against various isolates such as *Bacillus subtilis*, *Streptococcus faecalis*, *E. coli*, and *Staphylococcus aureus.* The main active constituents responsible for the antimicrobial activity of the crude extract might be linked to the presence of shogaols, diarylheptanoids, volatile oils, and gingerols. The authors also validate the presence of phytochemicals present in the crude extracts. It was discovered that the crude extract exhibits some level of antimicrobial activity against all the tested isolates. The authors suggested that the utilization of spices with a sustainable alternative could mitigate against all the adverse effect of medicine due to their high level of resistance. It will also minimize the high level of the poor people in these areas by minimizing the amount of money that would have been used in procuring expensive medicine.

Hassan et al. (2017) evaluated the antimicrobial effect of *Zingiber officinale* used in Iraq and determined the various chemical constituents responsible for its antimicrobial effect using HPLC. The antimicrobial activity was performed using agar well diffusion techniques against the fungal and bacterial isolates. The active components were extracted using soxhlet techniques with n-hexane and methanol as a solvent. The result shows the presence of seven components by the analysis performed by HPLC. Moreover, it was discovered that two extracts exhibit antimicrobial activity while methanol shows more effectiveness in comparison to n-hexane extract when tested against all the isolates. Their study shows that the plant of *Zingiber officinale* possessed antifungal and antibacterial activity which shows that it could be of great benefit in several sectors particularly source of active component with wild application in preservation of foods, pharmaceutical, and industry.

Pratap et al. (2017) wrote a comprehensive review on the potential of ginger as a source of neutraceutical, pharmaceutical, and cosmetic components. They stated that ginger possessed several active ingredients which might be volatile (zingiberene) and non-volatile (oleoresin) and some phenolic constituents like paradol, gingerol, zingerone, and shogaol. They also stated that there is the presence of several nutritional components like saccharides, coloring matter, starch, trace minerals, crude fiber, wax, or lipid. Moreover, they also highlighted some of the pharmacological benefits of ginger like anti-diabetic, antioxidant, gastroprotective, anti-inflammatory, and antibacterial.

Hassan et al. (2017) evaluated the effect of rhizome essential oil of *Zingiber officinale* against several isolates such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella*

pneumoniae, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*. The antimicrobial activity was performed using cup plate agar diffusion techniques. The result obtained reveals that the essential oil dissolved in methanol extract exhibits a very high antimicrobial activity against the following isolates *P. aeruginosa* (27 mm), *S. aureus* (23 mm), *P. vulgaris* (20 mm), *B. subtilis* (20 mm), and *E. coli* (21 mm) while moderate activity was observed against *C. albicans* (16 mm) and *K. pneumoniae* (15 mm) and no antimicrobial activity was recorded against *Aspergillus niger*. Moreover, the result obtained from the minimum inhibitory concentration assay performed using the agar diffusion technique showed high level of sensitivity against all the tested isolates.

Rigane et al. (2017) isolated and purified 6-shogaol (N) from rhizome of *Zingiber officinale* Roscoe with the aid of methanol as the solvent. This was performed by elution using a silica cartridge with 150 mL of *n*-hexane-diethyl ether (70/30, v/v). The structural elucidation of 6-shogaol was further confirmed using ^{1}H-NMR and ^{13}C-NMR, MS, and UV. The antimicrobial and the antioxidant properties of the active components were performed using standard procedure. The result obtained shows that the crude extract exhibited a high antimicrobial activity against gram negative and gram positive while the minimum inhibitory concentration of the active component varies from 0.312 to 2.5 mg/mL. Their study shows that 6-shogaol is a very active biological compound that has several benefits most especially because of its antimicrobial properties.

Candida albicans has been highlighted as an opportunistic pathogen which causes several reported cases of candidiasis. *C. albicans* possess the capability to develop biofilms on the abiotic and biotic surface, and these biofilms can form systemic and local infections. Moreover, it has been observed that *C. albicans* biofilms exhibit more resistance to its free yeast when compared to antifungal agents without any harmful effect to host immune responses. One of the most crucial stages during the development of biofilm formation is the transition of yeast cells to hyphal cells which is a significant virulence factor. In view of the aforementioned, Lee et al. (2018) evaluate the antivirulence and antibiofilm activity against a fluconazole-resistant *C. albicans* strain using six-component extract from ginger. It was observed that 6-shogaol, 6-gingerol, and 8-gingerol exhibited a high level of inhibition against the development of biofilm. Specifically, concentration of 10 μg/ml from 6-shogaol minimized *C. albicans* biofilm formation without any adverse effect on planktonic cell growth. Moreover, 6-shogaol and 6-gingerol prevented development of hyphal embedded colonies and free-living planktonic cells and obstructed the development of cell aggregation. Additionally, 6-shogaol and 6-gingerol minimized *C. albicans* virulence in a nematode infection model without any adverse effect from all the tested concentrations. The transcriptomic analysis performed using qRT-PCR and

RNA-seq exhibited that 6-shogaol and 6-gingerol prompted numerous transporters including *CDR1*, *RTA3*, and *CDR2* but suppressed the expressions of numerous hypha/biofilm associated genes (*HWP1* and *ECE1*), which buttressed detected phenotypic alterations. Their study shows that 6-shogaol and 6-gingerol possess a high level of antibiofilm and antivirulence activities against a drug-resistant *C. albicans* strain.

Khiralla (2015) tested the antibiofilm, antimicrobial, and anti-adhesive effect of several components of ginger including 6-, 8-, and 10-shogaols and 6-, 8-, and 10-gingerols after extracting with fresh and cooked ginger water extracts (FGE and CGE, respectively). The result obtained shows that the two extracts exhibit a high level of antimicrobial activity against three strains of *Escherichia coli* O157: H7 and six strains of *Salmonella typhimurium*, respectively. Also, the highest effective minimum inhibitory concentration from the two extracts was 25 mg DTSS /mL. Moreover, it was observed that the fresh ginger extract exhibits a very high antibiofilm and anti-adhesive capability when compared to the cooked ginger extract, while the utilization of 20 mg DTSS/mL from the fresh ginger extract led to the minimization of adhesive properties of the tested bacterial cells to HEp-2 cells up to 50%. The authors concluded their study by stating that soaking of 4–5 g fresh ginger rhizome in 200 mL in cold aqueous water (20–25 DTSS) for a period of 12 hours could produce an antibiofilm and anti-adhesive agent against food-related pathogens used during this study most especially *E. coli* O157:H7 and *S. typhimurium*.

9.3 Anticancer Effect of Shogaol

6-Shogaol [1-(4-hydroxy-methoxyphenyl)-4-decen-one] is a monomethoxybenzene and the most abundant shogaols found in ginger. The anticancer mechanism of 6-shogaol observed from human cancer cells involved the suppression of multiple signaling pathways (Weng et al. 2010; Saha et al. 2014; Warin et al. 2014). The objective of the review was to mine the anticancer effect of 6-shogaol. Natural products play an important role in the context of anticancer drug discovery (Newman and Cragg 2016).

Cancer is an aberrant growth of the normal cells into malignancy or metastasis from the initial point (Ferlay et al. 2015). There are more than 277 different types of cancer diseases (Hassanpour and Dehghani 2017), which are classified based on the extent of the metastasis, and it could be grouped into four main types: carcinoma, sarcoma, lymphoma, and leukemia. Due to the multiple strategies used by cancer cells such as high glycolytic flux, redox signaling, and modulation of autophagy, which involve systemic interactions, the context of the systemic level anticancer drug has been noted (Fatoki et al. 2018), and efforts are directed on the exploration of medicinal plant for safe bioactive compounds.

9.4 In Vitro Studies

9.4.1 Anticancer Activity of Shogaol

One of the most common cancers worldwide is colorectal cancer (Siegel et al. 2015). Qi et al. (2015) studied anti-proliferation effect of 6-shogaol using human colorectal cancer cell lines HCT-116 and SW-480 which were seeded in 96-well plates and allowed to stand for 24 h before 6-shogaol at varying concentrations was introduced into the medium. Their results showed that 6-shogaol inhibited the growth of HCT-116 and SW-480 cells at 50% inhibitory concentration (IC50) of about 7.5 and 10.0 μM, respectively.

Zhu et al. (2013) evaluated the anticancer effect of 6-shogaol and its synthetic metabolites through cell growth inhibition of four cancer cells, namely, HCT-116 human colon cancer cells, H-1299 human lung cancer cells, CCD-18Co human colon fibroblast cells, and IMR-90 human lung fibroblast cells, by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay, in 96-well microtiter plates for 24 h at 37 °C. TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay was then carried out on HCT-116 and H1299 cells which were seeded in 6-well plates and incubated at 37 °C in 5% CO_2 incubator, to detect the DNA break characteristic of cells undergoing apoptosis. The result of their work showed that two synthetic metabolites of 6-shogaol designated M2 and M13 have comparable cytotoxic and inhibitory effect as 6-shogaol. M2 which was cysteine conjugated metabolite of 6-shogaol has IC50 values of 24.43 and 25.82 μM for HCT-116 and H-1299 cells, respectively, while M13 which was 5-glutathionyl-6-shogaol has IC50 values of 45.47 and 47.77 μM for HCT-116 and H-1299 cells, respectively, whereas the parent compound, 6-shogaol, has IC50 values of 18.20 μM and 17.90 μM for HCT-116 and H-1299 cells, respectively. The result of TUNEL assay at 6 hours of incubation showed a similar level of the apoptotic effect of 6-shogaol and the effects of M2 at 20 and 40 μM and M13 at 40 mM, while M13 was significantly more potent than 6-shogaol at 20 μM. This fantastic result of these metabolites is liable to fast in vivo turnover, absence in ordinary food contents, and possibility of toxicity at higher dosages (Karakaya 2004), whereas 6-shogaol has been reported to be extensively metabolized in normal and cancer cells with over 90% conversion to form the metabolites (Chen et al. 2012), thus making these metabolites stable and exert functional bioactivities in biological process (Wild et al. 2001; Zhu et al. 2013).

Zhang et al. (2019) investigated the bioavailability and hepatoprotective effect of free 6-shogaol and 6-shogaol loaded in micelles (SMs) at different concentration by in vitro cell viability through MTT assay on HepG2 human hepatoma cells which were seeded in 96-well plates at a density of 4000 cells/well for 24 h at 37 °C in a 5% CO_2 incubator. The result showed that the proliferation of HepG2 cells was inhibited by both free 6-shogaol and SMs in a dose-dependent manner. However, the cell viability of free 6-shogaol on HepG2 cells was found higher than that SMs at low concentration between 5 and 50 μg/mL; this enhanced inhibition of cancer cell in vitro was attributed to the possible different cellular uptake mechanism by SMs such as carrier-mediated endocytosis, while free 6-shogaol entered the cell by passive diffusion (Zhang et al. 2016; Hou et al. 2017). Moreover, 6-shogaol has been reported to attenuate tumor cell propagation and induce tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated cell death in liver cancer cells (Nazim and Park 2018).

Breast cancer is the second leading cause of cancer-related mortality among females worldwide (Jemal et al. 2009). Breast cancer has an important effect on society and life quality of women; so, it becomes life-threatening condition such as premature death and reduced productivity (Ferlay et al. 2001). Ling et al. (2010) evaluated the effect of shogaols (6-, 8-, and 10-shogaol) on the MDA-MB-231 human breast cancer cells using the Matrigel invasion assay as well as suppressive effects of 6-shogaol on phorbol 12-myristate 13-acetate (PMA)-induced matrix metalloproteinase-9 (MMP-9) gelatinolytic activity and nuclear factor-kB (NF-kB) activation. They found that shogaols (6-, 8-, and 10-shogaol) inhibited MDA-MB-231 cell invasion, while NF-kB transcriptional activity and MMP-9 gene activation were greatly reduced by 6-shogaol. The inhibition was attributed to a possible involvement of 6-shogaol on activator protein-1 signaling pathway which is regulated by mitogen-activated protein kinases (Overall and Lopez-Otin 2002; Woo et al. 2004; Shin et al. 2007; Lin et al. 2008).

Hsu et al. (2015) investigated the ability of 6-shogaol to impair development and metastasis of lung and breast cancer. The results of the in vitro study showed complete inhibition of the secretion of inflammatory mediator CC-chemokine ligand 2 (CCL2) derived from tumor-associated dendritic cells (TADCs) facilitated by human lung cancer A549 and breast cancer MDA-MB-231 cells in in vitro assay. Lung cancer (newly called adenocarcinoma in situ) is one of the leading causes of mortality throughout the world (Fatoki et al. 2018; Travis et al. 2015). Kim et al. (2014) investigated whether 6-shogaol could affect the growth and induce apoptosis of non-small cell lung cancer (NSCLC) cells in vitro. The cell proliferation was conducted on human NSCLC cell lines (NCI-H1650, NCI-H520, and NCI-H1975); treated with different concentrations of 6-shogaol, 6-gingerol, or 6-paradol; and then measured by MTS assay. They further studied which direct target in PI3-K/Akt signaling pathway amplification associated with NSCLC pathogenesis was affected by 6-shogaol. They found that 6-shogaol showed the most significant effect on the viability of NCI-H520, NCI-H1650, and NCI-H1975 cells compared with 6-gingerol or 6-paradol. They reported that 6-shogaol greatly decreased the kinase activities of Akt1 and Akt2 in an ATP-independent binding mode but no impact on kinase activities of PI3-K.

Saha et al. (2014) studied the effect of 6-shogaol on the growth of prostate cancer cells. In an in vitro experiment, cell viability of human prostate cancer cells (LNCaP, DU145, and PC-3) and HiMyc mouse prostate cancer (HMVP2), treated with different concentrations of 6-shogaol, 6-gingerol, or 6-paradol, was measured by MTT assay. Their finding showed that 6-shogaol inhibited the growth and survival of both cultured human (LNCaP, DU145, and PC3) and mouse (HMVP2) prostate cancer cell lines. They found that 6-shogaol inhibited the phosphorylation of STAT3 in both LNCaP and DU145 cells in dose-dependent manner with subsequent decrease level of cyclin D1, survivin, and c-Myc and modulated mRNA levels of cytokine, chemokine, and apoptosis regulatory genes (IL-7, CCL5, BAX, BCL2, p21, and p27).

9.5 In Vivo Studies

9.5.1 Anticancer Activity of Shogaol

Qi et al. (2015) investigated the in vivo anticancer activity of 6-shogaol using a xenograft model of human colon cancer cells using HCT-116-Luc cells in 50 μl PBS injected into the flanks of each female athymic nude mice in groups, and 6-shogaol at 15 mg/kg was administered intraperitoneally after 24 h consecutively for 4 weeks. Their results showed that 6-shogaol mediated inhibition of xenograft tumor growth significantly ($p < 0{:}01$), though with no complete elimination of the tumors. Also, the G2/M phase of the cell cycle of the HCT-116 cells was arrested by 6-shogaol in a dose-dependent manner with induction of apoptosis through the upregulation of p53-dependent pathway, which led to increase in the level of $p21^{waf1/cip1}$, as well as by the downregulation of cdc2 and cdc25A, and these results corroborated the previous studies (Liu et al. 2000; Timofeev et al. 2009).

Zhang et al. (2019) investigated the bioavailability and hepatoprotective effect of free 6-shogaol and 6-shogaol loaded in micelles (SMs) at a different concentration by in vivo experiment which was tested in Kunming mice using CCl_4-induced hepatotoxicity model. They reported that SMs showed better hepatoprotection by higher activities of glutathione peroxidase and total superoxide dismutase as well as lower levels of malondialdehyde in the liver when compared to free 6-shogaol and the positive control group. The mechanism of 6-shogaol hepatoprotection was attributed to the phenolic structure which conferred antioxidant and anti-inflammatory properties (Li et al. 2012; Zhang et al. 2013).

Hsu et al. (2015) investigated the ability of 6-shogaol to impair development and metastasis of lung and breast cancer. In the in vivo study of the mediation of CC-chemokine ligand 2 (CCL2) derived from tumor-associated dendritic cells (TADCs) by 6-shogaol, 4 T1 mouse mammary tumor cells were implanted into mammary fat pads of BALB/c mice groups, and Lewis lung carcinoma (LLC) cells were implanted into C57BL mice groups. The 6-shogaol-treated group was given intraperitoneal injection (ip) daily for 14–21 days. They reported that 6-shogaol decreased CCL2 levels in TADCs of the lungs of mice with LLC transplantation as well as mammary tissue of the mice with 4 T1 tumors, and also the metastasis of both the breast and lung cancers was reduced. The mechanism of CCL2 inhibition by 6-shogaol in both breast and lung cancers was found associated with inhibition transcription factor STAT3 pathway activation in TADCs. Hsu et al. were the first to report involvement of TADCs in lung and breast metastasis and the inhibitory action of 6-shogaol on the production of TADCs. Moreover, another study reported that 6-shogaol was found effective in mortifying both breast cancer monolayer cells and spheroids at doses that were not toxic to noncancer cells (Ray et al. 2015).

Kim et al. (2014) investigated whether 6-shogaol could affect the growth and induce apoptosis of non-small cell lung cancer (NSCLC) cells in vivo. In the xenograft mouse model study, NCI-H1650 cells were injected into the subcutaneous on the right hind flank of groups of female BALB/c mice and were given 6-shogaol after 6 days of implantation. They found that [6]-shogaol reduced constitutive phosphorylation of STAT3 in NCI-H1650 cells and subsequent dose-dependent decrease in the level of cyclin D1, cyclin D3, and c-Myc which are gene products of STAT3 (Zhang et al. 2005). Furthermore, they found that 6-shogaol greatly reduced the growth of xenograft tumors. Also, Hung et al. (2009) have previously reported that 6-shogaol induced autophagic cell death in A549 human lung cancer cells.

Saha et al. (2014) studied the effect of 6-shogaol on the growth of prostate cancer cells. In an in vivo experiment, Matrigel which contained spheroids from HMVP2 cells was injected subcutaneously into the flank of syngeneic FVB/N male mice groups, and 6-shogaol was administered intraperitoneally daily for 32 days. They found that 6-shogaol decreased the growth and induced apoptosis in HMVP2 cells by inhibiting both constitutive and IL-6-induced phosphorylation of STAT3 and subsequently decrease the level of pSTAT3, cyclin D1, and survivin. Moreover, 6-shogaol was found inhibiting the activation of both STAT3 and NF-kB signaling in hepatocellular carcinoma cells and exhibiting antitumor activity in vivo via oxidative stress (Hu et al. 2012; Chen et al. 2007). However, it has been demonstrated that 6-shogaol could portend induced apoptosis and G2/M phase arrest in human cervical cancer HeLa cells and that endoplasmic reticulum stress and mitochondrial pathway were involved in 6-shogaol-mediated apoptosis (Liu et al. 2012).

9.6 Anti-ulcer Effect of Shogaol

Ulcers are defects in the gastrointestinal mucosa, stomach, as well as duodenum. Ulcers occur as dysfunction of the acid or peptic activity in gastric juice and are often chronic in nature (Fauci et al. 2008; Agbaje and Doe 2015). Peptic ulcer disease is an important cause of morbidity, and the epidemiology has been linked with two major etiological factors, nonsteroidal anti-inflammatory drug (NSAID) usage and *Helicobacter pylori* infection (Agbaje and Doe 2015). However, the main mechanism of gastroprotection is by inhibition of both the proton pump and *Helicobacter pylori* growth and also increased mucin secretion (Siddaraju et al. 2010).

Several factors which have been reported to play vital roles in the causation of gastric mucosal damage and its subsequent development include gastric microcirculation, gastric acid and pepsin secretion, prostaglandin E2 (PGE2) content, tumor necrosis factor (TNF), and pro-inflammatory cytokines interleukin (IL)-1 (Santucci et al. 1995; Appleyard et al. 1996; Wang et al. 2007; Wallace 2008; Laine et al. 2008). Also, an increase in nitric oxide synthase (NOS) activity has been reported to involve in the pathogenesis of mucosal damage (Muscara and Wallace 1999; Wallace and Miller 2000).

Several anti-ulcer compounds have been isolated from ginger, including 6-gingesulfonic acid (Yoshikawa et al. 1992), 6-shogaol, and curcumene (Ghayur et al. 2005). 6-Shogaol [1-(4-hydroxy-methoxyphenyl)-4-decen-one] is a monomethoxybenzene and the most abundant shogaols found in ginger. The objective of the review was to mine the anti-ulcer effect of 6-shogaol.

9.7 In Vivo Studies of Anti-ulcer Activity of Shogaol

Wang et al. (2011) investigated the anti-ulcer effects of ginger in aspirin-induced gastric ulcer model male Wistar rats. The groups of rats with stomach surgery were orally given 3 mL of 1% carboxymethylcellulose (CMC) in water, ginger powder suspended in 3 mL of 1% CMC in water, or aspirin suspended in 3 mL of 1% CMC in water. After 4 h of administration, the area covered by hemorrhagic ulceration in the stomach was measured. Another experiment which makes use the same procedure was carried out on 6-gingerol and 6-shogaol, and the doses were determined by those found in normal ginger according to the method of Schwertner and Rios (2007). The result showed no pathological changes in the normal cellular architecture in the gastric mucosa of rat treated alone with ginger powder. There was a significant increase in gastric mucosal inducible NOS (iNOS) activity in the aspirin-induced ulcerated rats when compared to that of the control or ginger administered rats. 6-Gingerol and 6-shogaol also showed no adverse effects on the stomach and inhibited the ulcer by increasing the mucosal iNOS activity and plasma TNF- and IL-1 levels.

Zaman et al. (2014) evaluated the anti-ulcerogenic effect of *Zingiber officinale* root powder in albino rats. The group of rats were treated with indomethacin to induce gastric damage and were orally given distilled water, ginger powder, or omeprazole. The NSAID-induced gastric damage in the rats by indomethacin was examined after 6 h. The results showed that the gastric damage induced by indomethacin was significantly inhibited by ginger root extract ($p < 0.05$) and possessed gastroprotective efficacy when compared to omeprazole, a proton pump inhibitor. They suggested that the mechanism of the anti-ulcer effect of ginger could be due to inhibition of 5-lipoxygenase which is one of the key enzymes in the arachidonic acid metabolic pathway.

6-Shogaol and few other bioactive components of ginger such as sesquiphellandrene and bisabolene have been reported for anti-ulcer activity in HCL-/ethanol-induced gastric lesions in rats (Yamahara et al. 1988, 1992; Yoshikawa et al. 1994). Yamahara et al. (1990) studied the effect of orally administered acetone extract of ginger, 6-shogaol, as well as 6-, 8-, and 10-gingerol on the gastric motility (charcoal meal) in mice. They found that the administered ginger and its components enhanced gastric motility. The result was later corroborated by the study by Sharma and Gupta (1998), who found that orally administered aqueous, acetone, and 50% ethanolic extracts of ginger reversed cisplatin-induced delay in gastric emptying in rats.

It is important to note that 6-gingesulfonic acid, which showed weaker pungency, has been found to have more potent anti-ulcer activity than 6-gingerol and 6-shogaol (Yamahara et al. 1988; al-Yahya et al. 1989; Yoshikawa et al. 1994).

9.8 Antioxidant Activity

Antioxidants are organic substances that wage war against oxidative processes, thereby inhibiting or suppressing oxidative stress which is either induced or generated. Hence, the search for natural antioxidant from a safety point of view had made human resort to the use of ginger as a spice.

Wei et al. (2017) documented that 6-paradol and 6-shogaol, the pungent compounds of ginger, promote glucose utilization in adipocytes and myotubes and 6-paradol reduces blood glucose in high-fat diet-fed mice. They evaluated the antihyperglycemic activity of ten ginger active components comprising 6-, 8-, and 10-paradols; 6-, 8-, and 10-shogaols; 6-, 8-, and 10-gingerols; and zingerone, and it was found that among all the constituents tested, 6-paradol and 6-shogaol exhibited a more potent activity in stimulating utilization of glucose by 3T3-L1 adipocytes and C2C12 myotubes. Furthermore, in an in vivo study carried out, the authors reported that 6-paradol is a major metabolite of 6-shogaol responsible for the lowering of glucose, cholesterol, and body weight in high-fed diet mice. The mechanism of action of shogaol was attributed to the increase in 5’adenosine monophosphate-activated protein kinase phosphorylation in 3T3-L1 adipocytes.

Suk et al. (2016) investigated the effect of 6-shogaol as a bioactive compound present in ginger on the adipogenesis and lipolysis in 3T3-L1 adipocytes. It was observed in their findings that the 6-shogaol anti-adipogenic effects were significantly higher than that of gingerol another major active constituent of ginger due to its ability to inhibit the expression of key regulators like PPARγ and C/EBPα involved in adipogenesis and also stirred up lipogenesis in mature 3T3-L1 adipocytes. Also, an experiment carried out by Prasad and Tyagi (2015) on the role of ginger and its constituents in the prevention and treatment of gastrointestinal cancer observed that 6-gingerol and 6-shogaol exert anticancer activities against GI cancer. The pharmacological activity of these constituents as an anticancer is ascribed to its capacity to modulate many signaling molecules like NF-κB, STAT3, MAPK, PI3K, ERK1/2, Akt, TNF-α, COX-2, cyclin D1, cdk, MMP-9, survivin, cIAP-1, XIAP, Bcl-2, caspases, and other cell growth regulatory proteins (Prasad and Tyagi (2015)).

In a study on comparative antioxidant properties of some gingerols and shogaols, and the relationship of their contents with the antioxidant potencies of fresh and dried ginger (*Zingiber officinale* Roscoe) performed by Guo et al. (2014), it was reported that 6-, 8-, and 10-gingerols, as well as 6-, 8-, and 10-shogaols, exhibited substantial in vitro antioxidant activities. It supported the result of the DPPH, ABTS, and FRAP assays which revealed that the antioxidant activities of 6-shogaol are the greatest. Mošovská et al. (2015) also reported that ginger extract is a good source of polyphenolic compounds like gingerols, shogaols, paradols, and gingerdions which manifest as a very good scavenger of ABTS radical cation and DPPH radical, respectively. Guo et al. (2014) attributed the high antioxidant abilities of 6-shogoal to the presence of the unsaturated ketone moieties.

In an attempt to determine if optimized heat treatment enhances the anti-inflammatory capacity of ginger, Ho and Su (2016) reported that 3 h of treatment of ginger at different temperature ranges reduces the content of gingerols drastically, thereby increasing the shogaol content, especially at 150 °C. The authors also ascertain that heat processing may perhaps quicken the conversion of gingerol into shogaols. It was also documented that the tumor necrosis factor-α, prostaglandin E2, and nitric oxide inhibitory capacities of ginger could be increased by heat treatment, but it was observed that only TNF-a inhibitory ability of ginger was the utmost susceptible to heat. Meanwhile, the heat-derived shogaols is responsible for the enhancement of prostaglandin E2 inhibitory capacity (Ho and Su 2016).

Seow et al. (2017) studied 6-shogaol, a neuroactive compound of ginger (jahe gajah) which induced neuritogenic activity via NGF responsive pathways in PC-12 cells. In this experiment to determine the cytotoxic effect of 6-shogaol, the neuritogenic activity was assessed. 6-Shogaol (500 ng/ml) was used to induce neuritogenesis similar to NGF (50 ng/ml) but cytotoxic to PC-12 cells. 6-Shogaol induced low level of NGF biosynthesis in PC-12 cells, showing that 6-shogaol stimulated neuritogenesis possibly by inducing NGF biosynthesis and also acting as a substitute for nerve growth factor (NGF mimic) in PC-12 cells. The inhibitors of Trk receptor (K252a), MEK/ERK1/2 (U0126 and PD98059) and PI3K/AKT (LY294002), attenuated the neuritogenic activity of both NGF and 6-shogaol, respectively. It was concluded that 6-shogaol thus induced neuritogenic activity in PC-12 cells through activation of MEK/ERK1/2 and PI3K/AKT signaling pathways. It therefore suggests that 6-shogaol could act as an imitation of NGF activity by inducing biosynthesis of NGF in PC-12 cells; this may be of substantial benefit in the prevention and treatment of neurodegenerative diseases (Seow et al. 2017).

An experiment was carried out by Ali et al. (2018) to determine the total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus, and callus treated with some elicitors using petroleum ether (PE) and a mixture of chloroform and methanol (CM) as the extraction solvent. The quantitative determination of each extract reveals that total phenolic and flavonoids were detected in the rhizome as the antioxidant present; meanwhile, total phenolic has the highest amount (Ali et al. 2018). Also, Ali et al. (2018) have observed that the bioactive constituents displayed high antioxidant activity in both assays as IC_{50} 4.85 + 0.58_{DPPH} and 5.35 ± 0.33_{ABTS} g/mL for gingerol and IC_{50} 7.61 ± 0.81_{DPPH} and IC_{50} 7.05 ± 0.23_{ABTS} g/mL for shogaol. Further action on callus with elicitors like salicylic acid exhibited significant effects in improving the phenolic content and allied antioxidant activity. The investigator therefore concluded that elicitation of ginger cultured tissues in phenolic accumulation might be of immense benefit to pharmacological, cosmetic, and agronomic industries (Ali et al. 2018).

9.9 Neuroprotective Effect of Shogaol

Ha et al. (2012) observed that pathogenesis which is responsible for various neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease, and cerebral ischemia is caused by inflammation of the brain and this neuroinflammation primarily originates from the stimulation of astrocytes and microglia which are regarded as the inhabitant of the immune cells of the brain which have different roles where the microglia's function is for immune supervision while astrocyte's function is to maintain the survival of the neurons by secreting nerve growth factors and diminishing the actions of neurotransmitters.

The author's aim of carrying out this investigation was to assess the neuroprotective effect of 6-shogaol through microglial stagnation in primary cortical neuron-glial culture. Deduced from their investigation, anti-inflammatory and neuroprotective ability of ginger has been a long time practiced by Asians as these people use ginger to ameliorate inflammatory situations and the pains that accompany the situation. The author's evaluation revealed that ginger extract has been known to be an inhibitor of synthesis of NO and pro-inflammatory cytokines in LPS-excited BV-2 microglial cells through NF-kB pathway; their study further reveals the ability of 6-shogaol block to excite LPS which induces iNOS and COX gene expression which is an illustration that 6-shogaol, as well as 6-gingerol, has the ability to control neuroinflammation through inhibition of microglial activation in the brain.

The author's investigation revealed that 6-shogaol has the potential of exerting its anti-inflammatory effects as opposed to 10 mM of 6-gingerol which had no remarkable inhibitory action on NO synthesis and iNOS expression in microglial cells. Furthermore, it could be deduced from their study that in cortical neuron-glial culture and in a systemic inflammatory model, 6-shogaol could not downregulate the microglial activation. However, from their experiment, it could be deduced that 6-shogaol had a remarkable neuroprotective potential in the cortical neuron-glial culture of ischemia model which was accrued to have resulted from anti-inflammatory effects through inhibition of microglial activation. Deduced from their study, the authors observed that in the central nervous system (CNS), inflammatory processes are responsible for neuronal cell death in neurodegenerative diseases, but they further observed that brain inflammation has no ability to influence neuronal cell death which in cases where the brain inflammation is not moderated has the potency of causing damage which is similar to that caused by several inflammatory diseases. With the understanding that brain inflammation is revolved mainly by activated microglia, in cases of persistent neurodegeneration, the situation is supplemented by an inflammatory response described by cautious activation of the microglial cells in the CNS.

Ha et al. (2012) study also affirmed that neurodegenerative diseases such as Alzheimer's and PD, stimulation of microglial was observed the process. This shows that a regime that can be used to ameliorate neurodegenerative diseases is inhibition of microglial activation. Evaluating the in vitro and in vivo anti-neuroinflammatory activity of 6-shogaol, the authors observed that 6-shogaol inhibited the expression of NO and PGE2 via arresting iNOS and COX-2 protein spreading, respectively; however, they deduced from their study that 6-shogaol also arrest the activation of NF-kB and MAPK. Their further investigation to ascertain 6-shogaol and its ability to activate NF-kB is linked to the fact that NF-kB is a concern with inflammatory responses to diseases as well as several chronic diseases. Furthermore, the authors deduced from their study that NF-kB transcription factor plays an active role in the synthesis of pro-inflammatory cytokines that are recognized as an active agent for curing inflammatory diseases.

However, 6-shogaol has the potential to inhibit the spread of iNOS and COX-2 via blocking LPS-induced NF-kB activation in macrophages. Investigating the effect of 6-shogaol on the NF-kB pathway, the authors observed that NF-kB activation in microglial enables 6-shogaol decreased activation of NF-kB via blocking IkB phosphorylation as well as future degradation of IkB which shows that for 6-shogaol to express its anti-neuroinflammatory activity, it must inhibit NF-kB in primary microglia. The authors verified that exposure of 6-shogaol to LPS has an effect on the primary microglial cells and stimulates all MAPKs (p38, ERK, and JNK). They further observe that phosphorylation of p38 and JNK in feedback from LPS had an effect by decreasing the regime of 6-shogaol without 6-shogaol causing any change on ERK. Deduced from their study, from their experiment to final result, 6-shogaol has been observed to have an anti-neuroinflammatory ability and/or response protecting the brain from neurodegenerative diseases, and this anti-neuroinflammatory effect occurs with the ability of 6-shogaol to impede p38 MAPK and JNK in microglial cells.

Shim et al. (2011) investigated 6-shogaol and its anti-inflammatory properties. The author's investigation revealed that the anti-inflammatory agents present in 6-shogaol are necessary for preventing cell damage when looking at aging and age-related neurodegenerative diseases. They further observed that some disease conditions have the ability to trigger astrocytes which are being referred to as the main origin of pro-inflammatory cytokines which cause neuronal dysfunction.

Deduction from their investigation shows that astrocytes play a vital role in moderating inflammatory processes in the brain and spread of IL-1 which are adhesion molecules revealing that primary rat astrocyte culture could be a good modeling system for showcasing different neuroinflammatory agents that are linked with neurodegenerative diseases. Hence, their study validated that 6-shogaol has a vital role in manipulating primary rat astrocyte stimulation which is revealed via modulation of histone acetylation. The author's observation that LPS is an activator of glial cells could cause pro-inflammatory cytokines in cultured glial cells such as IL-1b, IL-6, NF-kB, iNOS, and COX-2, and these cells were remarkably arrested in primary rat astrocytes after a regime of 6-shogaol revealing that 6-shogaol is a necessary inhibitor of pro-inflammatory cytokines in neuronal cells and has the tendency of showcasing an important role in controlling neuroinflammation.

The authors further verified that in neurodegenerative diseases, HDAC inhibition and histone H3 acetylation are seen as a probable mechanism for progressing therapeutic approaches in several neurological disease conditions. Deduced from their investigation, 6-shogaol had a remarkable effect in inhibiting HDAC1 expression and increased histone H3 acetylation. They also evaluated that 6-shogaol strongly elevates HSP70 spread in primary cortical astrocytes, followed by increased histone acetylation levels. According to the authors, these effects observed show that 6-shogaol works as HDAC inhibitor and HSP70 induces neuroprotection. Investigating the 6-shogaol HDAC inhibitory effect, the authors observed that 6-shogaol could effectively arrest LPS-induced HDAC upregulation and as well as elevate HSP70 secretion and histone H3 acetylation in primary rat astrocytes. This shows that 6-shogaol and HDAC inhibitors which include trichostatin A and MS275 motivate the spread of HSP70 in primary rat astrocytes. Also, the author's findings revealed that upregulation of HSP70 through 6-shogaol is linked with transcriptional stimulation which includes histone acetylation. Furthermore, their study also demonstrated that inhibition of class 1 HDAC is strongly implicated in HSP70 induction. Deduced from their study, HSP70 upregulation could be associated with the restorative target used for curing neurodegenerative diseases associated with neuroinflammation. Hence, 6-shogaol has a neuroprotective ability.

Gaire et al. (2015) investigated on the neuroinflammatory ability of 6-paradol. Form the author's research, they could deduce that their result gotten from in vitro and in vivo experiment showcases that 6-paradol is a non-pungent metabolite of 6-shogaol, an unconventional active constituent of *Zingiber officinale* which has a curative ability on transient focal cerebral ischemia that could be through inhibition of neuroinflammatory responses in activated microglia. Deduced from their study, a strong elevation in NO synthesis and pro-inflammatory cytokines which are IL-6 and TNF-α was obstructed by exposure to 6-paradol which is a symbolic indication that 6-paradol has the properties of a neuroprotectant; thus, it can protect the brain and decrease inflammatory responses. To investigate the in vitro neuroprotective effect of 6-paradol, experimental animals were used, induced with cerebral ischemia and treated with 6-paradol. According to the authors, the treatment revealed that 6-paradol has a curative benefit by decreasing microglial activation and spread of TNF-α. From this result, the authors ascertain that paradols are olefin-reduced form of shogaols which are the main constituent of ginger extract when extracted through heat and found naturally and as a biological metabolite of shogaols. Deduced from their study, the authors observed that among the paradols found in shogaol, 6-paradol was proven to be the most effective in decreasing inflammatory responses in activated microglia which they found to be in consonance with several studies whose research produces the same result that 6-paradol is an effective compound for ameliorating and regulating obesity, platelet aggregation, or 12-O-tetradecanoylphorbol13-acetate-induced alterations. From the author's investigation, 6-paradol decreases neuroinflammatory responses in activated microglia which includes decreased productivities of NO, prostaglandins, and pro-inflammatory cytokines. The authors observed the in vitro effects of 6-paradol on microglial activation. Using an experimental animal model of the mouse, induced with cerebral ischemia, an M/R-challenged brain, they observed that microglial activation was confirmed in the experimental model; however, microglial activation induced by M/R was ameliorated through the administration of 6-paradol which also ameliorated the ischemic brain damage. The authors measured the microglial responses of 6-paradols, and they observed that after 3 days of administration of 6-paradol, the microglial response was still active after M/R induced effect and the site of the effect was seen in the peri-ischemic regions where the penumbra lies. This shows that 6-paradol influence on microglial reaction is an indicator that it has the ability to salvage peri-ischemic zone. The authors observed that the neuroprotective ability of 6-paradol was still effective after reperfusion which shows that 6-paradol has a restorative ability to ameliorate cerebral ischemia. Furthermore, the authors investigated on the in vivo neuroprotective ability of 6-paradol, and they observed that 6-paradol is linked to the decrease in the spread of iNOS and TNF-α which are recognized as the pathogenic constituent of cerebral ischemia. Their investigation also revealed that iNOS and TNF-α are produced from various cell types such as microglia, astrocytes, or infiltrated immune cells. Furthermore, the author's study revealed that 6-paradol decreases the synthesis of NO followed by downregulation of iNOS spread while the synthesis of TNF-α in LPS activates microglia, which buttress their point that neuroprotective ability of 6-shogaol in cerebral ischemia could be accrued to decrease in spread levels of iNOS and TNF-α in microglia. Deduction from their study shows that 6-paradol is a non-pungent metabolite of shogaol and also possesses anti-inflammatory properties.

9.10 Conclusion

This review has shown the potential multisystemic effects of 6-shogaol which is a by-product from thermal dissociation of gingerols which has a lots of potential that portends them as a sustainable solution that could mitigate uncountable health challenges facing mankind. With this understanding this review has pointed out several nutritional and medical health benefits of shogaol. This shows that constant usage of ginger could serve as a permanent replacement to several synthetic drugs that have been reported to develop a high level of resistance; the fact that it is cheap and readily available will make it more easier for individuals to live a healthy and normal life most especially poor people in developing countries that cannot afford cost of medical and hospital bills. Moreover, more research needs to be conducted to establish the modes of action through which the 6-shogaol a derivative of ginger performs its activity when tested in different in vitro and in vivo animal studies. This will give a better insight and proper understand on how it executes its mechanism of action.

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Exopolysaccharides Derived from Beneficial Microorganisms: Antimicrobial, Food, and Health Benefits

10

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Abstract

Microbial polysaccharides are natural biopolymer which is produced in the extracellular space which possesses numerous biological functionalities including surface adherence, cellular interactions, and environmental protection. They have been observed to exhibit multiple functions which might be linked to their variation in their structure which include structural polysaccharides or exopolysaccharides and intracellular polysaccharides. The exopolysaccharides derived from microbial have been utilized in several sectors in the industry, food, agriculture, and medical. Their wide application might be linked to their biocompatibility and eco-friendly nature. Some of their uncountable medical benefits include their application as anticancer drugs, antimicrobials, antioxidants, cardiovascular, antiulcer, and neuroprotective among many. Also, the application of exopolysaccharides has application in the production of fermented food such as water-binding agents, thickeners, gelling, stabilizers, and emulsifiers. Therefore, this chapter discusses the recent advances in the application of exopolysaccharides obtained from beneficial microorganism and their wide application in the medical and food industry. Moreover, more insight into the application of nanotechnology was the emphasis as an effective tool for the delivery of the active ingredient present in exopolysaccharides which possess

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the capability to synthesize and effectively stabilize metal nanoparticles which are the process involved in the synthesis of nanoparticles.

Keywords

Exopolysaccharides · Food sector · Biomedical application · Antimicrobial · Antiulcer

10.1 Introduction

Microbial exopolysaccharides have been recognized as biopolymer that is produced by microbial cells into an extracellular environment or medium which could lead to the development of a capsule or slime-like substances which are gradually released into the cell surface (Rehm 2009). The biological biopolymer might come in the form of soluble or insoluble in nature depending on the species of microorganisms. The biopolymer is effectually released into the environment which might build up outside the cells (Ates 2015; Moscovici 2015). The biopolymer might later get attached to the fermentation medium or to the surface of the microbial cell (Ates 2015.)

Moreover, it has been highlighted that the production of exopolysaccharides is essential for their survival which enables them to perform numerous roles like cell-cell interactions, cell protection, cell aggregation, and attachment to solid surfaces (Nicolaus et al. 2010). Production of polymer from microbial sources could be derived from diverse microorganisms including microalgae (Underwood and Paterson 2003), cyanobacteria (Laurienzo 2010), bacteria (including marine and extreme and bacteria) (Nicolaus et al. 2010), fungi, and yeasts (Mahapatra and Banerjee 2013).

Several studies have shown that the most reported exopolysaccharides include pullulan, hyaluronic acid, alginate, gellan, cellulose, dextran, succinoglycan, xanthan, and curdlan (Donot et al. 2012; Freitas et al. 2011; Mishra and Jha 2013). There are several factors that are used in the classification of various exopolysaccharides which includes redox-active, constructive or structural, informative, sorptive, redox-active, and surface-active and nutritive (Flemming and Wingender 2010).

Exopolysaccharides structurally consist of various components such as non-carbohydrate substituents like phosphate, protein, succinate, lipids, acetate, pyruvate, as well as monosaccharides. The microbially produced exopolysaccharides have been documented for several applications because of their uniqueness in several fields which include metal removal in mining and industrial waste treatment, adhesives, pharmaceuticals, textiles, food additives, and oil recovery; industries for the production of thickeners, gelling agents, stabilizers, and emulsifiers; and medical and food sectors (Decho 1990; Linton 1991; Sutherland 1998)

The application of current trends in application of exopolysaccharides has potentiate their usage in the production of bacterial alginate in cell microencapsulation, which includes the application of microsphere vectors for drug delivery, the utilization of dental impressions, and their application as anti-reflux therapies and delivery of active ingredient in absorbent dressings (Flemming and Wingender 2001). The utilization of synthetic drugs which has been highlighted with numerous adverse effects such as the development of drug resistance and low effectiveness has necessitated the introduction of a natural compound of significant interest that could be used as a permanent replacement to synthetic drugs. The usage of exopolysaccharides from microorganism could be used for the management of chronic and tropical diseases.

The exopolysaccharides obtained from microorganism have been documented for several unique features which include antitumor, platelet aggregation inhibition, antimicrobial, colony-stimulating factor synthesis, anti-inflammatory, and an inducer for interferon. Moreover, some of the unique and special features which potentiate the application of exopolysaccharides from microbial origin include their biodegradability, non-toxicity, and eco-friendly. Moreover, the application of nanobiotechnology might contribute to enhancing the activities of exopolysaccharides derived from microorganisms.

Therefore, this chapter intends to write on the significant of exopolysaccharides derived from beneficial microorganism most especially for their application in the food, industry, and their application in the medical sector.

10.2 Antimicrobial Activity

Benattouche et al. (2018) optimized and standardized the best fermentation conditions that could lead to an increase in the production of exopolysaccharides from *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. The authors also validate the antimicrobial and the antioxidant effects of the exopolysaccharide produced in an in vitro assay. The result obtained showed that the microorganism which was lactic acid bacteria isolated from yogurt produced more cell growth but enhanced exopolysaccharide was obtained from the pure of *Streptococcus thermophiles*. The antimicrobial assay showed that the purified exopolysaccharide containing between 62 and 1000 g/mL exhibited more antimicrobial effectiveness against *Staphylococcus aureus* ATCC 250923 and *Escherichia coli* ATCC 250922, respectively. The exopolysaccharide showed positive antioxidant effectiveness on DDPH radical scavenging. Their study showed that the antioxidant produced from these two strains portends that capability to be utilized in the food industry because of their enhanced antimicrobial and antioxidant activities.

Nwodo et al. (2012) wrote a comprehensive review on the functional and structural diversity and valuable significant of exopolysaccharides produced from bacteria. It has been observed that these polysaccharides had various structural diversities ranging from heteropolymeric to homopolymeric which might have a molecular weight varying from 10 to 1000 kDa. The authors also highlighted the significance of exopolysaccharide in the industry and medical sectors. Their wide application includes the production of several products most especially in the production of herbicides, nutraceutical, insecticides, pharmacological, cosmeceutical, and functional food, while prospects include uses as immunomodulation, antimicrobial, anticoagulant, bioflocculants, antithrombotic and anticancer.

Ghalem (2017) evaluated the antioxidant and antimicrobial efficacy of exopolysaccharide produced from yogurt starter. The antimicrobial effectiveness was performed using diffusion techniques against *Staphylococcus aureus*, *Proteus* spp., the yeast of *Candida albicans*, *Escherichia coli*, and *Streptococcus* D, while the antioxidant properties were determined using DPPH-free radical trapping. The result obtained revealed that the exopolysaccharide exhibited some level of antimicrobial activity against all the tested isolates with zones of inhibition that varies from 9 to 13 mm. The antioxidant assay carried out showed that the exopolysaccharide could minimize the level of free radical (DPPH) to diphenylpicrylhydrazine a yellow-colored at 24.25% exhibiting antioxidant activity when compared to ascorbic acid which had 69.79%.

El-Essawy et al. (2016) produced an exopolysaccharide from *Klebsiella* sp. isolated from a marine environment. The structural composition of the exopolysaccharide was performed using quantitative and qualitative paper chromatography from the complex hydrolysis product. The result obtained showed that the monosaccharide composition of the exopolysaccharides contained glucose 32%, uronic acid 10%, galactose 16%, fucose 22%, and fructose 20%. The antimicrobial properties of the exopolysaccharides were determined using standard procedures. It was observed that exopolysaccharides exhibited a higher antibacterial effect against *E. coli* and *S. aureus*, but there was no antifungal effect against *Candida albicans*. The minimum inhibitory concentration obtained against all the tested isolates was 15 mg/dl. Their study showed that the exopolysaccharides consist of two new monosaccharide moieties containing uronic acid and fructose which potentiates their capability for various industrial uses.

Orsod et al. (2012) obtained exopolysaccharides from marine bacteria obtained from Asian sea bass. The antimicrobial efficacy of the exopolysaccharides was performed using Kirby-Bauer techniques against gram-negative and gram-positive bacteria. The result of the 16S rRNA analysis showed that the two obtained bacteria obtained from Asian sea bass (*Lates calcarifer*) from aquaculture farm. The result showed that the bacterial isolate exhibited ors2 had 96% similarity with *Brachybacterium* sp., while ors1 had 99% identity to *Bacillus cereus*. The bacterial isolates exhibit same and different characteristics in terms of microscopic observation and biochemical reactions. The composition of the exopolysaccharides reveals that it contained 24% fatty acids, 50% carbohydrates, and 26% protein. The various functional groups present were detected by the FTIR analysis. The exopolysaccharides exhibited a various form of antimicrobial inhibition against all the tested isolates. Their study showed that the exopolysaccharides could be utilized as a source of antimicrobial agents toward the development of potent drugs derived from a marine environment.

Li et al. (2014) validate the antimicrobial and antioxidant properties of exopolysaccharides obtained from *Lactobacillus plantarum* R315 (L-EPS) and *Bifidobacterium bifidum* WBIN03 (B-EPS). The result obtained from the antimicrobial activity using Agar diffusion assay showed that the exopolysaccharides exhibit a very high level of antimicrobial activity against all the tested isolates including *Bacillus cereus*, *Cronobacter sakazakii*, *Escherichia coli*, *Shigella sonnei*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*. The most effective concentration tested was 300 μg/mL. The antioxidant effects of the exopolysaccharides were determined by the techniques superoxide radical scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and hydroxyl radical scavenging. The author also performed lipid peroxidation and inhibition of erythrocyte hemolysis, respectively. The result revealed that the two strains exhibit enhanced scavenging potential against superoxide radicals and 1,1-diphenyl-2-picrylhydrazyl when tested at higher concentration. Their study shows that the exopolysaccharides obtained from the two strains could be utilized for various applications in the food industry because of their high level of antioxidant and antimicrobial activity.

Patel et al. (2018) assessed the antimicrobial effectiveness of bacteria capable of producing exopolysaccharide from sugarcane field soil. It was discovered that KPEP3 and KPEP4 were established as the best producing strains out of the nine exopolysaccharides producing strains evaluated. The result of the antimicrobial result carried out showed that exopolysaccharides exhibited a high level of antimicrobial activity against all the tested isolates including *V. cholera*, *E. coli*, *B. subtilis*, and *B. cereus*. Moreover, it was discovered that isolates KPEP4 and KPEP3 exhibit higher antimicrobial effectiveness against all the isolates, while the minimum inhibitory concentration obtained from the exopolysaccharides showed antimicrobial activity. The biochemical characterization carried out showed that these isolates belong to the genera *Pseudomonas* and *Bacillus*.

Adebayo-Tayo and Popoola (2017) perform the antimicrobial effect of synthesized silver nanoparticle from the exopolysaccharide obtained from the following lactic acid bacteria strains such as *Lactobacillus fermentum* (LPF6) and *Lactobacillus casei* (LPW2E) against some pathogenic bacteria. The exopolysaccharide produced from these isolates shows that the exopolysaccharide produced varies from 256 to 640.9 mg/L. The nanoparticles were produced from exopolysaccharide of these strains. The functional groups present in the nanoparticle were determined using FTIR analysis and showed the presence of carboxyl, aldehydes, hydroxyl, and ester. The level of inhibition observed against the tested isolates varies from 12 to 26 mm. The presence of the antimicrobial activities might be linked to the various functional groups present in the exopolysaccharides. This might also be responsible for the stabilization, reduction, and capping effect of the synthesized nanoparticles.

Mohamed et al. (2018) evaluated the antimicrobial effect of 13 different marine bacteria obtained from the surface of algae rocks in biofilm-forming device in the Red Sea. The various isolated strains were tested for their capability to produce exopolysaccharides. The most active strain used for the production exopolysaccharides was characterized molecularly using 16S rDNA analysis. The result obtained reveals that isolate 2D shows a *Halomonas saccharevitans* AB2 with 99% similarity. The result obtained from the Plackett-Burman experimental design showed that the maximum yield of exopolysaccharides containing 138 gl-1 with 1.6-

fold increase was obtained using Box-Behnken design. The highest level of antimicrobial activity of the exopolysaccharides showed that exopolysaccharides exhibited a maximum inhibitory effect against *Aspergillus niger* ATCC 16404 and *Vibrio fluvialis*.

10.3 Antioxidant Effect of Exopolysaccharide

Madhuri and Prabhakar's (2014) study revealed that exopolysaccharide could be produced from probiotic bacteria with significant importance in the lives of human by maintaining the activities in the ecosystem and in the gastrointestinal tracts of animals which give a clear division of exopolysaccharide significant effect into nutritional, physiological, and therapeutic effects. It was deduced from their study that exopolysaccharide had antioxidant as well as free radical scavenging properties. Their study validated exopolysaccharide free radical scavenging property is essential in inhibiting oxidation of vegetable oils, and separation of exopolysaccharide antioxidant and free radical scavenging from *Rhizobium meliloti* revealed that RPS has the capacity to inhibit lipid oxidation present in sunflower oil emulsions.

Zhang et al. (2013) researched on the antioxidant activity of exopolysaccharide which was separated from *Lactobacillus plantarum*. The authors deduced from their study that oxidative stress is a result of variation linking the formation of reactive oxygen species (ROS) and the ability of body systems to detoxify or repair resulting damage. The authors validated that exopolysaccharide formed from lactic acid bacteria has an essential physiological and rheological characteristics which promote their usefulness in the food industry as either viscosifying, gelling, or emulsifying agents. Their further investigations validate that bowel inflammatory diseases and ischemic reperfusion injury are disease conditions associated with oxidative stress and exopolysaccharide a probiotic occurring as live microbial feed is important to the host having these disease conditions through stimulating the immune system to act against any infectious agents, balancing intestinal flora, lowering serum cholesterol levels, and risk associated with tumors. These beneficial effects of exopolysaccharide according to the authors represent its antioxidant activities. The authors investigated that exopolysaccharide is extracellular polymers which are found in either the surfaces of LAB or secreted from any surrounding environment of LAB making the union of exopolysaccharide and LAB to possess protective properties, protecting bacteria cells from desiccation, phagocytosis, bacteriophage, and phagocytosis. Their study revealed exopolysaccharide from LAB is very significant in searching for nontoxic substances with free radical scavenging and antioxidant activities. The author's study revealed that exopolysaccharide used during their study was obtained from *Lactobacillus plantarum* C88 which revealed significant protection against H_2O_2-induced oxidative stress damaged in Caco-2 cells to understand the mechanism behind this; the authors observed that LPC-1 reacted to H_2O_2-induced oxidative stress through elevation of enzymatic and nonenzymatic intracellular antioxidant defense mechanism. The authors validated that protective ability was traced to its ability to elevated antioxidant enzyme activities such as SOD which act to elevate nonenzymatic antioxidant levels such as MDA and T-AOC. Deduced from their study, exopolysaccharide and LPC-1 are derivatives of *Lactobacillus plantarum* C88 where LPC has a high hydroxyl radical and DPPH radical scavenging activity owing to the strong antioxidant activity possessed by exopolysaccharide and LPC-1.

Sasikumar et al. (2017) investigated the importance of exopolysaccharide obtained from *Lactobacillus plantarum* BR2. During their investigation, the authors analyzed the sugar composition of EPS using HPLC which revealed that exopolysaccharide from *Lactobacillus plantarum* based on its character is a heteropolysaccharide consisting of a glucose and mannose sugar residues with the thermal stability of 260 °C. Deduced from their study, the authors observed that that exopolysaccharide in vitro had 29.80% DPPH radical scavenging activity at 2 mg/ml attributed to the presence of hydroxyl group and other functional groups of exopolysaccharide having the ability to transform free radical into a more stable form. Using ascorbic acid as control, the authors observed that the total antioxidant capacity of *Lactobacillus plantarum* BR2 exopolysaccharide was observed to be 18.98%. Their study revealed that the antioxidant, antidiabetic, and cholesterol-reduced properties of exopolysaccharide are associated with the presence of high molecular weight glucomannan type of exopolysaccharide.

10.4 Antidiabetic Effect of Exopolysaccharide

Hwang et al. (2005) validated that immersed mycelia culture of *Phellinus baumii* has the potency of producing exopolysaccharide that is composed of two separate heteropolysaccharides and two proteoglycans. The authors validated that EPS can be extracted from mushrooms which have medicinal value; deduced from their study, *Phellinus linteus* was among the other four mushrooms with medicinal value. Their studies revealed that EPS extract gotten from *Phellinus linteus* is associated with increased body weight, while mushroom *Phellinus baumii* which was linked has properties that reduce glucose level mediated through a significant reduction of food consumption linked to the availability of specific chemical compound present in the plant. Their further investigation revealed the hypoglycemic mechanism of *Phellinus baumii*. They observed that *Phellinus baumii* has the ability to mend impaired pancreatic β-cells and well enhanced the synthesis of insulin which has the potential of reducing glucose level present in plasma. Their study further explained that hypoglycemic activity of EPS was through elevated glucose consumption with a corresponding increase in insulin secretion in the pancreas. Deduced from their study, it was observed that extract from mushroom has both extrapancreatic and pancreatic roles in controlling plasma glucose level resulting from β-cell protection against any cytotoxic action. Their study verified that *Phellinus baumii* EPS is associated with a significant reduction of hyperglycemia; this implies that *Phellinus baumii* has the ability to reduce excessive glucose in the body revealing it as an antidiabetic agent.

Yamac et al. (2009) validated EPS could be extracted from *Cerrena unicolor*, *Coprinus comatus*, and *Lenzites betulina*; however, diabetes induced in adult rats during the administration of the EPS extract from these species of mushroom was reduced with almost similar glucose level present in the control animals. Deduced from this study, the authors observed that the hypoglycemic activity of the mushroom plant could be traced to an increased fiber, protein, and fat content in the plant. Investigation on each of the mushroom, the authors observed that *Cerrena unicolor* having a value of 61% had the high reducing ability of serum plasma glucose level. Their further investigation proves that in cases of chronic diabetes complication, *Cerrena unicolor* will be an effective mushroom with a high EPS content to lower glucose level present in the blood. During their investigation, the authors observed that fruiting body and intracellular and extracellular polysaccharides of mushrooms are all associated with decreased serum glucose level in diabetic animals, while comparing their results with previous studies, the authors validated that the mushrooms *Cerrena unicolor*, *Coprinus comatus*, and *Lenzites betulina* used during the previous study administered either intravenously or intraperitoneally had the highest serum glucose reducing ability of 7.5%; Yamac et al.'s (2009) studies revealed that glucose level was reduced ranging from 42.78% and above. Their study affirms that STZ is a broad-spectrum antibiotic gotten from the extraction of *Streptomyces* acromogenes and is able to inhibit insulin secretion in the pancreas by selective destruction of β-cells present in the pancreatic islets during their biochemical and histological analysis; the authors observed that aside reducing the serum glucose level. EPS present in the mushrooms used also elevated the size and number of Langerhans islets suggesting that the EPS present in the mushroom is necessary restoring and repairing a normal structure and function of damaged pancreatic β-cells which in turn aid insulin synthesis which lowers serum glucose level in the blood.

Cho et al. (2007) experimented on the hypoglycemic ability of EPS which was extracted from two mushrooms, namely, *Tremella fuciformis and Phellinus baumii*. The mycelial culture of these mushrooms revealed the presence of antidiabetic potential due to the presence of EPS which had a significant effect on reducing blood glucose levels. Their study observed that administration of oral EPS present in *Tremella fuciformis* and *Phellinus baumii* led to elevation of plasma insulin level in the experimental models, and deduced from their experiment, they observed that the mechanism behind this was the ability to elevate pancreatic secretion of insulin from already existing β-cells which ameliorate insulin or create an increased effect of insulin in plasma. Using OGTT, they observed that treatment of diabetic mice with EPS for 52 days resulted in a remarkably increased glucose removal from the body system which was associated with an elevated level of insulin sensitivity. The authors' observation revealed significant decrease in triglyceride level as well as an insignificant increase in total cholesterol level after treatment with EPS indicating insulin sensitivity caused by adenosine monophosphate-activated protein kinase (AMPK) which has the ability to improve triacylglycerol lipase activity necessary for elevation of glycogen synthesis in the liver that further improves glucose uptake in skeletal muscle and adipocytes. Deduced from their study, it was observed that EPS extracted from *Tremella fuciformis* and *Phellinus baumii* has hypoglycemic.

10.5 Neuroprotective Effect of Exopolysaccharide

Sun et al. (2005) validated how EPS2 an exopolysaccharide could protect the pheochromocytoma line PC12 cells against oxidative stress a major pathology that affects the central nervous system leading to neurodegenerative diseases including Parkinson's and Alzheimer's diseases. During their investigation, the authors validated that oxidative stress on PC12 caused by H_2O_2 was impaired by EPS2 after preincubating PC12 cells in EPS2 for about 24 h. Their further investigation revealed that for EPS2 to protect the PC12 cells from oxidative damage, it can be achieved in a dose-dependent manner. The authors observed the discharge of LDH into the culture medium indicating cellular damage and cytotoxicity revealing an elevated level of MDA which is an indicator of lipid peroxidation; they observe during their study that preincubating PC12 cells in a dose-dependent manner impaired elevated levels of MDA and LDH activity that was triggered by the administration of H_2O_2. Further evaluation of the protective function of EPS2, the authors observed that treatment of PC12 cells with H_2O_2 resulted in a reduced action of GSH-Px and CAT; they observed that preincubation of PC12 cells with EPS2 in a dose-dependent manner resulted in reduced activity of GSH-Px and CAT. Deduced from their study, PC12 has been an effective component of the central nervous system to detect drug that could be used to treat neurodegenerative diseases based on the ability of PC12 cells to showcase phenotypic characteristics of both adrenal chromaffin cells and sympathetic neurons; oxidative stress induced by H_2O_2 had detrimental effect on the PC12 cells which was ameliorated by EPS2 revealing that EPS2 an exopolysaccharide has neuroprotective ability against neurodegenerative diseases caused by oxidative stress.

Rahman et al. (2015) validated the neuroprotective effect of mushroom with emphasis on Alzheimer's disease, a rapid neurodegenerative disease in the globe; the authors looked critical on each activity through which mushroom uses in ameliorating Alzheimer's disease. The authors validated that administration of aqueous *G. lucidum* extract of 500 mg/ml concentration in vitro has the potential effect of reducing the rate of synaptic degeneration also known as loss of synaptic connection which they emphasized as a vital sign signifying pathogenesis of Alzheimer's disease. According to the authors, the mechanism in which *G. lucidum* undergoes to repair synaptic density proteins, synaptophysin in cortical neurons, is to inhibit phosphorylation of c-Jun N-terminal kinase (JNK), c-Jun, and p38 MAP kinase. They further investigated how *G. lucidum* could retrogress neuronal cell death (apoptosis) associated with AD pathogenesis resulting from arousement of caspase-3 activity by Ab through an apoptotic signaling pathway that encompasses protein kinase pathways such as JNK c-Jun and p38 MAP kinase. Deduced from their study, aqueous *G. lucidum* extract was able to inhibit Ab-induced neuronal cell apoptosis through impeding the protein kinase pathways. Deduced from the author's observation, *G. lucidum* has the potential of reducing neuronal cell apoptosis in a dose-dependent manner through a mechanism that involves downregulation of caspases-3-8 and -9 as well as Bax expression with impede depletion of Bcl-2 impression and, hence, a change in Bcl-2/Bax ratio. Their further investigation revealed that *G. lucidum* in powered form at various percentages significantly reduced Ab deposition in the brain as well elevating anti-oxidative enzyme levels which in turn help to enhance learning skills associated with memory. The authors validated that *G. lucidum* mycelial extract has the ability to imitate the activities of nerve growth factor (NGF) and stimulation of phosphorylation of ERK 1/2 and PKC and participate during the signaling cascades of PI3K and ERK. The authors further investigated the restorative effect of *G. lucidum* in ensuring neurite outgrowth and neurogeneration; the authors observed that neurotrophic factors which include brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and neurotrophin 3 (NT-3) have the function of maintaining and differentiation of neurons. Deduced from their study, cyrneines A and B sequestered from *Sarcodon cyrneus* have the ability to instigate neurite outgrowth from PC12 and NG108-15 cells and NGF production in 1321N1 cells. Verifying the effect of *Sardon glaucopus*, the authors observed that glaucopine C can be extracted from *Sardon glaucopus* and has the ability to stimulate NGF generation with the sole purpose of generating neurite outgrowth and NGF production. They further investigated on *H. erinaceus* mushroom observing its role in neurite outgrowth and NGF production deduced that *H. erinaceus* has an essential exopolysaccharide responsible for neurite outgrowth promotive effect in PC12 cells. Their further investigation on pheochromocytoma (PC) 12 neuronal cell lines in experimental models revealed that *G. lucidum* in vitro enhances neurite outgrowth and variation in the neuronal cells which are an indicator that *G. lucidum* a mushroom has neuroprotective effect against Alzheimer's disease. The author's study observed that the mechanism of action of *G. lucidum* is through Ras/extracellular signal-regulated kinase (Erk) and signaling cascade with cAMP-response element-binding protein. They also observe that the lipophilic part of *G. lucidum* was responsible for improving neurite outgrowth, impels NGF activity, and reinforces PC 12 neuronal differentiation. Observing the anti-oxidative and memory improving potential of mushroom, the authors observed that *G. lucidum* cultivated on brown rice had both in vitro and in vivo anti-oxidative abilities. Deduced from their study, they noted that *G. lucidum* elevated anti-oxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels. Using the powdered form of *G. lucidum*, reduction of Ab level in the brain of experimental animals was observed which is an indication free radical scavenging, and anti-oxidative potential of *G. lucidum* is responsible for the neuroprotective potential against neurodegenerative diseases.

10.6 Cardiovascular Effect of Exopolysaccharide

Xu et al. (2017) validated the protective effect of exopolysaccharide extracted from Lachnum YM130 on the cardiovascular system, which they experimented on diabetic-induced rats. Deduced from their study, the authors revealed that diabetes mellitus is a risk factor associated with an elevated level of oxidative stress in relation to influx of hyperglycemia. The authors validated that elevated levels of oxidative stress will trigger antioxidant enzymes such as SOD, GPx, and CAT which act using their antioxidant defense mechanism on oxygen species, and when these enzymes are inhibited, antioxidants which make the myocardial membrane susceptible to oxidative damage are built up. The authors deduced that the use of exopolysaccharide as treatment averted oxidative stress by fixing the potential effect of SOD, GPx, and CAT in the cardiovascular system while safeguarding the myocardial membrane from inducing oxidative impairment proving that LEP-2a as an exopolysaccharide found in Lachnum YM130 has the potential of protecting the heart against oxidative stress. Their further investigation revealed that MDA a by-product of lipid peroxidation is used to detect structural impairment produced in the presence of elevated levels of un-scavenged free radicals whose effect is associated with production of harmful substances that destroy beneficial protein and inactivation of membrane-bound enzymes; they further validated that administration of polysaccharide LEP-2a had the potential to reduce elevated level of MDA proving that polysaccharide has the ability to protect the heart through enhancing glycometabolism and lipid metabolism in diabetic mice. The authors observed elevated levels of markers of cardiovascular disease which includes AGEs, hs-CRP, and slCAM-1, which they deduce AGEs as an end product of complex glycation associated with cardiovascular diseases; they further observed that these biomarkers of cardiovascular disease were significantly ($P < 0.01$) elevated in diabetic mice; however, treatment with LEP-2a significantly ($p < 0.05$ or $p < 0.01$) reduces the levels of AGE, hs-CRP, and sICAM-1 as well as ameliorating damages to induce diabetes to the heart. Deduced from their study, LEP-2a has the potential to alleviate cardiovascular disease by improving antioxidant enzyme and reducing oxidative stress as well as reducing biomarkers that have the ability to make the cardiovascular system susceptible to injuries.

Zhang et al. (2016), during their study, attested that cardiovascular impediment is a leading cause of death in diabetes mellitus patients resulting from oxidative damage, inflammation, and fibrosis with oxidative stress as a major pathogenic impediment factor in diabetes mellitus. During the cause of diabetes mellitus, the authors observed that antioxidant enzymes such as SOD, GPx, and CAT which are considered to be the first line of the antioxidant defense system, with a significant importance of protecting cardiovascular tissue against oxidative damage, were impeded resulting to build up oxidants with potential effect of causing myocardial cell membranes prone to oxidative damage. However, the authors deduced that treatment using *Ophiopogon japonicus* (OJP1) has the potential effect of improving the impaired activities of antioxidant enzymes such as SOD, GPx, and CAT in the heart as well as protecting myocardial membrane from the oxidative damage suggesting that OJP1 has a defense mechanism against oxidative stress by enhancing cellular anti-oxidative defense enzymes. The authors further validated that MDA levels were elevated in the heart tissues of diabetic rats indicating impairment to protein levels and inactivation of membrane-bound enzymes which are characteristic features of cellular damage and cytotoxicity. They validated that the regime of OJP1 has the potential of reducing elevated levels of MDA in the heart tissues by improving antioxidant enzyme activities, scavenging free radicals, and protecting the heart from disease conditions associated with diabetes. To further understand the cardioprotective effect of OJP1, the authors validated that AGE, sICAM-1, and hs-CRP were used in the cause of their study, and they observed that AGE and sICAM-1 were significantly ($P < 0.01$) deducing that their (AGE and sICAM-1) elevation has the potential of causing tissues damage. The regimen with OJP1 according to their study had a significant reduction ($P < 0.01$) in serum levels of AGE and sICAM-1 to ameliorate heart impairment. The authors also validated hs-CRP had a significant elevation in diabetic animals buttressing that hs-CRP is the most accepted biomarker to indicate risk cardiovascular disease. However, treatment with OJP1 according to their study ameliorated the risk caused by hs-CRP by reducing its levels in the heart proving that OJP1 has the ability to protect the heart from damages caused by diabetes. The authors further investigated the cardioprotective effect of OJP1 using certain factor such as NO and ET-1 which they observed that NO secreted by nitric oxide synthase (NOS) enzymes is an essential signaling messenger present in the cardiovascular system whose vital role is to protect the heart from the baseline and advancement of cardiovascular disease; hence, decrease in NO in their diabetic animals leads to reduction of certain vital role such as regulation of blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion, and prevention smooth muscle cell proliferation and signifies defect of the cardiovascular system, whereas the authors identified Endothelin-1 (ET-1), as a peptide produced mainly by vascular endothelial cells which are exhibited in the vasculature and regarded as the most effective vasoconstrictor whose oversecretion advances to high blood pressure and heart disease. Deduced from their study, the authors observed a significant decrease and increase in NO and ET-1 levels, respectively, indicating that the stability connecting vasoconstriction and vasodilation sustained by endothelin and nitric oxide might have shivered; however, administration of OJP1 significantly ($P < 0.05$ or $P < 0.01$) caused an elevation and reduction in NO and ET-1 levels, respectively, which rebuilt the stability connecting vasoconstriction and vasodilation as well as protecting the cardiovascular physiology of the heart. The authors during their histopathological investigation observed that OJP1 has the ability to impede the deterioration of myofibrillar tissue and cellular infiltration suggesting that it has cardioprotective ability against heart diseases incurred by diabetes mellitus. Deduced from their study, *Ophiopogon japonicas* a polysaccharide has protective potential against cardiovascular impairment.

10.7 Conclusion and Future Recommendations

This chapter has an emphasis on the application of exopolysaccharides produced from the microbial origin for their utilization as a sustainable solution to mitigate the problem facing mankind in the area of food and medical sector. However, there is a need to explore more beneficial microorganisms most especially from unutilized areas like microorganisms present in the marine area because most of them might be a source of a novel compound that might be of great benefit in the industry, food and biomedical sector. This might even serve as a permanent replacement to several drugs that have been reported to develop a high level of resistance. Moreover, more research needs to be conducted to establish the modes of action through which the exopolysaccharides obtained from microbial origin perform their activity when tested in different in vitro and in vivo animal studies. Also, it has been observed that the application of recent biotechnology techniques like nanotechnology will go a long way toward the development of enhanced biological products of high relevance in food and biomedical sector.

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11 Biofilm Threat for Food Industry: An Approach for Its Elimination Using Herbal Food Components

Ankita A. Singh, Raghvendra Raman Mishra, O. P. Verma, and Anshuman Mishra

Abstract

Biofilm formation is an alliance of microorganisms in which microbial cells constitute an alternative lifestyle and embrace a multicellular behavior that facilitates and/or prolongs survival in adverse environmental niches. In nature, biofilms are present everywhere, and they can be found in places like waste water channels, labs, bathrooms, industrial places, and hospital settings and frequently occur on hard surfaces that are immersed in or exposed to an aqueous solution. It can also be formed as buoyant on surface of liquid; biofilm formation comprises a substitute lifestyle in which microorganisms espouse a multicellular behavior that smooth the way and/or promote prolong survival in diverse environmental niches. Cells in a bacterial biofilm communicate via quorum sensing which is a multistep process that starts with the attachment of cells to a surface and then formation of microcolony that further leads to the formation of three-dimensional structure and finally ending with maturation followed by dispersion or detachment. According to National Institutes of Health (NIH), about 65% of all microbial infections and 80% of all chronic infections are associated with biofilms. Bacterial biofilm is less

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attainable to antibiotics and human immune system and thus gives rise to ultimatum to public health because of its participation in variety of infectious diseases. A greater understanding of steps leading to biofilm formation on surfaces and within eukaryotic cells, pointing several medically important pathogens, for the development of novel, effective control strategies aimed at biofilm prevention and/or elimination.

Keywords

Biofilm · Antibiotic · Immune system and eukaryotic cells

11.1 Introduction

Formation of a biofilm permits single-cell organisms to reckon a short-term multicellular lifestyle, in which "group behavior" enables survival upon living and inanimate surfaces under unfavorable environments. These surfaces may happen to be in numerous forms, for example, including those that are found in soil and aquatic systems, those on indwelling medical devices, and those of living tissues such as tooth enamel, heart valves, or the lungs. The indwelling medical devices that enable the microorganisms to adhere on their surfaces (in most cases) are associated with many bloodstream (circulatory) infections and urinary tract infections. The most common feature of this adhered growth state is that the cells develop a biofilm. Biofilms have great importance for public health, because biofilm-associated microorganisms display substantially decreased susceptibility to antimicrobial agents.

11.2 Biofilm and Its Composition

Biofilm is an association of microorganisms on a living or nonliving surfaces that produce a matrix of extracellular polymeric substance (EPS) which is composed of proteins (<1–2%) including some enzymes, DNA (<1%), polysaccharides (1–2%), and RNA (<1%), and apart from these components, water plays the most important part in theis biofilm formation (up to 97%); it is responsible for the flow of nutrients inside the biofilm matrix (Fux et al. 2005). Antibiotics and human immune system are incapable of accessing the bacterial biofilms. Biofilms-associated microorganisms have embellished probability of counteracting antimicrobial agents that results in tedious treatment. Bacteria within a biofilm trigger some genes due to certain changes like cell density, nutritional, temperature, pH, and osmolarity that activate the expression of gene stress which in turn switch to resistant phenotypes (Hall-Stoodley et al. 2004).

11.3 Development of Biofilm

During the formation of biofilm, bacteria of different species are capable of interacting with one another via particular mechanism called quorum sensing. Development of a biofilm is a multistage procedure, which starts with the binding of microbial cells to a surface (living or nonliving), and then the formation of microcolony which further leads to the formation of three-dimensional structure and eventually finishing with maturation followed by detachment (dispersal) (Fig. 11.1).

11.4 Historical Background of Biofilm

Bacterial cells manifest two types of growing approach, i.e., planktonic cell and sessile or sedentary aggregate which is also known as the biofilm. Bacteria themselves produce a substance on a surface, known as extracellular polymeric substance. Inside this substance, microorganisms come together, and cells stick to each other (Costerton et al. 1999).

Antonie van Leeuwenhoek, a Dutch researcher, abraded the plaque biofilm from his teeth and for the first time observed "animalcule," by using a simple microscope, and this was considered as microbial biofilm discovery. Characklis, subsequently in 1973, stipulated that biofilms are not only sedulous but reveal higher resistant to disinfectants, e.g., chlorine. In 1978, Costerton invented the term biofilm and notifies the world about the significance of biofilm (Naves et al. 2010).

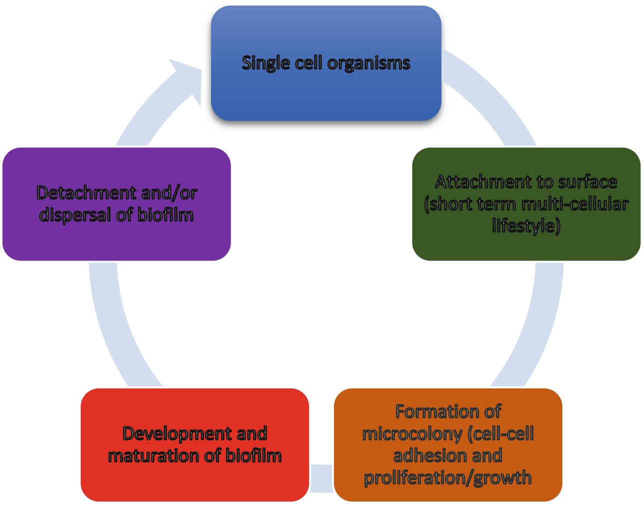


Fig. 11.1 Representation of biofilm life cycle

11.5 Biofilm-Forming Bacteria

Microorganisms are capable of producing extracellular polymeric substance (EPS) and form a biofilm, whenever they bind to a surface. The biofilm formed by these microorganisms poses a great threat for public health due to its resistant nature to antibiotics and disease linked with indwelling medical devices (Sekhar et al. 2009). A vast variety of microorganisms nearly 99.9% have the ability to form biofilm on a wide range of surfaces, i.e., inert and biological surfaces. In a study it was observed that *H. influenza* has the ability to form biofilm in human body, and not only this, but they can even escape from human immune system (Dethlefsen et al. 2008). A few biofilm-forming bacterial species have been enlisted below (Table 11.1).

11.6 Biofilm Resistance to Antimicrobial Agents

Typically antibiotics are either bacteriostatic (that prevents the bacterial cell division or inhibits bacterial growth) or bactericidal (that kills the cells). Antibiotics have been proven critical in abolishing bacterial pathogens, enormous evidence stipulate that they damage the host microbiota considerably and generate an environment where opportunistic pathogens can reign, and they increase the selective pressure toward antibiotic resistance (Nickel et al. 1985).

Significantly, sessile bacterial cells, with respect to medicine, can withstand host immune responses, and they are very less vulnerable to antibiotics than their nonattached individual planktonic counterparts (Stewart 1998). It is probably because the biofilms avoid antimicrobial challenges by multiple mechanisms.

One of the main mechanisms of biofilm resistance toward antimicrobial agents is the lack of success of an agent to pierce the full depth of the biofilm. Diffusion of antibiotics is retarded by the polymeric substances (acting as a physical barrier) that are known to make up the matrix of a biofilm and generally solutes diffuse at slower rates within biofilms than they do in water (Hogan and Kolter 2002).

Several other mechanisms which have been explored that are contemplated to be an important factors in high-resistance nature of biofilms toward antibiotics are:

Table 11.1 List of common biofilm-forming bacterial species (Khan et al. 2014)

S.no.	Biofilm-associated bacterial species
1.	*Escherichia coli*
2.	*Pseudomonas aeruginosa*
3.	*Staphylococcus epidermidis*
4.	*Staphylococcus aureus*
5.	*Staphylococcus epidermidis*
6.	*Enterobacter cloacae*
7.	*Klebsiella pneumonia*
8.	*Actinomyces israelii*
9.	*Haemophilus influenzae*
10.	*Burkholderia cepacia*

1. Limited diffusion
2. Neutralization caused by enzymes
3. Heterogenous functions
4. Slow growth rate
5. Presence of nondividing (persistent) cell
6. Biofilm phenotype such adaptive mechanisms, e.g., efflux pump and membrane alteration (Poole 2002; Böttcher et al. 2013)

11.7 Natural Agents for the Elimination of Biofilm

Biofilms are protective shields that microbes use to protect themselves from the host's immune system. The traditional approach to eliminate biofilm infection would be to administer prescription antibiotics. Research has shown that this approach is not very effective. As a matter of fact, prescription antibiotics have often been isolated as a benefactor to biofilm formation (Hammer et al. 1999).

Hereunder, research has highlighted some natural alternatives that are able to address biofilm gently without creating deeper issues. Essential oils are known to be the natural antibiotic formulations with broad-spectrum activities against virus, fungi, and bacteria (Kavanaugh and Ribbeck 2012). *Cassia*, *Peru balsam*, and *red thyme* (their essential oils) when compared to ofloxacin and gentamicin have high biofilm-eradicating effect against biofilms of *Pseudomonas* and *S. aureus* (Nostro et al. 2007a). Oregano essential oils, *carvacrol* and *thymol*, were used against *S. aureus* for the inhibition of biofilm formation (Kwiecinski et al. 2009).

An essential oil (tea tree oil) extracted from the leaves of *Melaleuca alternifolia* has been found to eradicate biofilm in *S. aureus*, including MRSA by damaging ECM (extracellular matrix) and subsequent removal of the biofilm from the surface (Artini et al. 2012).

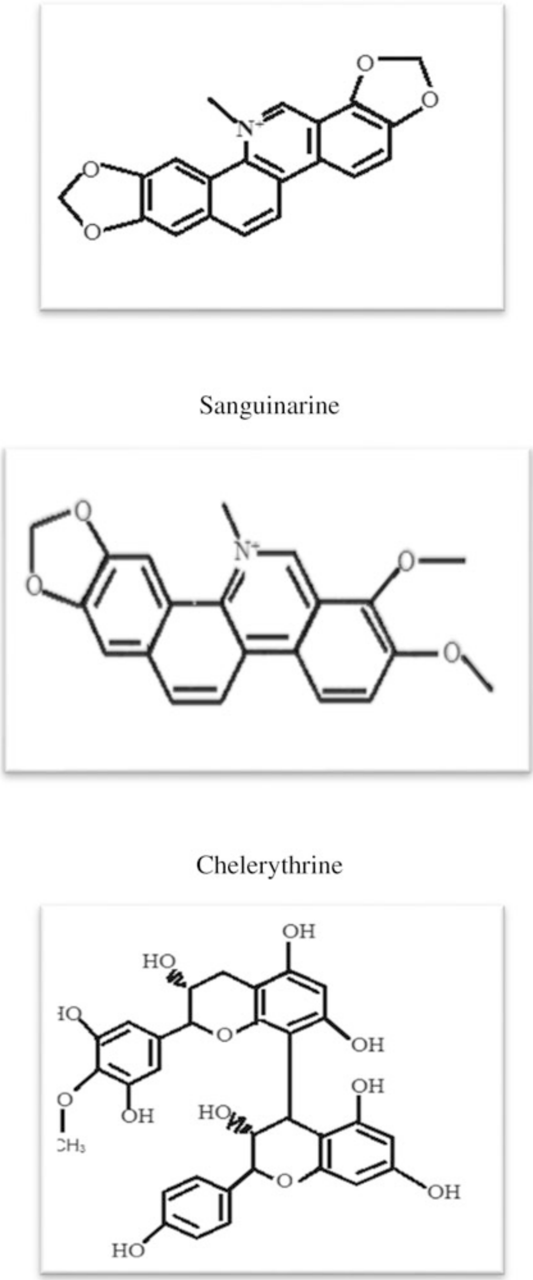
Four compounds are yielded from the extracts of *Karmeria*, *Aesculus hippocastanum*, and *Chelidonium majus*. The compounds are sanguinarine, chelerythrine, dihydroxybenzofuran, and proanthocyanidin (Fig. 11.2). These compounds have been reported for exerting inhibition of biofilm formation in *S. aureus* (Zorofchian Moghadamtousi et al. 2014).

Here are some common natural herbal drugs that are prominent in the elimination of biofilm.

1. *Curcumin*: Curcumin is the primary active constituent of turmeric and is one of the most diversely beneficial natural compounds currently known. In 2014, a reviewed study acknowledged curcumin as a potential antifungal, antiviral, antibacterial, as well as antiparasitic agent. On top of all these properties, curcumin has considered remarkably effective at disrupting biofilm (Magesh et al. 2013).

   Another study carried out in 2013 found out that out of 35 different compounds observed, curcumin alight itself as one of the top six biofilm disrupting-agent (Bjarnsholt et al. 2015).

Fig. 11.2 (**a**) Sanguinarine, (**b**) chelerythrine, and (**c**) proanthocyanidin



2. *Apple cider vinegar*: Apple cider vinegar is one of the ancient tonic, used as a biofilm disruptor. Apple cider vinegar contains acetic acid, which has shown to kill unwanted bacteria and is also capable of cutting through mature biofilms in chronic infections (Nostro et al. 2007b).
3. *Oregano*: Oregano's active constituent carvacrol has been known in terms of pathogen eradication. Oregano (oregano oil especially) has been used for a long time to naturally eliminate unwanted pathogens from the gastrointestinal tract (GIT). Carvacrol has been known to inhibit antibiotic-resistant bacteria, viruses, parasites, and fungi. Moreover, this powerful compound also inhibits the release of harmful toxins released by these pathogens including biofilm (Friedman 2014; Rutherford and Bassler 2012).
4. *Garlic*: Garlic's active constituent allicin is capable of destroying antibacterial resistant bacteria and has also reported to inhibit biofilm formation as well. Garlic is known to be a powerful broad-spectrum anti-pathogenic food. Bacteria form biofilm in order to survive the adverse environments; the formation of biofilm takes place with the help of bacterial communication called quorum sensing (Ta and Arnason 2016). For bacteria, this is an important survival mechanism. Fortunately, allicin in garlic has been shown to disrupt this bacterial communication process and biofilm growth (Kumar et al. 2015).
5. *Berberine*: Berberine is isolated from Oregon grape, goldenseal, and a few other herbs; this compound has an impressive list of benefits. Berberine is a potential antimicrobial agent with few side effects, other than this berberine is also found to consists an antidiabetic and anticancer properties (Kumar et al. 2015).

11.8 Conclusion

Biofilm formation is the strategy that is adopted by the microorganisms for their survival in the harsh/unfavorable environment, which makes their eradication particularly difficult. The age/maturation and composition of a biofilm are the major factors that influence the susceptibility of the colonized microorganism within the biofilm. Metabolism and growth rate majorly get affected as the biofilm matures and accumulation of EPS combined with oxygen and nutrient gradient; this leads to reduced penetration of antimicrobial agents which further results in antimicrobial-resistant pathogens. Novel strategies have been designed and adopted for the elimination of biofilms such as use of natural herbal drugs and anti-adhesion agents, uses of bacteriophages, and many more. Although these natural agents were effective and showed enormous potential in the treatment of biofilm-associated infections, their mechanisms of action remain unclear. The molecular pathways and animal model studies of these potential agents could provide a clearer view on the pathways affected. Another approach is to look into the synergistic effect of combinations of these agents and antibiotics to eliminate the infections associated with biofilm formation. Among these strategies natural herbal drugs have shown a promising result in eliminating biofilms.

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12 Cyanobacterial Exopolysaccharide as Natural Sources for Food Packaging Applications

Pragya Mishra

Abstract

Edible packaging materials (films and coatings) have received considerable attention over nonedible synthetic packaging materials as the aid in reducing environmental pollution. These edible films/coatings are promising systems to be used as carrier for active ingredients (antioxidants, antimicrobials, nutraceuticals, additives, and flavoring agents) which may help to improve the mechanical integrity, handling, and quality of food products. Furthermore, algal or cyanobacteria have promising prospects to be developed as alternative sources for delivery of ingredients that play an important role in the development of bioactive edible packaging film/coating. Nevertheless, further studies at molecular and assessment microbiological safety measures are necessary before using such type of edible film/coatings as an active packaging for preserving food products, assuring its quality as well as a prolonged shelf-life.

Keywords

Edible food packaging material · Cyanobacteria · Antioxidants · Nutraceuticals · Additives

12.1 Introduction

Synthetic packaging materials pose serious problems for the environment and spurred the development of biodegradable and edible films produced from natural and renewable sources. Renewable, biodegradable, and active packaging is currently

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considered to be a very important part of the research and development in packaging industry. According to the new social trends, the consumers are continuously searching for more natural and healthy food, based on minimally processed, easily prepared, ready-to-cook (RTC), and ready-to-eat (RTE) fresh products, which has resulted in an increase in consumer requirements for safe and high-quality food with longer shelf-life. Primary packaging that is in direct contact with food should prevent or reduce product damage and food spoilage, reduce or eliminate the risk of adulteration, and maintain the hygiene and should appeal smartly, for example, film wrappers, bottles, trays, or bags. Over the past 40 years, water-soluble microbial polysaccharides have gained its commercial importance as packaging material. Most commonly used packaging materials such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS) are non-biodegradable posing threats to human health as well as to the environment with a crucial problem of acting as largest contributor (63%) of plastic waste.

As defined in the early 1940s to be introduced in the food processing sector, "Polysaccharides are water-soluble biopolymers (also referred to as hydrocolloids or gums), derived from diverse renewable sources such as seeds, fruits, vegetables, plant exudates, microorganisms, and animals, that can meet most of these requirements for food additives." Naturally originating marine polysaccharides are considered edible, safe, and an effective means to impart many appealing properties to foods including modified texture, stability, foam and emulsion capacities, water retention, fat replacement, microbial protection, rancidity control, and fiber content enhancement. The best-known sources for extracellular polysaccharides (EPS) are some of the commercially cultivated microalgae including cyanobacteria *Chlorella*, *Spirulina*, *Phormidium*, and *Anabaena* (Gates 2012). Among all existing cyanobacteria, *Spirulina* has high potential to be used as an active agent that can be incorporated into films due to presence of natural polyphenols with multiple biological activities (Balti et al. 2017). Some other microalgae such as *Chroococcus submarinus*, *Johannesbaptista pellucida*, *Rhabdoderma* cf. rubrum, *Geitlerinema* (Oscillatoria) sp., *Lyngbya aestuarii* (Mertens) *Liebman*, and *Plectonema* (Leptolyngbya) cf. *golenkinianum* (Gates 2012) are also known to produce exopolysaccharide. Some gelatinous lichens are also reported to produce polysaccharide sheath along with photobiont cyanobacteria, which contributes to water retention (Prieto et al. 2008). For example, Jensen et al. (2010) isolated colleman (heteropolysaccharide) from a cyanobacterium present in the lichen *Collema flaccidum*. The cyanobacterial EPS has wide applications as food coatings, emulsifying and gelling agents, flocculants, hydrating agents, etc., possibly due to its anionic and sulfated nature which may be responsible for inhibitory properties against various types of viruses and tumors (Ohki et al. 2014). Exopolysaccharides are high-molecular-weight polymers that are composed of sugar residues and are secreted by a microorganism into the surrounding environment which contributes to the protection of these organisms in adverse conditions. Biotechnological applications of algal polysaccharide as emulsifiers, thickeners, and laxatives have led to the screening and selection of certain saline/alkaline inhabitant such as filamentous cyanobacteria from different parts of developing countries like India.

The aim of this study is to explore the application of exopolysaccharide extracted from different cyanobacterial strains for development of edible bioactive film. It also provides an overview of the application of biodegradable polymers from renewable resources like cyanobacteria in packaging materials. A wide range of different cyanobacterial polysaccharides; their physico-chemical, structural, and antioxidant properties; and their state of the art in research and commercial fields for further usage as food packaging application are described and discussed hereunder.

12.2 Edible Packaging

The packaging material made from polysaccharides, proteins, and lipids is called as edible polymeric packaging materials. Edible film/coatings are used as primary packaging because the process includes direct coating of thin layer of edible material on the food or direct wrapping of film over the food and without altering the original constituents or the processing method. Thin layer of material which can be consumed and provides a barrier to moisture, oxygen, and solute movement for the food is referred to as edible films. The material can be complete food coating or can be disposed as a continuous layer between food components (Guilbert 1986). A simple differentiation between edible films and edible coatings is that films are pre-formed separately and applied to food surface or sealed into edible pouches. Coatings are formed and cover directly onto the food surface. Both the terms are used as synonyms. Currently their involvement in packaging has been increased due to their good barrier properties against gases (such as oxygen, carbon dioxide) and lipids along with their great mechanical properties (tensile strength and elongation at break). Edible films and coatings showed promising systems to be employed as active ingredient carriers; however, the mechanisms of deterioration of each food and the mode of action of each package should be understood and related in order to assess a successful application of these materials as food packaging (Salgado et al. 2015). Mainly the three polymeric constituents used to produce edible films are polysaccharides, proteins, and lipids (Pascall and Lin 2013). To produce composite edible films/coatings, a combination of two or all of these ingredients is blended which may help to minimize the disadvantages (Table 12.1) of the individual components.

12.3 Biodegradable Versus Non-biodegradable Polymers

Biopolymers are polymeric materials derived totally from renewable resources. For any of the polymer industry along with their respective consumers, it is important to distinguish between biodegradable and non-biodegradable biopolymers. According to the American Society for Testing and Materials (ASTMStandard D-5488-94d), a biodegradable material is defined as “material capable of undergoing decomposition into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be

Table 12.1 Major polymeric constituent with their packaging properties and biotechnological applications

Polymeric constituents	Characteristic nature	Packaging property	Application	Example
Polysaccharide	Hydrophilic and provide strong hydrogen bonding	Good oxygen but poor moisture barrier properties	Bind with functional additives such as flavors, colors, and micronutrients	Chitosan, carrageenan, cellulose, pectin
Protein	Hydrophilic and have good mechanical strength	Poor moisture barrier	Used on fruits to reduce injuries during transportation	Collagen, gelatin, caseins, whey proteins, corn zein, wheat gluten, soy protein
Lipid	Hydrophobic nature	Good moisture barrier but low mechanical properties	High level of antimicrobial activity against different strains of Gram-positive and Gram-negative bacteria, delivery of active agents such as natural antimicrobials, nutraceuticals, antibrowning agents, and natural flavor	Oregano essential oil

measured by standardized tests, in a specified period of time, reflecting available disposal condition" (Steinbuchel 2003). Natural polymers are materials of natural origin with properties of biocompatibility, low cost, availability, and lack of toxicity.

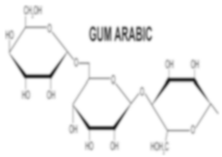
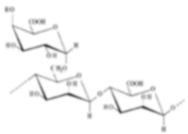
12.4 Sources of Exopolysaccharides

Gums and mucilages in the form of exopolysaccharides are sourced from the endosperm of plant seeds, plant exudates, tree or shrub exudates, seaweed extracts, fungi, bacteria, and animal sources, where they perform a number of structural and metabolic functions as given in Table 12.2.

12.5 Cyanobacterial Exopolysaccharides

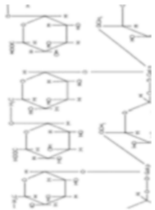
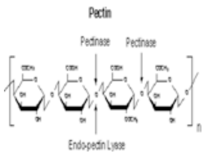
Exopolysaccharides are diverse in nature due to genetic rearrangements of the glycosyltransferase genes and other genes involved in translocation of the repeating units. Exopolysaccharides are those molecules having various structures and functions and also provide different types of advantages to their producing

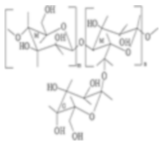
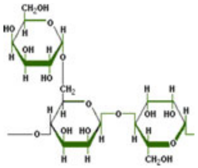
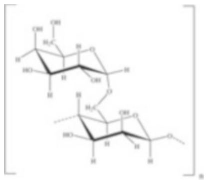
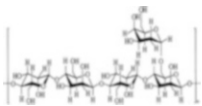
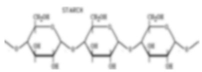
Table 12.2 Summarized list of exopolysaccharides from different sources with their chemical structure, type, properties, and biotechnological applications

Source	Name	Structure	Type	Properties	Biotechnological applications	Reference
Tree gum exudates	Arabic gum		Arabinogalactan	Emulsifier, stabilizers, and binders	Act as carrier in control- released matrix system, soothing of irritating mucous membrane	Deogade et al. (2012)
	Prosopis alba gum		Arabinogalactan	Clear, water soluble, emulsifying capacity, and stabilizing properties	Fish oil encapsulation	Vasile et al. (2016)
	Acacia tortuosa gum	–	Arabinogalactan	Clear and very soluble in water, viscoelastic properties, shear thinning non-Newtonian flow properties	Used as emulsifier and stabilizer in food emulsion	Martínez et al. (2015)
	Almond gum	–	Arabinogalactan	Good flow characteristics and higher mineral content than gum arabic. Emulsification and foaming properties	Used as edible coatings, adsorbent	Rezaei et al. (2016)
	Albizia stipulata gum	–	Arabinogalactan	Reddish-brown gum, non-Newtonian flow, antioxidant properties	Used as a substitute for gum Arabic, controlled-release matrix tablets	Thanzamia et al. (2015)

(continued)

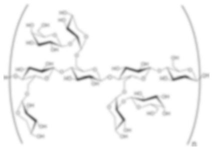
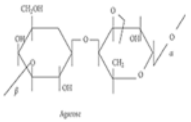
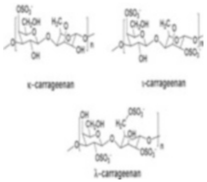
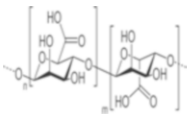
Table 12.2 (continued)

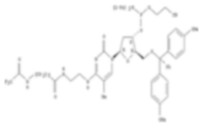
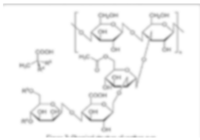
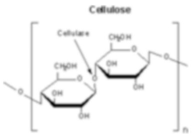
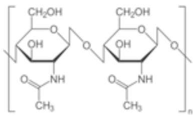
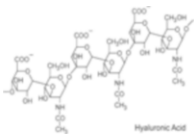
Source	Name	Structure	Type	Properties	Biotechnological applications	Reference
	Acacia senegal gum		Arabinogalactan	High solubility and low viscosity, good emulsifying characteristics and non-toxic nature	Used as a safe food additive, stabilizers, film formers, thickeners, flocculants, suspending agents, and emulsifiers	Gashua et al. (2015)
	Cashew gum	–	Galactan with glucose, arabinose, rhamnose, mannose, and glucuronic acid units	Clear glassy masses, color ranges from dark brown to pale yellow, highly hydrophilic, good physico-chemical properties, and high levels of minerals (calcium, sodium, and potassium). Non-toxic and biodegradable	Used for preparation of layer-by-layer films with potential application in nanobiomedical devices; also used as thickening agent, a gelling agent, and a colloidal stabilizer; agglutinant for capsules and pills	Souza et al. (2010)
Extracts	Pectin		Galacturonic acid with rhamnose, arabinose, galactose, xylose, and glucose units	Soluble in pure water, non-Newtonian flow, pseudoplastic behavior characteristics, ability to form gels	Carrier of a variety of drugs for controlled-release applications, pectin-based delivery systems, especially ionotropic gelation and gel coating	Lefsih et al. (2016)

Seeds	*Cassia spectabilis* gum		Galactomannan	Viscoelastic and critical shear rate	Thickening agent and gelling agent	Kapoor et al. (1998)
	Guar gum		Galactomannan	Dissolves in polar solvent on dispersion and form strong hydrogen bonds, pseudoplastic or shear-thinning behavior	Used as thickener and stabilizer, also used in food products for supplementation as dietary fiber	McCleary et al. (1981)
	Fenugreek		Galactomannan	Form of oil-in-water emulsions with small droplet size (2–3 μm) and long-term stability, forms thick interfacial film	Nanocomposite films and biodegradable films	Brummer et al. (2003)
	Locust bean gum		Galactomannan	Soluble in water, high gelling capacity	Used as film coating, binder, biopharmaceutical application	Dakia et al. (2008)
	Starch		Glucan	Strong hydrophilicity, poor mechanical properties, low viscosity, high stability, and high transparency, excellent film-forming ability, binding	Used for fabrication of biodegradable films	Liu et al. (2014)

(continued)

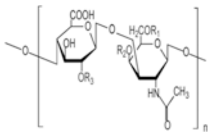
Table 12.2 (continued)

Source	Name	Structure	Type	Properties	Biotechnological applications	Reference
				properties, and biodegradable		
	Hymenaea courbaril gum		Xyloglucan	Storage polysaccharides, hydrophilic polyelectrolyte, good polymer-solvent interaction	Controlled drug release and texture modifier	Arruda et al. (2015)
Red Sea weeds	Agar		Galactan, D- and L-galactopyranose	Thermoreversible, biocompatibility, and bioinert nature	Growth medium for microorganisms, agar films for food packaging	Yarnpakdee et al. (2015)
	Carrageenans		Galactan	Good water vapor barrier and mechanical properties, edible and biodegradable	Used to produce pills and as potent inhibitors of herpes and human papillomavirus (HPV), edible film preparation	Prajapati et al. (2014)
Brown seaweeds	Alginate		Mannuronic acid with guluronic acid units	Transparent, colorless, noncoagulable on heating, wide viscosity range	Microencapsulation of nutraceuticals and drugs	Rinaudo (2007)

Cyanobacteria	Lectins		High-mannose N-glycans	Ability to bind glycoconjugates, antiviral (anti-HIV and anti-influenza virus activity) and antibacterial	Film, forms affinity matrix	Praseptiangga (2017)
Microbial	Xanthan gum		Glucan	Soluble in cold and hot water, non-Newtonian and highly pseudoplastic, biodegradable	Used as stabilizer for suspensions and emulsions, improves the cohesion of starch granules, contributes to the structure, and increases shelf-life due to moisture retention.	Silva et al. (2009)
	Cellulose		Glucan	Insoluble to water, tough, flexible, biodegradable, good tensile strength	Form a gel network, food-grade film	Mirhosseini and Amid (2012)
Animal	Chitin		Glucosamine	Highly impermeable to gases, exhibit rather high water vapor permeability, antimicrobial and antioxidant activity	Drug delivery system	Kumari and Rath (2014)
	Hyaluronic acid		Glucosamine with glucuronic acid units	Main component of cellular matrix, low stability, weak mechanical strength, and fast degradation speed, promising	Scaffolding for tissue engineering, dermatological fillers, and viscosupplementation	Fakhari and Berkland (2013)

(continued)

Table 12.2 (continued)

Source	Name	Structure	Type	Properties	Biotechnological applications	Reference
				coagulation property, cell compatibility and antibacterial property		
	Chondroitin sulfate		Galactosamine with glucuronic acid units	Structural component of cartilage, good moisturizing properties, forms hydrogel films with high toughness and anti-adhesion property	It is a chemical used for osteoarthritis, make complex with iron, utilized for polysaccharide composite film scaffolds	Yataka et al. (2020)

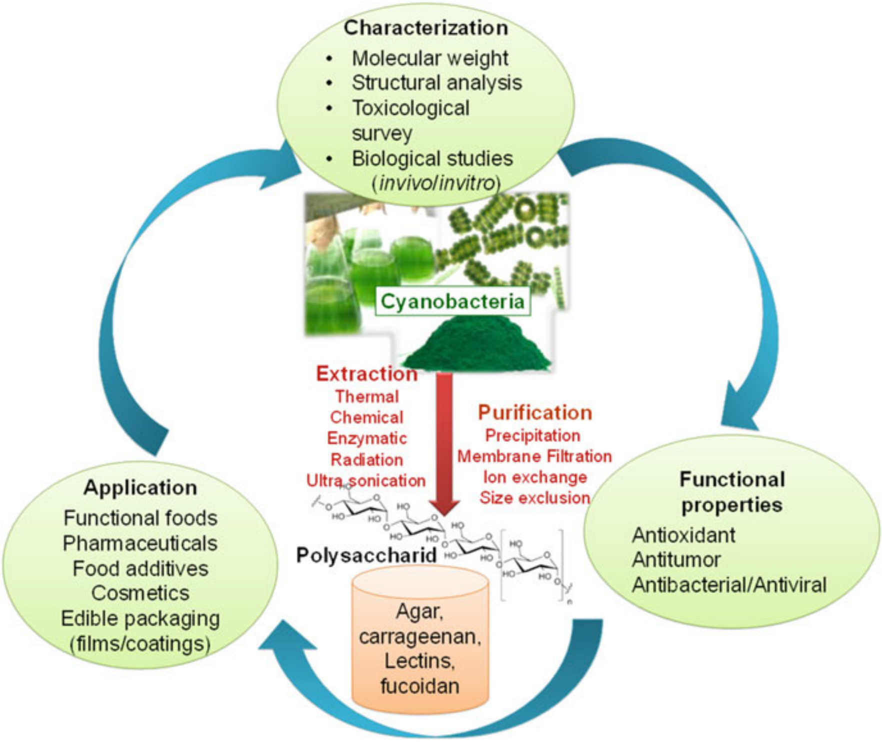


Fig. 12.1 Schematic diagram of extraction, purification, characterization, biological activities, and applications of cyanobacterial exopolysaccharides

microorganisms, including surface variability, resistance to innate and acquired immunity mechanisms, the ability to adhere to different surface and cell types, and resistance to antibiotic activity. The environmental conditions have pronounced impact on microorganisms, and in consequence it is expected that new productive microorganisms can be isolated. Owing to the increasing interest in the exploitation of microorganisms for various industrial applications (Sutherland 2001), the attention toward the polysaccharide-producing cyanobacterial strains greatly increased, in particular toward the strains that possess abundant capsule or slime and also release large amounts of extracellular polysaccharide (Fig. 12.1).

Cyanobacteria have been known to produce large amounts of exopolysaccharide since prehistoric time (Drews and Weckesser 1982). There are three types of exopolysaccharide: (1) sheath (thin uniform external layer immediately next to the outer membrane), (2) capsule or slime (capsular or slime polysaccharide, CPS) which is the outer unstructured zones, and (3) soluble polysaccharides (released polysaccharide, RPS), which are released by many cyanobacteria as response against stress condition into the media (Bold and Wynne 1985; Bertocchi et al. 1990). Both

Table 12.3 List of some cyanobacteria along with their exopolysaccharide types

Cyanobacteria	Polysaccharide types
Microcystis flos-aquae	Slime polysaccharide
Nostoc commune	Released and capsule polysaccharides
A. halophytica	Rigid/extra-rigid rod-type polysaccharide glucose, arabinose, fucose, mannose, and glucuronic acid with branch points at mannose and the remaining glucose and glucuronic acid
Mastigocladus laminosus	Branched pentadecasaccharide
Microcystis wesenbergii	Released polysaccharide (RPS, mainly composed of uronic acid)
S. platensis	Galactose
C. capsulate	Released polysaccharide (RPS), comparable to that of alginate (branched decasaccharide or octasaccharide repeating unit)
Mastigocladus laminosus	Capsule polysaccharide (CPS)
Anabaena sp. ATCC 33047	Released polysaccharide (RPS)
Anabaena sphaerica RPS	Released polysaccharide (RPS) (*galactose*)

sheath and capsular polysaccharides are difficult to differentiate. Some of the cyanobacteria along with their exopolysaccharides are categorized in Table 12.3.

12.6 Conclusion

In recent times, there is a trend of using renewable, biodegradable, and active packaging as important part of the research and development in different packaging industry. Among all existing biopolymers (proteins, lipids, and polysaccharides), seaweed-based biopolymers such as alginates and carrageenans have been frequently used in development of edible film and coatings. Cyanobacteria are considered as a good source of natural polyphenols with multiple biological activities; hence they have potential to be used as an active agent to provide functionality when incorporated into films/coatings.

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Microbial Laccase Production and Its Industrial Applications

13

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Abstract

Pollution remains a major problem to be dealt with. Laccase is multicopper oxidase enzyme belonging to the group of blue oxidases that are widely distributed among plants, insects, fungi, and bacteria. As pollution increases day by day, various industries mainly textile, pulp, and paper discharge huge quantity of waste in the environment, and the disposal of this waste is a big problem. Work has been done toward an enzyme, which can detoxify these wastes without harming the environment, and this increases the interest in its use to replace conventional non-biological methods. Laccase uses oxygen and produces water as by-product. Laccases have shown potential properties to act on a range of substrates and also to detoxify a range of pollutants which have made them to be usable for several purposes in many industries like for bleaching process in paper and pulp industries, scouring and decolorization in textile

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industries, dough making and softening in food industries, and clarification and removal of haze formation in beer, wine, and juices industries, as well as in bioremediation process for degradation of pesticides and herbicides and removal of toxic pollutant which are the major areas where laccase enzyme plays active role and become interesting area of research for researchers.

Keywords

Laccase · Biodegradation · Bioremediation · Decolorization · Oxidation · Enzyme

13.1 Introduction

The enzyme involved in the cross-linking monomer, the cleavage ring of aromatic compounds, degradation of the polymer and belongs to the blue multicopy oxidase known as laccase. Laccases (EC1.10.2.3) are enzymes that oxidize multicopper varieties of aromatic and non-aromatic compounds with molecular oxygen. Laccase is of major importance in commercial and biotechnological applications due to its low specificity to reduce the substrate. Laccase is widely present in nature – found in a variety of higher plants, fungi, and some bacteria.

Laccase is a particularly promising enzyme for enzymatic oxidation in a variety of industrial sectors, including wood pulp, paper, textiles, and the food industry (Rodriguez Couto and Toca Herrera 2006, 2007; Mendonça et al. 2008). Laccases (CE 1.10.3.2) are oxidoreductases that oxidize diphenols and use oxygen as electron acceptor (Thurston 1994; Viswanath et al. 2008). Utilization of laccases in synthesis reaction has been difficult because of their unavailability. But recently these things are gaining momentum (Baldrian and Šnajdr 2006; Riva 2006; Joo et al. 2008). Main reactions that are being catalyzed by said enzyme are polymerization and depolymerization reactions. However different functions in different organisms are also reported. Reaction mechanism starts with monoelectronic oxidation using free or reactive electron. Copper ions help in carrying out the reduction and oxidation process.

In our work laccases are of prime concern because of their ability to oxidize variety of substances. Laccases have wide potentials that prove them as ecological green enzymes (Baldrian and Šnajdr 2006; Riva 2006; Singh and Chen 2008; Wong 2008).

13.2 Laccase Occurrence

Laccase is most widely distributed in a panoramic view of higher plant, insect, fungi, and bacteria (Diamantidis et al. 2000).

Plant: Laccase in plants have been identified in tree (mango, pine, etc.), cabbages, turnip, beets, apples, asparagus, potatoes, pears, and various vegetables. In the embryo of maize (*Zea mays*), laccase has been expressed recently.

Insect: The insect laccase is a long amino-terminal sequence characterized by unique domain consisting of several conserved cysteine, aromatic, and charged residues. In dozen of insects of genera which include *Bombyx*, *Calliphora*, *Diploptera*, *Drosophila*, *Lucilia*, *Manduca*, *Musca*, *Oryctes*, *Papilio*, *Phormia*, *Rhodnius*, *Sarcophaga*, *Schistocerca*, and *Tenebrio*, laccases are found (Xu 1999). Recently, two isoforms of laccase 2 gene have been found to catalyze larval, pupal, and adult cuticle tanning in *Tribolium castaneum* (Arakane et al. 2005; Sharma and Kuhad 2008).

Fungi: Fungal laccases have higher redox potential than bacterial or plant. Most of the laccase described in literature was isolated from higher fungi. Laccases have been isolated from ascomycetes, deuteromycetes, and basidiomycetes fungi (Assavanig et al. 1992). The peroxidative activity was shown by the first laccase isolated from *Monicillium indicum* which was characterized from an ascomycetes (Thakker et al. 1992). In fungi, ascomycetes and deuteromycetes have not been a focus for lignin degradation studies as much as the white-rot basidiomycetes. Most common laccase producers are the wood-rotting fungi *Trametes versicolor*, *Trametes hirsuta*, *Trametes ochracea*, *Trametes villosa*, *Trametes gallica*, *Cerena maxima*, *Coriolopsis polyzona*, *Lentinus tigrinus*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *etc.* (Morozova et al. 2007).

Bacteria: Bacterial laccase was first reported in *Azospirillum lipoferum* (Givaudan et al. 1993); it plays a role in cell pigmentation and oxidation of phenolic compounds (Faure et al. 1994). Other names are *E. coli*, *Bacillus subtilis*, *S. lavendulae*, *S. cyaneus*, *Marinomonas mediterranea*, *Aquifex aeolicus*, *Bacillus* sp., *Bacillus halodurans*, *Leptothrix discophora* SS1, *Oceano bacilusiheynesis* (cotA), *Alpha-proteobacterium* SD21, *Gama-proteobacterium JB*, *Pseudomonas fluorescens GB-1*, *Pseudomonas maltophilia*, *Xanthomonas campestris* (copA), *Pseudomonas putida* GB-1 (cumA), *Pseudomonas syringae* pv. *tomato* (copA), *Pseudomonas aerophillum* (pae1888), *Streptomyces antibioticus*, *Streptomyces griseus* (epoA), *Thermus thermophilus* (HB27), *Streptomyces psammoticus* MTCC 7334, etc. (Sharma et al. 2008).

13.3 Laccase Reaction Mechanism

Laccases occur often as isozymes with monomeric or dimeric protein structures (Thurston 1994). Most monomeric laccase molecules contain four copper ions in their structure that can be classified in three groups using UV/visible and electron paramagnetic resonance (EPR) spectroscopy (Leontievsky et al. 1997). The type I copper (T1) is responsible for the intense blue color of the enzymes at 600 nm and is EPR-detectable, the type II copper (T2) is colorless but EPR-detectable, and the type III copper (T3) consists of a pair of copper ions that give a weak absorbance near the UV spectrum but no EPR signal. The T2 and T3 copper sites are close together and form a trinuclear center that are involved in the catalytic mechanism of the enzyme (Solomon et al. 2001; Quintanar et al. 2005; Ferraroni et al. 2007; Augustine et al. 2008). The three-dimensional structures of a few fungal laccases (Hakulinen et al. 2002; Piontek et al. 2002) and the CotA laccase from *Bacillus subtilis* (Enguita et al. 2003) have been characterized. All fungal laccases show a similar architecture consisting of three sequentially arranged domains of beta-barrel-type structure (Fig. 13.1).

The active site is well conserved with four copper sites. T1 is located in domain 3 with copper lying in a shallow depression, and trinuclear copper cluster is at the

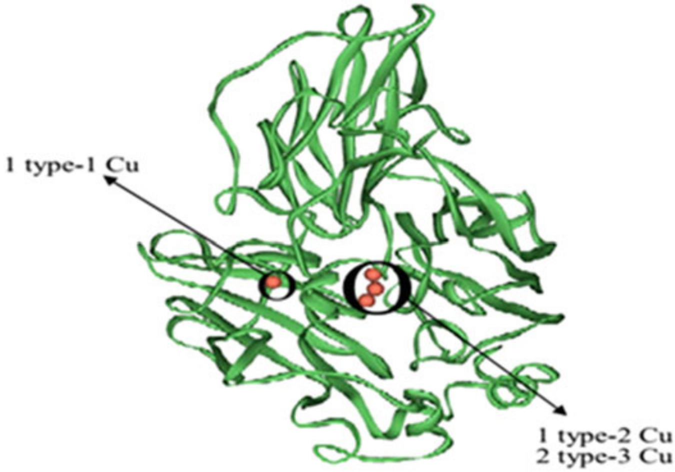

Fig. 13.1 Ribbon representation of the X-ray crystallographic structure of *Trametes versicolor laccase*. (Modified from figure atwww.chem.ox.ac.uk/id/faagroup/fuelcell.html)

interface between domains 1 and 3 with each domain providing ligand residues at the coordination of copper ions. The T1 copper is coordinated with His-N and Cys-S as conserved equatorial ligands. The axial position has Leu or Phe that does not participate in the coordination. The copper-thioether bond and noncoordination residue strongly influence the redox potential of the enzyme. A wide range of redox potentials is displayed by different sources of laccase. The T1 site of laccase of *T. versicolor* shows a high redox potential of 780–800 mV (Piontek et al. 2002) where the plant *R. vinifera* enzyme has a value of 420 mV (Reinhammar 1984). The T1 site is the primary electron acceptor site where the enzyme catalyzes four 1e oxidations of a reducing substrate (Huang et al. 1999). The T2/T3 trinuclear site is where the reduction of molecular oxygen takes place by accepting electrons from T1 site. Elucidation of the nature coordination of the copper sites in laccase by spectroscopic and DFT studies (Quintanar et al. 2005) reveals that the T2 copper site is coordinated to two His-N and one oxygen atom as OH^- while each of the T3 coppers coordinates to three His residues. Further, the open coordination positions toward the center of trinuclear cluster with the negative protein pocket (four conserved Asp/Glu residues) are shown by both T2 and T3 copper sites. Reduction of oxygen takes place via the formation bound oxygen intermediates.

Removal of one electron from phenolic hydroxyl groups of lignin to form phenoxy radical is promoted by laccase (Fig. 13.2). The degradation of lignin proceeds by phenoxy radical that leads to either oxidation at C^{α}-carbon or cleavage of bond between C^{α}-carbon and C^{α}-carbon. This oxidation results in an oxygen-centered free radical, which can then be converted in a second enzyme-catalyzed reaction to quinone. The quinone and the free radicals can then undergo polymerization (Thurston 1994). The presence of electron-withdrawing substituents at phenoxy groups and bulky groups makes it more difficult to be oxidized. Oxidation

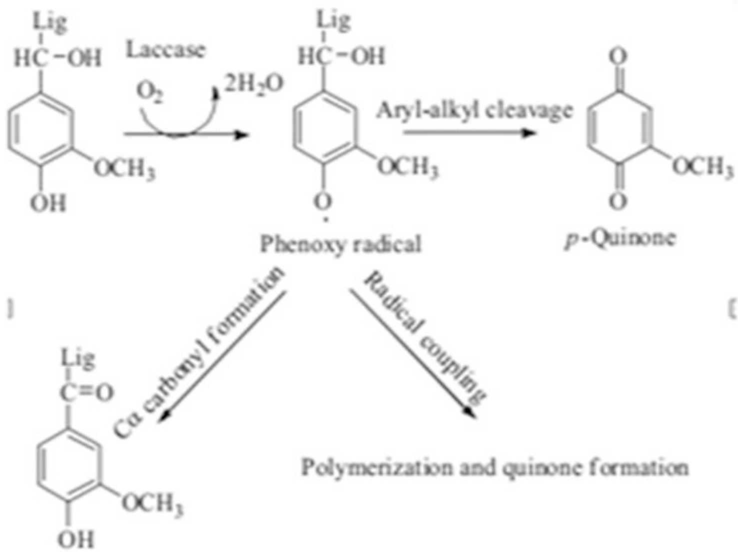

Fig. 13.2 Oxidation of phenolic subunits of lignin by laccase

of phenols, anilines, and benzene is catalyzed by laccase which correlates with the redox potential difference between laccase's T1 copper site and the substrate. Laccases have been found to oxidize non-phenolic compounds and lignin in the presence of mediators −2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS), I-hydroxybenzotriazole (HBT), and 3-hydroxyanthranilic acid (Bourbonnais et al. 1995). In presence of ABTS, oxygen uptake of laccase is faster than in HBT, and widening of the substrate range of laccase to non-phenolic subunits of lignin by the inclusion of a mediator such as ABTS is shown in Fig. 13.3. ABTS-mediated oxidation of non-phenolic substrates proceeds via electron transfer mechanism through formation of ABTS++. Further investigation is warranted on the precise role of small molecule mediators in the catalytic mechanism of laccase.

13.4 Molecular Biology of Laccases

Isozymes of laccases in lignolytic organisms are encoded by multiplicity of laccase gene sequences (Mander et al. 2006). For example, the number of laccase genes in *Rhizoctonia solani* is four, five in *Trametes* species (Yaver et al. 1996), three in the Basidiomycete I-62 (Mansur et al. 1997), at least two in *Agaricus bisporus* (Smith et al. 1998), at least three in *Pleurotus* species (Palmieri et al. 2003), four in *Podospora anserina* (Fernandez-Larrea and Stahl 1996), two in *Lentinula edodes*, or three in *Gaeumannomyces graminis* (Litvintseva and Henson 2002). For the first time, laccase genes were isolated and sequenced about 20 years ago from the fungi *Neurospora crassa* (Germann and Lerch 1986), *Aspergillus nidulans*, *Coriolus*

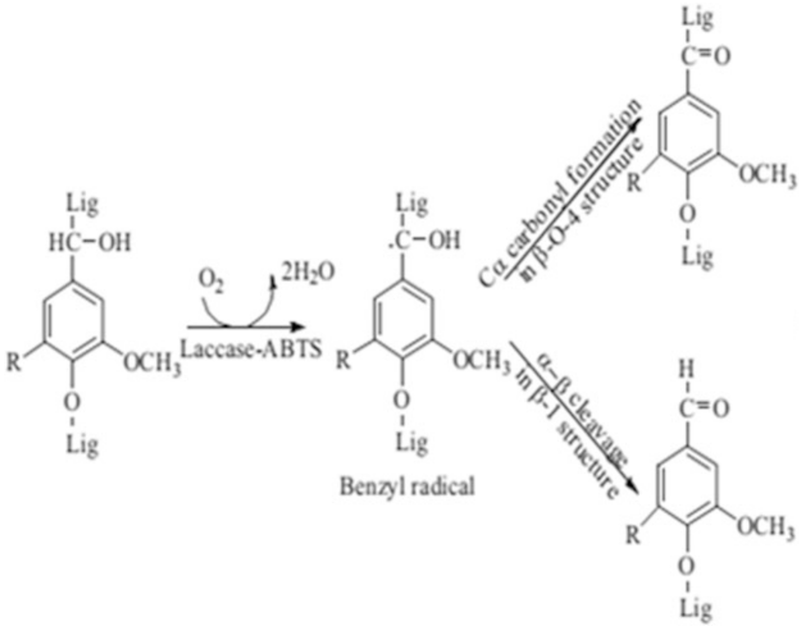

Fig. 13.3 Oxidation of non-phenolic lignin subunits by laccase and ABTS

hirsutus, and *Phlebia radiata*. Since then, sequencing of laccase genes has increased considerably. Laccase genes in lignolytic fungi have been cloned and characterized; Yaver et al. 1996). The overall intron-exon structure of all three laccase genes in G. graminis (Litvintseva and Henson 2002) and in *P. ostreatus* is distinct. Analysis of laccase genes in different organisms points out conserved sequences in genes that encode copper-binding regions of N-terminal domain of laccase enzyme. These sequences are laccase gene specific and have become basis for PCR-based screening for the presence of the laccase genes in organisms. However, the corresponding protein products of the number of laccase genes which have been experimentally characterized are significantly lower. To date, there are about 50 such enzymes, most of which are fungal laccases (Morozova et al. 2007). SDS-PAGE determines that a protein of 500–600 amino acids with a molecular weight usually ranging from 60 to 90 kDa is coded by a typical laccase gene. The difference between the molecular weight predicted from the peptide sequence and the experimentally obtained molecular weight is caused by glycosylation, which typically accounts for about 10–20% of the total MW. Although laccase production in organisms is known to be influenced by a number of physiological and environmental factors, little work has been done to examine the regulation of expression of laccase genes at molecular level (Fernandez-Larrea and Stahl 1996; Mansur et al. 1997).

Laccase production is subject to complex regulation by nutrients in the culture medium during the fungal growth. Nitrogen-limiting condition regulates the expression of laccase in some fungi, while in others nitrogen sufficiency results in enhanced enzyme production. The effect of various nutrient nitrogen concentrations on expression of lcc genes at molecular level in *T. versicolor* was examined. There was a direct correlation between concentration of nitrogen nutrient in growth medium and the level of lcc expression by *T. versicolor*. In Basidiomycetes 1-62 under nonlimiting nitrogen conditions, lcc1 and lcc2 transcript levels increased 100-fold over limiting conditions. The use of 1% cellulose as a carbon source increased both lcc1 mRNA and lcc2 mRNA of *Lentinula edodes*. A medium with high nitrogen has been shown to induce transcription of laccase genes in the Basidiomycetes I-62 (CECT 20197) and in *Pleurotus sajor-caju*. The defense mechanism against oxidative stress caused by free copper ions has been related to a laccase often a strong inducer of laccase gene transcription (Fernandez-Larrea and Stahl 1996; Litvintseva and Henson 2002. In addition to copper, other metal ions such as Mg^{2+}, Cd^{2+}, or Hg^{2+} can stimulate laccase expression. Metal response elements (MRE) with consensus sequences are found in the promoter regions of two fungal laccase genes of *P. anserina* (Fernandez-Larrea and Stahl 1996) and the basidiomycete PM 1. Involvement of these elements on copper induction of expression of lcc genes needs to be further assessed.

Certain aromatic compounds that are structurally related to lignin precursors, such as 2,5-xylidine or ferulic acid, have also been shown to increase laccase gene transcription in *Trametes villosa*, *T. versicolor*, and *Pleurotus sajor-caju* (Yaver et al. 1996. Current understanding of differential expression of laccase genes in lignolytic organisms is fragmentary and needs to be further improved.

13.5 Heterologous Production of Laccases

The lack of sufficient enzyme stocks and the cost of redox mediator are the important obstacles in commercial application of laccases. In order for laccases to be effective in environmental detoxification, large amounts of active enzyme are needed. Since an inexpensive source of laccase must be obtained for some potential applications to become a reality, there have been several reports of heterologous expression of recombinant laccases (Table 13.1). Laccase genes from *Myceliophthora thermophila*, *Trametes versicolor*, *Coriolus hirsutus*, and *Melanocarpus albomyces* in *Saccharomyces cerevisiae* were expressed. Similarly, expression of laccase genes from *Pleurotus sajor-caju* (Soden et al. 2002), *Trametes trogii*, and *Pycnoporus cinnabarinus* was in *Pichia pastoris*, those from *Pleurotus ostreatus* in *Kluyveromyces lactis*, *Trametes versicolor* laccase genes in *Yarrowia lipolytica*, the laccase gene of *Coriolus hirsutus* and *Lentinula edodes* in tobacco, *Myceliophthora* laccase gene in *Aspergillus oryzae*, and the *Pycnoporus* laccase gene and *Trametes versicolor* in *Aspergillus niger*. Laccase production levels have often been improved significantly by expression in heterologous hosts, but the reported levels are still rather low for industrial applications (Table 13.1). The common problems associated with heterologous expression of fungal enzymes are incorrect folding of the polypeptide and inefficient codon usage of expression organisms, resulting in non-functional or low yields of enzyme. The incorrect

Table 13.1 Laccase production in heterologous hosts

Laccase gene	Production host	Laccase production ($mg l^{-1}$)	Reference
Ceriporiopsis subvermispora lcs-1	*Aspergillus nidulans* *Aspergillus niger*	1.5 1.5	
Myceliophthora thermophila lcc1	*Saccharomyces cerevisiae*	18	
Pleurotus sajor-caju lac4	*Pichia pastoris*	4.9	Soden et al. (2002)
Pycnoporus cinnabarinus lac1	*Aspergillus niger*	70	
Pycnoporus cinnabarinus lac1	*Aspergillus oryzae*	80	
Trametes versicolor	*Yarrowia lipolytica*	2.5	
Trametes versicolor lac1 & lac2	*Pichia pastoris*	2.8	
Trametes trogii lac1	*Pichia pastoris*	17	

substitution of carbohydrate residues during glycosylation of proteins, which is due to preferential utilization of specific carbohydrates by the expression organism, may pose an additional problem to heterologous expression. These problems are being overcome by using more advanced organisms as expression hosts whose codon usage and molecular folding apparatus are suitable for correct expression of these proteins. For proper laccase folding, the adequate copper concentration has a vital role which was further supported by studies in which two genes related to copper-trafficking in *T. versicolor* were overexpressed in *S. cerevisiae* expressing *T. versicolor* lcc3 gene; the heterologous laccase production by *S. cerevisiae* was improved up to 20-fold. The effect was suggested to result from more efficient transport of copper to the Golgi compartment. Directed evolution has also been used for improving heterologous laccase production. A site-directed mutation/ random mutation in the *Myceliophthora thermophila* laccase gene accompanied by evolution resulted in the highest reported laccase production level in S. cerevisiae but also enhanced Kcat of the enzyme. A similar strategy with *Fome lignosus* yielded mutant laccase enzyme with four substitutions. This mutant enzyme exhibited improved kinetic properties due to rapid movement of water along water channel in the enzyme molecule. A thermostable laccase is the genetic product of five rounds of directed evolution in *Saccharomyces cerevisiae* that tolerates high concentrations of solvents. Thus, efforts have to be made in order to achieve cheap overproduction of this biocatalyst in heterologous hosts and also their modification by chemical means of protein engineering to obtain more robust, active, and tailormade enzyme.

13.6 Industrial Applications

In biotechnology field laccase is used which has oxidation ability toward the phenolic and non-phenolic compounds. Other applications of laccase include the cleaning the industrial effluents, mostly from industries like paper, pulp, textile, and petrochemical industries. Laccase is also used in the medical diagnostics and for cleaning herbicides, pesticides, and some explosives in soil. Laccase has enormous uses in agricultural, medicinal, and industrial areas. Laccases are also used to clean the water in many purification systems. It has also applications in medical side to prepare certain drugs like anticancer drugs, and it is added in cosmetics to minimize their toxic effects. In bioremediation laccase is used which has a potential to remove xenobiotic substances and to produce polymeric product. Now research is ongoing on enzymatic synthesis of organic compounds, laccase-based bio-oxidation, bio-transformation, and biosensor development. The yield of laccase can be increased by optimizing different cultural conditions (Fig. 13.4).

13.6.1 Decolorization of Dyes

Laccases on which the development of processes is based provide a solution because of their potential in degrading dyes having a different chemical structure which includes synthetic dyes that are currently employed in the industry (Hou et al. 2004). Laccase in the textile industry is used to decolorize textile effluents, bleach textiles, and synthesize dyes. *Flavodon flavus* decolorized several synthetic dyes like Azure B and Brilliant Blue R in low-nitrogen medium. Alternatively, laccase, along with stabilizers, may be suitable for treatment of wastewater. Partial decolorization of two azo dyes and complete decolorization of two triphenylmethane dyes (bromophenol blue and malachite green) was achieved by cultures of Pycnoporus sanguineus producing laccase as the sole phenoloxidase. Dyes like triarylmethane, indigoid, azo, and anthraquinonic which are used in dyeing textiles are easily degraded by *Trametes hirsuta* and a laccase purified from the fungus as well as 23 industrial dyes. The purified laccase of white-rot fungi *Pleurotus ostreatus* and *Stereum ostrea* decolorized textile dyes, i.e., Remazol black-5, Remazol blue-19, and Remazol orange-16. More than 90% of decolorization activity was observed after 16 h incubation with laccase at 20 nkat/ml for all the textile dyes used in their study. The degradation of azo dyes, which are the most widely used colorants, has been studied most.

13.6.2 Degradation of Xenobiotics (Bioremediation)

One of the most efficient processes to remove pollutants from environment is through biodegradation of xenobiotics. It is the process by which living organisms degrade or transform hazardous organic contaminants into less toxic compounds. Screening of indigenous microbes from the pollutant contaminated site for their

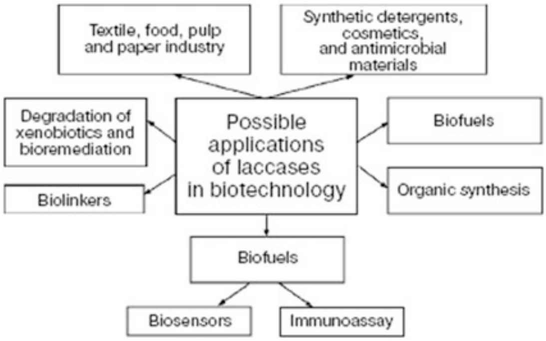

Fig. 13.4 Scheme of applications of laccase (Morozova et al. 2007)

degradation potential is one way to approach the problem. Thus, microorganisms that can degrade various pollutants have been isolated with the eventual goal of exploiting their metabolic potential for the degradation of polluted sites. Laccases are able to oxidize a broad range of xenobiotic compounds including chlorinated phenolics, pesticides, and polycyclic aromatic hydrocarbons. Moreover, polycyclic aromatic hydrocarbons, which arise from natural oil deposits and utilization of fossil fuels, were also found to be degraded by laccases. Laccase purified from a strain of *Coriolopsis gallica* oxidized carbazole, N-ethylcarbazole, fluorine, and dibenzothiophene in presence of 1-hydroxybenzotriazole and 2.2′-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid as free radical mediators. Established the effective role of laccase of *Pleurotus ostreatus* HP-1 in bioremediation of PAHs contaminated sites. Laboratory experiments have demonstrated that phenols and aromatic amines may be removed from water by the application of laccase. The underlying mechanism of the removal involves enzymatic oxidation of the pollutants to free radicals or quinones that undergo polymerization and partial precipitation.

13.6.3 Effluent Treatment

A biotechnological application of industrial effluent treatment uses laccases from fungi. As they exhibit broad substrate specificity, they can bleach Kraft pulp or detoxify agricultural by-products including olive mill wastes or coffee pulp. Laccase of an isolate of the fungus, *Flavodon flavus*, was shown to decolorize the effluent from a Kraft paper mill bleach plant. Laccase purified from white-rot basidiomycete, *T. villosa*, degrades bisphenol A, an endocrine-disrupting chemical. Nonylphenols have increasingly gained attention because of their potential to mimic the action of natural hormones in vertebrates. They result from incomplete biodegradation of nonylphenol polyethoxylates (NPEOs), which have been widely used as non-ionic surfactants in industrial processes. Both nonylphenols and NPEOs are discharged into the environment, mainly due to incomplete removal of wastewater treatment facilities. Nonylphenols are more resistant to biodegradation than their parent compound and hence are found worldwide in wastewater treatment plant effluents and rivers. Due to their hydrophobicity, they tend to absorb onto surface water particles and sediments and accumulate in aquatic organisms. Consequently, nonylphenols represent a serious environmental and human health risk. Laccases from aquatic hyphomycete, *Clavariopsis aquatica*, has proved to degrade xenoestrogen nonylphenol. In addition to the potential role of such degradation processes for natural attenuation processes in fresh-water environments, laccase also offers new perspectives for biotechnological applications such as wastewater treatment.

13.6.4 Food Industry

13.6.4.1 Wine Stabilization

Laccase enhances the quality of drinks and stabilizes certain perishable products containing plant oils (Morozova et al. 2007). In food industry, wine stabilization is the main application of laccase. Polyphenols have undesirable effects on wine production and on its organoleptic characteristics, so their removal from the wine is very necessary. Many innovative treatments, such as enzyme inhibitors, complexing agents, and sulfate compounds, have been proposed for the removal of phenolics responsible for discoloration, haze, and flavor changes, but the possibility of using enzymatic laccase treatments as a specific and mild technology for stabilizing beverages against discoloration and clouding represents an attractive alternative. Since such an enzyme is not yet allowed as a food additive, the use of immobilized laccase might be a suitable method to overcome such legal barriers as in this form it may be classified as technological aid. So laccase could find application in preparation of must and wine and in fruit juice stabilization.

13.6.4.2 Baking Industry

In the bread-making process, laccases affix bread and/or dough-enhancement additives to the bread dough; these result in improved freshness of the bread texture, flavor, and machinability. Laccase is also one of the enzymes used in the baking industry. Oxidizing effect of laccase enzyme in baking process improves the strength of structures in dough and/or baked products. Many characteristics of the baked products including an improved crumb structure, increased softness, and volume are provided by laccase. A poor-quality flour can also be used in this process by using laccase enzyme.

13.6.5 Pharmaceutical Industry

Pharmaceutical industry uses laccase for the synthesis of several products. The first chemical of the pharmaceutical importance that has been prepared using laccase enzyme is actinocin that has been prepared from 4-methyl-3-hydroxyanthranilic acid. This compound has anticancer capability and works by blocking the transcription of DNA from the tumor cell. Another example of the anticancer drugs is vinblastine, which is useful for the treatment of leukemia. The plant *Catharanthus roseus* naturally produces vinblastine. This plant produces small amount of this compound. Katarantine and vindoline are the precursors of this pharmaceutically important compound. Katarantine and vindoline precursors produced in higher quantities are easy to purify. The conversion of these precursors into vinblastine is made through the use of laccase. Through the use of laccase, a 40% conversion of these precursors into the final product is obtained. The preparation of several important compounds with useful properties, like antibiotics, has been possible through the uses of laccase in such conversion reactions (Pilz et al. 2003). Catechins have the antioxidant ability and laccases can oxidize catechins. These catechins consist of small units of tannins, and these are important antioxidants found in tea, herbs, and vegetables. Catechins have the tendency to hunt free radicals, and their property makes them useful in preventing several diseases including cancer, inflammatory, and cardiovascular diseases. The property of less antioxidant ability of catechins can be increased by using laccase which has been resulted in the conversion of catechins into several products with enhanced antioxidant capability. Hormone derivative uses the application of laccase in their synthesis. Intra et al. (2005) and Nicotra et al. (2004) have reported that laccase has the ability to separate innovative dimeric derivatives of the β-estradiol and of the phytoalexin resveratrol. New dimeric derivatives are produced through isoeugenol oxidation coniferyl alcohol and totarol and a mixture of dimeric and tetrameric derivatives (Shiba et al. 2000) respectively, whereas the oxidation of substituted imidazole has resulted in the production of even more complex substances. These new formed imidazoles or oligomerization products (2–4) can be used for pharmacological purposes. Laccase-based oxidation application helps in the conversion of aromatic and aliphatic amines into 3-(3,4-dihydroxyphenyl)propionic acid. The derivatives have the antiviral natural activity and can be used for pharmaceutical purposes.

13.6.6 Biosensors

The laccase of the ectomycorrhizal fungus *Xerocomus chrysenteron* responds to DDT stress in various ways, suggesting a large potential of biodegradation or mineralization of DDT. Biodegradation of 2,4-dichlorophenol using response surface methodology by the laccase of *Pleurotus* sp. is one of the recent applications demonstrated by Bhattacharya and Banerjee (2008). The mechanism(s) of bisphenol A (BPA) to induce cell proliferation and the occurrence of its bioremediation by treatment with laccase are reported by Bolli et al. (2008). BPA, a naturally occurring pollutant that can be used as a model of environmental estrogen action complexity promotes human cancer cell proliferation via ER-alpha-dependent signal transduction pathways. BPA oxidation by laccase impairs the binding of this environmental estrogen to ER-alpha losing all its ER-alpha-dependent effect on cancer cell proliferation. Moreover, the laccase-catalyzed oxidation of BPA reduces the BPA cytotoxic effect (Bolli et al. 2008). Recently, aqueous solutions polluted by BPA have been bioremediated by using laccase (*Trametes versicolor*) immobilized on hydrophobic membranes in non-isothermal bioreactors. Mohamad et al. (2008) described the design of a laccase of *Trametes versicolor* with broader substrate spectrum in bioremediation. Application of evolutionary trace (ET) describes the analysis of laccase at the ligand-binding site for optimal design of the enzyme. In this attempt, class-specific sites from ET analysis were mapped onto known crystal structure of laccase. These findings give a base to the design of laccase which has broader substrate spectrum for further expansion of laccase application in industry and bioremediation.

13.7 Future Possibilities

Laccases (blue copper proteins) catalyze oxidation reactions which are coupled to four-electron reduction of molecular oxygen to water. Because of the versatility of potential substrates, laccases are highly interesting as novel biocatalysts in various industrial processes. This review provided information on distribution and physiological functions of laccases with their biotechnological applications with special reference to bioremediation with recent account. In addition, deeper understanding of the biochemistry of laccase will facilitate the development of novel and more economical laccase applications.

13.8 Conclusion

Laccases are produced through various sources (fungi, bacteria, insects, etc.). They have many industrial applications because of their innate ability of oxidation of a broad range of phenolic and non-phenolic compounds. Laccase is used in drinking industry to improve the quality of drinks and stabilization of some perishable products which are having plant oils. Laccases have the potential for the synthesis of several useful drugs in pharmaceutical industry because of their high value of oxidation potential. Oxidation occurs in laccase which is harmful for industrial products that belongs to those enzymes, which have instinctive properties of immediate radical production. Laccase enzyme has the property to act on a range of substrates and to detoxify a range of pollutants, which has made it to be useful in many industries including paper, pulp, textile, and petrochemical industries.

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Contribution of Lichens in Rectification of Alcohol-Induced Liver Damage: Preventive or Curative

14

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Abstract

Although lichens have a large range of pharmacological activities, even then they are underestimated by many researchers due to their slow growth. Though their artificial culture is slow progressing, soon we will be completely culturing lichens in laboratory and take benefit of the potent secondary metabolites synthesized by them. Lichens possess great potential which needs to be completely investigated. Many recent researches suggest many major ailments like alcohol-induced liver damage can be managed using these lichens or their secondary metabolites. In this chapter we focus on to it. Lichens are highly unexplored with reference to their medicinal properties and pharmacologically active constituents found in them.

Keywords

Lichen · Pharmacological activities · Secondary metabolites · Liver damage · Alcohol

14.1 Introduction

Lichens are nonvascular cryptogams and brilliant examples of symbiotic association. They possess composite thallus comprised of mycobiont and photobiont. The number of species found all over the world is 20,000 (Asplund and Wardle 2017). The association of algae and fungi in lichens is highly selective and has evolved

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through many ages (Aptroot et al. 2015). Along with mosses, they cover major part of ecosystem, around 10% of the earth's terrestrial habitat. Lichens having cyanobacterial symbionts contribute greatly to nitrogen fixation in forest. Lichens have several traditional folk uses, but important uses of lichens include monitoring pollution and in dating of rocks (Zambare and Christopher 2012).

Medicines from the natural origin are being used increasingly in hepatic disorders because of the safety and efficacy of currently used therapy (Vetriselvan et al. 2010). Antioxidant properties are reported in many secondary metabolites of lichens such as polyphenols and terpenoids. These secondary metabolites have been reported to have a significant role in prophylaxis against several diseases. Lichen substances possess a huge variety of biological actions including analgesic, antipyretic, antibiotic, hepatoprotective, antiproliferative and antiviral. Although these varied activities of lichen have been recognized, their pharmacological potential is quite unexplored. Medicinal importance of lichens is due to the presence of a vast range of secondary metabolites produced by them (Boustie and Grube 2005).

Till date structures for more than 1050 diverse lichen substances have been recorded. Lichen substances are secondary metabolites synthesized by lichens. These are of huge variety and often possess unique structures, not found in other fungi or higher plants. Lately, a huge attention has been paid on the biological activities of lichen secondary metabolites. Many of them have been already reported for the activities like antitumor, antiviral, anti-inflammatory, antifungal, antibacterial and antioxidant (Yousuf et al. 2014; Fernández-Moriano et al. 2015). However, most of the lichens tested for the given activities are not edible (no record found stating that lichens are edible), and hence, while utilizing these lichens in food or pharmaceutical industry, special attention has to be paid to this fact. But working with edible lichens is easier that non-edible ones. Also most of the lichen extracts reported for antioxidant activities are not fractionated, and hence the compounds responsible for their activities are not clear (Cardile et al. 2017).

Alcoholic Liver Disease (ALD) is not a single problem; it is a broad-spectrum disorder, covering fatty liver, cirrhosis and alcoholic hepatitis; and in extreme untreated condition, hepatocellular carcinoma (HCC) may also develop. More than 90% of heavy drinkers develop fatty liver, followed by mild steatosis in zone 3 hepatocytes, which also affect zone 2 and even zone 1 hepatocytes under severe liver damage. Although only 30% of heavy drinkers develop severe forms of ALD, e.g. fibrosis and cirrhosis, patients having ALD and heavy alcohol consumption usually develop superimposed alcoholic hepatitis. Alcoholic hepatitis may lead to dangerous complications connected to liver failure, portal hypertension and increased mortality. Present therapy for ALD employs disorder-dependent treatment to achieving long-term abstinence. The medicines used in the symptomatic treatment of alcohol-dependent liver damage cause a large range of side effects. It indicates towards the requirement of newer treatment therapy which overcome ALD.

Lichens are among the important source of novel substances or chemicals with vast range of activities. To meet the increasing demands of newer pharmacologically active compounds, it has forced the pharmaceutical industry to develop newer compounds. For this lichens prove to be the large source of the same as they possess antimutagenic, antioxidant, antiherbivore, antiprotozoal and many other activities (Fernández-Moriano et al. 2016) (Zaini et al. 2017) (Shcherbokova et al. 2015). Although diverse activities of lichens have been recognized, their therapeutic potential remains pharmaceutically unexploited (Shcherbokova et al. 2015). Hence, they can be the potential source of such compounds which can be used in the treatment of alcohol liver disease. The current chapter focuses on the role of lichen in liver ailments chiefly in alcohol-induced liver damage.

14.2 What Happens to Liver on Alcohol Consumption?

Alcohol-induced liver damage is divided into phases depending upon the intensity of consumption (Fig. 14.1). Damage to the liver after short-term consumption of alcohol can be classified as under (Rehm et al. 2017):

1. **Steatosis:** These are fatty changes due to alcohol intake and could be reversed after strict abstinence.
2. **Acute alcoholic hepatitis:** This condition arises due to the consumption of large amounts of alcohol for longer durations. This would involve abnormality in liver functions and development of hepatic encephalopathy.
3. **Cirrhosis:** In the liver with severe injury, normal hepatocytes are replaced by fibrotic tissue and nodules, resulting in cirrhosis. The precise mechanism behind cirrhosis and alcoholic hepatitis is not known yet.

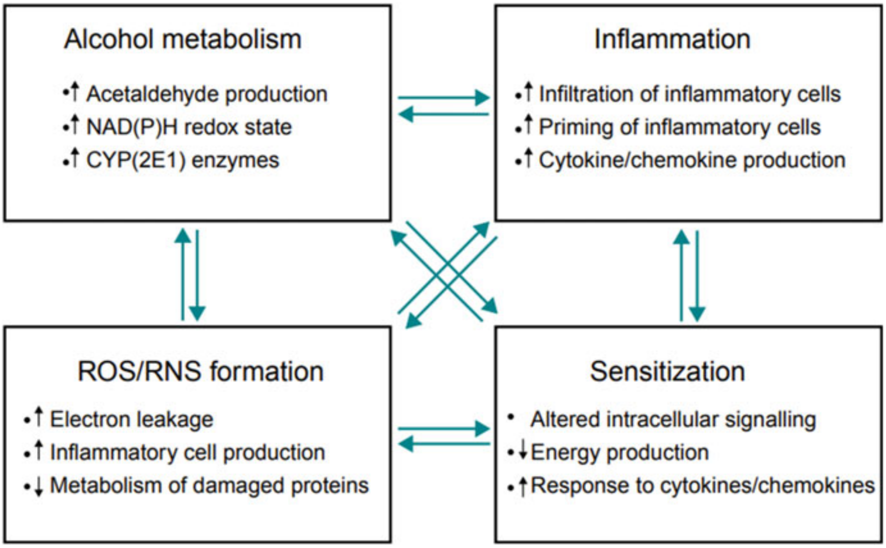

Fig. 14.1 A brief mechanism followed by alcohol liver disease

4. **End-stage ALD:** This stage ends up in death, the reason being hepatic encephalopathy, copious GI bleeding, infections, renal failure and hepatocellular cancer (Lachenmeier et al. 2014).

14.3 Lichens and Their Secondary Metabolites Against Liver Ailments

In current time, lichens are being taken up by many researchers of different fields due to their wide application in pharmaceuticals. Lichens possess a huge range of medicinal properties, e.g. antimicrobial, antioxidant, antiviral, hepatoprotective, anticancer, antigenotoxic, anti-inflammatory, analgesic and antipyretic activities. Lichens and their secondary metabolites have reported a variety of hepatoprotective effects (Table 14.1).

Substances being produced by these lichens are also unique in their nature and properties but again less explored. With increasing understanding and importance of biomolecules, more and more attention is drawn to lichens. Since the lichens can prove to be the promising source of newer chemical entities with pharmaceutical

Table 14.1 Hepatoprotective effect of various species of lichens

S. No.	Lichen	Use in liver ailments
1.	*Cladonia pleurota*	To cool liver (Wang and Qian 2013)
2.	*Usnea diffracta*	Cures fevers of the liver (Clark 1995)
3.	Parmelioid lichens	Cleansing liver (Pieroni 2000)
4.	*Lobaria pulmonaria*	Liver diseases (De Grey 1639; Willemet 1787; Drummond 1861)
5.	*Peltigera canina*	Tonic and medicine for liver complaints (Subramanian and Ramakrishnan 1964)
6.	*Usnea ceratina*	Hepatitis (Wang and Qian 2013
7.	*Evernia divaricata*	Used for hepatitis (Wang and Qian 2013)
8.	*Xanthoria parietina*	Boiled with milk to treat jaundice (Tonning 1769)
9.	*Cladina rangiferina*	Jaundice (Wang and Qian 2013)
10.	*Usnea barbata*	Jaundice (Lightfoot 1777; Willemet 1787; Luyken 1809)
11.	*Usnea pectinata*	Jaundice (Luyken 1809)
12.	*Parmelia saxatilis*	Jaundice (Wang and Qian 2013)
13.	*Ramalina* spp.	Mixed with honey to treat jaundice (Bostock and Riley 1855; Yavuz 2013)
14.	*Peltigera canina*	Used for jaundice (Biswas 1956) and China (Wang and Qian 2013)
15.	*Polycauliona candelaria*	Boiled with milk to treat jaundice (Tonning 1769)
16.	*Parmelia perlata*	Hepatic dysfunction (Shailajan et al. 2014)
17.	*Usnea ghattensis*	Prevent oxidative liver damage (Verma et al. 2008)

importance, they can play a pivotal role in drug discovery (Zambare and Christopher 2012).

Knowledge of the medicinal uses of lichens is found due to the contribution of traditional people in these cultures. Different cultures emphasize different lichen genera, *Usnea* being the most common among them (Bates et al. 2011). Scientific taxonomy didn't match with folk taxonomy, which reflected the cultural values of the given lichens and traditional methods of identifying them (Lutzoni et al. 2001). In traditional medicines, lichens have been reported for various medicinal uses, e.g. for treating wounds, in skin disorders, in digestive and respiratory ailments and also in gynaecological and obstetric issues. They have been utilized for their storage carbohydrate as well as for their secondary metabolites. The secondary metabolites of lichens are highly stable. Even very old specimens of lichens in herbarium show negligible decrease in amount of lichen substances (Rundel 1978).

A number of secondary metabolites found in lichens possess liver-protecting activities. The diffractaic acid has been reported to be active against acute liver toxicity induced by CCl_4 (Karagoz et al. 2015). Atranorin is a secondary metabolite found in *Stereocaulon caespitosum* and has been reported for its tumour-suppressive activity in hepatocellular carcinoma (HCC). Atranorin accomplished this action by regulating the cell cycle, cell death and metastatic potential (Jeon et al. 2019). Sulphated polysaccharide α-D-glucan ($Glu.SO_4$) extracted from *R. celastri* was evaluated against liver granuloma. Treatment produced a statistically significant reduction in the number of granulomas, by 62% and 63%, respectively (Araújo et al. 2011). A pharmaceutical preparation possessing ramalin or one of its salts, which can be utilized as functional food for the management of liver disease, could inhibit liver cirrhosis (Yim et al. 2013).

14.4 Some Lichens Proved Efficient in Management of Alcohol-Induced Liver Damage

14.4.1 *Cladonia rangiferina*

It is a light-coloured fruticose lichen belonging to family Cladoniaceae (Fig. 14.2). It is also called as reindeer lichen or reindeer moss or caribou moss (but it's not moss and hence these names could be misleading). It can grow well in both hot and cold climates, provided the environment is open and well drained. It is extremely cold hardy and found in alpine tundra (Piercey-Normore 2004). Synonyms for this lichen are *Cladina rangiferina* and *Lichen rangiferinus*. It is a slow-growing lichen (3–5 mm/year) and takes decades to grow back again if overgrazed, burned or otherwise consumed (Kosanić et al. 2014).

Rigorous research is under process to identify newer compounds from lichens. One such study has reported two novel abietane diterpenoids in *C. rangiferina*, being recognized as hanagokenols A and B. This study also reported 15 known compounds from the same lichen, namely, sugiol, dehydrosugiol, junicedric acid,

Fig. 14.2 Image showing *Cladonia rangiferina*

ontuanhydride, montbretol, ciscommunic acid, atronol, homosekikaic acid, β-resorylic acid, 15-acetylimbricatoloic acid, didymic acid, imbricatolic acid, 7-α-hydroxysandaracopimaric acid, barbatic acid and condidymic acid. These compounds fall in either of these groups of compounds abietanes, dibenzofuran, diterpenoids, monocyclic aromatic compound, depsides and labdanes. Some of these reported compounds possess slight inhibitory properties against *Staphylococcus aureus* (methicillin resistant) and *Enterococcus* (vancomycin resistant) (Yoshikawa et al. 2008). Exposure to UV-B radiation stimulates the buildup of usnic acid and melanic compounds (Lafleur et al. 2016).

In the past, Monpa (the alpine region) of west Kameng district of Eastern Himalaya used this lichen in treating kidney stones. A study published in May 2011 reported that some lichen species including the *Cladonia rangiferina* can degrade the noxious prion by the enzyme serine protease (Gómez-Guzmán et al. 2011).

Recently a study reported *Cladonia rangiferina* (CR) to be helpful in the treatment of alcohol-induced hepatotoxicity and oxidative stress. The ameliorative action of CR in alcoholic liver damage may exist due to its antioxidant, anti-inflammatory and anti-apoptotic activities. Considerable evidence suggested that TNF-α and IL-6 contribute to the pathogenesis of liver inflammatory diseases by activating the NF-κB signaling pathway (Nanji et al. 1999). This lichen treatment corrected the disturbed levels of inflammatory mediators and brought them back to near normal (Shukla 2019).

14.4.2 *Flavoparmelia caperata*

It is a foliose lichen, usually called as common greenshield lichen. It has a very distinguishing pale yellow green upper cortex, when it's dry (Fig. 14.3). Generally it has patches of granular soredia arising from pustules. It has rounded lobes, measuring about 3–8 mm (0.1–0.3 in). In older specimens, the lobes are often wrinkled in appearance, but fresh specimens have smooth thallus. Its lower surface is black in colour with brown margin. Rhizomes found attached on the lower surface are also black coloured and unbranched. This lichen is found on trees, shrubs and fences in open areas and rarely on rocks. Few traditional uses of *F. caperata* include treating intestinal worms and application of its dried thallus on burns. This species also found its use in dyeing industry used to dyeing wools in Man Island. It also acts as a bio-indicator in determining levels of atmospheric pollution (Majumder et al. 2013).

The caspases were appreciably augmented in the case of alcohol consumption. This lichen treatment considerably reduced their activities. It also exerted its hepatoprotective potential by acting upon oxidative stress markers. Treatment with *Flavoparmelia caperata* significantly increased the reduced levels of glutathione and glutathione reductase in albino rats (Shukla 2019).

14.4.3 *Ramalina conduplicans*

It is an edible and fruticose lichen (Fig. 14.4) species usually found in central and southeastern Asian countries. Chinese people in the southwestern region cook lichen to prepare a traditional cold dish that is served in marriages, or they use it in fried pork (Luo et al. 2010). Rai and Limbu communities of East Nepal traditionally use it as food. In India it is used as spice at several places. A study in the past has reported about the presence of plenty of trace elements in this lichen, and hence it has a great

Fig. 14.3 Image showing *Flavoparmelia caperata*

Fig. 14.4 Image showing *Ramalina conduplicans*

nutritional value. However, the therapeutic uses of this lichen have not been evaluated before (Oh et al. 2006).

The effect of the lichen has been reported upon serum marker enzymes against ethanol-induced hepatotoxicity in rats. It restored the reduced levels of hepatic biochemical markers like AST, ALT and ALP. It also improved the levels of PON1 which are degraded by alcohol. It also corrected the levels of myeloperoxidase, which elevates due to alcohol consumption (Shukla 2019).

14.5 Conclusion

As lichens have both fungal and algal cells in the thallus as well as various sources of entophytes, these are a great source of secondary metabolites, and most of them are unique. They have chemically aliphatic and aromatic substances with relatively low molecular weight. More than 1000 secondary compounds have been identified to date. Traditionally many of the lichens have been used to treat a number of ailments. Hence these are the promising candidates for futuristic pharmacological research.

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Microbial Production and Applications of L-lysine

15

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Abstract

L-lysine is one of the nine essential amino acids and important for human and animal growth. L-lysine constitutes a crucial part of a billion dollar animal feed industry and represents the fastest growing amino acid segment. The global market for L-lysine has increased almost 20 times in the past 20 years. As a supplement in cereals, it forms a balanced feed for animals including poultry and other livestock. It is used in pharmaceuticals, cosmetics, dietary and feed supplement. L-lysine is used in overcoming *Angina pectoris* as key factor to clean arteries and also in cancer prevention. It is important for adequate calcium absorption and hence for maintaining bone health. It is also an integral component of musculature and has a role in antibody production. L-lysine, along with arginine, is shown to have a role in collagen synthesis and also the highest proportion of these two amino acids has been reported in histone nucleoprotein. Soybean meal, malt extract, corn steep liquor and molasses are rich source of L-lysine and are generally used as substrates for the industrial production of the amino acid. Various species like *Corynebacterium glutamicum*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*, *Corynebacterium lilium*, *Brevibacterium divaricatum* and *Escherichia coli* are used for industrial production of L-lysine.

More than 2.2 million tonnes of lysine salts are produced annually worldwide and the demands have been continuously increasing in recent years. Ajinomoto Co. Inc., Archer Daniels Midland Co. and Global Bio-chem Technology Group Company Ltd. are some of the leading manufacturers of L-lysine.

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Keywords

L-lysine · Amino acids · Fermentation · Microorganisms

15.1 Introduction

L-lysine is one of the nine amino acids essential for human and animal nutrition in adequate amounts. It is an essential amino acid which constitutes the basic building block of almost all proteins. L-lysine is preponderantly used as an additive in animal feed and its first isolation dates back to 1889 from casein. The demand of this amino acid has been firmly increasing in recent years. Globally more than 2.2 million tons of lysine salts are being produced by microbial fermentation annually. It is a polar amino acid commonly found on surfaces of proteins and enzymes with net positive charge at physiological pH values making it one of the three basic (with respect to charge) amino acid. On a molecular basis is incorporated into proteins at a rate of 7% as compared to other amino acids.

L-lysine assists in calcium absorption and is important for proper bone development and growth in children. In adults, it maintains a suitable nitrogen balance in the body. It also aids in the production of antibodies, enzymes and hormones and provides assistance in collagen formation and tissue repair. L-lysine is helpful for patients recovering from injuries and postoperation effects since it aids in building muscle protein and in maintaining healthy blood vessels. It also has been reported to assist in combating herpes and cold sores.

Industrially, strains of *Escherichia coli* and *Corynebacterium glutamicum* are being used as major lysine producers with their respective advantages (Van Dien et al. 2006). Considering the potential safety issues of using genetically engineered *E.coli* strains, the lysine product manufactured from such strains has been now confirmed to be safe (Efsa 2015). The strain employed for industrial fermentation is the key administrator of the entire process as it largely affects the economic and environmental performance of the entire biotechnological process. Based on the existing available knowledge of the genetic makeup, the metabolic engineering of L-lysine-producing *E.coli* strains has been broadly applied for several decades (Van Dien et al. 2006; De Graaf et al. 2001; Ikeda et al. 2006). To date, the best reported *E. coli* strain produced lysine at a concentration of 134.9 g/L, a yield of 45.4 % (lysine/glucose, w/w) and a productivity of 1.9 g/(L.h) (Ying et al. 2014). The maximum theoretical lysine yield by the *E. coli* strain is about 68.2 % (lysine/glucose, w/w) (Van Dien et al. 2006). Hence, the current lysine-producing *E. coli* strains are far from optimal stressing on the fact that our knowledge on metabolism and regulation of the *E. coli* strains is still deficient (Karp et al. 2007; Fu et al. 2011), making it arduous to further accurately optimize the high lysine-producing industrial strains by pure rational metabolic engineering.

Over the years, increased interests in amino acids production have resulted in development of wide variety of technologies. Kikunae and Ikeda, in 1907 at the Tokyo Imperial University, initiated his experiments by identifying and purifying the principle responsible for flavour enhancing in seaweed Konbu (*Laminaria japonica*). He discovered that the extract contained monosodium glutamate (MSG) (Kurihara 2009). Shortly after his discovery, Ajinomoto Co. extracted MSG from defatted soybean or acid-hydrolysed wheat gluten and sold it as flavour enhancer (Sano 2009). Kikunae and Ikeda, for the amino acid production industry, provided the bases and are considered as father of MSG. The development in applications of amino acids in various industries, such as feed supplements, pharmaceutical, food additives, polymer materials, cosmetics and agricultural chemicals, has led to rapid increase in the production of amino acid. Indeed, the total amino acids market in 2008 was estimated to be around USD 5.4 billion (März 2009) which is expected to worth over USD 9.4 billion by 2018 (Global Industry Analysis 2012–2018. 2013). However, the production of amino acids by industrial processes still needs to be optimized. Due to this reason, many academic institutions (Kumagai 2013) and companies (Kim 2010) began their research with the aim of finding better sustainable routes and cost-effective ways for amino acids production. Within a few years after the report of the L-glutamate fermentation, the company also found that a homoserine-auxotrophic mutant of *C. glutamicum* produced a large amount of L-lysine in a medium (Nakayama et al. 1961), which enabled industrial production of L-lysine by fermentation.

15.2 Amino Acid Production

The production process of amino acids is mainly classified as (i) extraction, (ii) chemical synthesis and (iii) microbial methods. The microbial methods of production are further subclassified into fermentation and enzymatic synthesis. The suitability and advantages of each method varies and depends majorly upon factors like availability of raw material, process economics, environmental regulation and market sizes. Thus the method to be adopted depends upon the type of amino acid to be produced.

The use of extraction method alone for amino acid production proves to be tedious because it depends upon the availability of natural protein-rich resources such as keratin, hair, feather or soybean. Therefore, this method is used in conjunction with other methods. Chemical synthesis produces D, L-forms of amino acids and hence to obtain biologically active L-isomers, an additional optical resolution step which needs to be incorporated which increases the production costs. Due to this reason only a few amino acids are being manufactured by this method commercially. The enzymatic method projects the advantage of producing optically pure higher concentrations of L and D amino acids with fewer by-products resulting in simpler and economic downstream processing. Various substrates have resulted in variety of enzymatic routes which are being developed to yield optically pure amino acids (Esaki et al. 1996). The commercial competitiveness of enzymatic method mainly depends upon factors such as the cost of the fermentation yield, purification yield, carbon source and productivity in the overall process. The improvement in the continuous process has resulted in the economic feasibility of the existing fermentation method, especially for large-scale productions of monosodium glutamate and L-lysine hydrochloride salt (L-lysine HCl).

15.2.1 Chemical Synthesis

The first amino acid synthesis is quoted back to 1850 by a German chemist Adolph Strecker and is known as Strecker synthesis. In this reaction, an aldehyde or ketone and an amine or ammonia react to get converted into α-amino acids in presence of metal cyanide, an acid catalyst and water (Strecker 1850). The major drawbacks of chemical synthesis are associated with the cost of catalyst and the hazardous effects of metal cyanide used (Zuend et al. 2009). Also, due to lack of enantioselectivity, Strecker synthesis can produce only a mixture of D- and L-amino acids (Gröger 2003). In order to overcome these shortcomings, Harada et al. in 1963 introduced a catalytic asymmetric Strecker-type reaction which involves the use of a chiral amino-thiourea catalyst compatible with the safer aqueous cyanide salts (Zuend et al. 2009). Moreover, such catalysts controlling hydrocyanation steps selectively increase the synthesis of particular enantiomeric form. Example of chemical synthesis is the ammonolysis of trichloroethylene to glycine (Inoue and Enomoto 1982). The most widely used chemical synthesis process for production of racemic amino acids in industries is Bucherer-Bergs method, a modification of the Strecker synthesis. In Bucherer-Bergs method, aldehydes or ketones react with sodium cyanide and ammonium carbonate to produce hydantoins, followed by hydrolysis of racemic amino acid mixture in a basic medium (Breuer et al. 2004). Biochemical methods are used to convert racemic mixtures into desired target compounds. For example, in order to obtain only the desired L-form, an additional optical resolution step is required, which may include either chemical process using chemical substances or enzymatic process using an enzyme such as from *Aspergillus fumigatus* (Singh et al. 2014). The drawbacks of Bucherer-Bergs method are the elevated temperatures and long reaction times (Breuer et al. 2004).

15.2.2 Microbial Pathways

Biological processes such as enzymatically catalysed synthesis and fermentation comprise the final route of amino acid production. Several enzymes or mixture of enzymes such as hydrolytic enzymes, NAD^+-dependent L-amino acid dehydrogenases, and ammonia lyases have been used for catalysing the production process (Pollegioni and Servi 2012). Microorganisms such as *Corynebacterium glutamicum*, *Escherichia coli*, *Pseudomonas dacunhae*, *Cryptococcus laurentii* and *Saccharomyces cerevisiae* are some of the sources for these enzymes. The production of higher concentrations of optically pure L- and D-amino acids and lesser by-products are some advantages of enzymatic method (Ikeda and Nakagawa 2003). Ramakrishnan et al. (2013) analysed the reaction time effect on the hydrolysis process. The highest amino acids yield was obtained in reaction time of 48 h with a combination of Alcalase and Neutrase enzymes. The main drawbacks include the limited stability and high cost of enzymes (Ikeda and Nakagawa 2003). Therefore, techniques for immobilization of biocatalysts have been developed to increase the performance and economic feasibility of the process. Fermentation route is currently the basis for amino acid production at industrial scale. Among the various advantages of this process is the production of only L-form of amino acids thus eliminating the need of further purification steps. The use of mild operating conditions reduces the chances of product degradation, and also maintenance costs are lower as compared to the extraction processes (Ugimoto 2010). Some drawbacks, on the other side, include high capital and operation costs due to the requirement of sterility and high energy consumption for oxygen transfer (for aerobic fermentations) and water addition as well as mixing. Also, large reactor requirements lead to higher capital investment as compared to the other production methods (Ivanov et al. 2013). Several criteria such as raw materials, available technologies, operation costs and capital investments, market size of the product and revenues as well as impact of each unit process on the environment are considered while selecting the best method for amino acid production. However, fermentation, due to its economic and environmental advantages, is currently the most widely used process for amino acid production at industrial scale (Ikeda and Nakagawa 2003). The pathway for the biosynthesis of L-lysine is shown in Fig. 15.2.

15.3 Fermentation Processes for L-lysine Production

15.3.1 Batch Production

Industrial fermentation processes have been developed for the very large-scale production of amino acids. On commercial level, airlift tank fermenters or aerated agitated tank fermenters in 50–500 kl size range are generally used for carrying out fermentation. The size of fermenters is gradually increased to fulfil the demands of amino acids reducing the cost. Figure 15.1 shows a process flow diagram of L-lysine amino acid fermentation.

The first seed tank (100–200 L size) is fed with the inoculum culture grown in a flask. After the appropriate growth of cells to a desired level, the first seed tank culture is transferred to the second seed tank (1000–2000 L size) which in turn provides the inoculum for the main tank fermenter. These seed steps are crucial to ensure highest fermentation yield within shortest possible time and better reproducibility of results. The cells activity and inoculum size often influences the growth rate and productivity in the fermentation. Therefore, strict and consistent preparation of the culture to maintain it in optimal condition for inoculation must be assured at each seed step.

Parameters such as aeration, tank pressure, agitation, substrate feeding rate (sugar), temperature and pH affects the production yield in the fermenter. Production scale-up to plant scale is primarily dependant on parameters such as aeration rate,

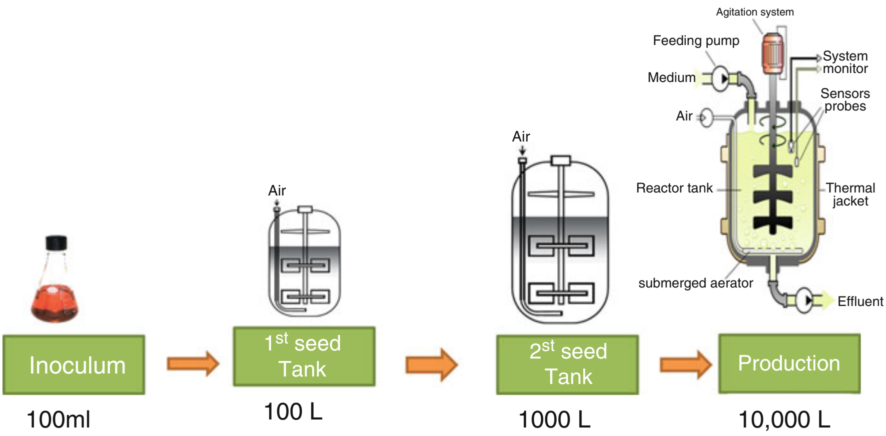


Fig. 15.1 Inoculum development for the production of L-lysine at industrial scale

oxygen transfer coefficient (kLa), mixing time, stirrer tip speed and power input (Sikyta 1983). However, production yields on an industrial scale often do not reflect the laboratory results mainly due to the substantial heterogeneity within the fermenters as a result of lower mixing efficiency and increase in the scale of operation (Buckland and Lilly 1993). Fed sugar and oxygen distribution, in specific, could affect cell physiology triggering undesirable stress responses such as switching biosynthesis from a desired amino acid to undesirable by-products like carbon dioxide, acids, and biomass. Thus, for the success of a scale-up, interactions among chemical and physical environments in the culture, culture conditions, microbial physiology as well as fluid dynamics in fermenters should be properly understood. Scale-down fermenters has been developed which can imitate the heterogeneity in a full-scale fermenter, and also the response of microorganisms to changes in ambient conditions are analysed. Recently, another technique to study the effects of poor mixing on amino acid fermentation in industrial scale has been proposed (Schilling et al. 1999). The study showed a decrease in L-lysine production through less enzyme activities due to inhomogeneous mixing involved in L-lysine biosynthesis.

15.3.2 Fed-Batch Production

Fed-batch operation is the most common mode used in the amino acid industry (Ikeda and Nakagawa 2003). Here only a small amount of medium and inoculum are required initially to start the process. The carbon source fed in the reactor is developed following a predefined feed profile in order to acquire higher yield. Both cells and product remain in the reactor, and no effluent is taken out during the process. The necessary nutrients like biotin and vitamins are fed at the starting with the inoculum (Hermann 2003). Additional nutrients are added either intermittently or continuously during cultivation until an optimal yield of product is obtained. The configuration is set up in a manner so as to satisfy the oxygen demand preventing oxygen limitation and consequent formation of undesired by-products resulting in an increased yield and productivity (Hermann 2003).

It has been demonstrated that the fed batch process outcompetes batch configuration with a higher productivity and reproducibility despite of the simple process control in case of batch processing (Longobardi 1994). The important rationale of fed-batch operations are not only the aforementioned increased process performance but also the increased process reproducibility and the reduced inhibition risk due to the high carbon content at the beginning of the fermentation (Gnoth et al. 2007).

The ability to control the concentration of nutrients in the culture is an important characteristic of the fed-batch process (Yamanè and Shimizu 1984). Despite of the easy process control and no requirement of an additional tank for nutrient feeding in case of batch processes, industrial processes prefer fed-batch systems. This preference can be attributed to several advantages of fed-batch process.

In most of the amino acid fermentation processes, high amounts of sugar (20% or more) are used in one run of cultivation in order to get high batch yields. Such high initial concentrations sometimes inhibit the growth of microorganisms, reduces yield and results in the production of lactate and acetate like by-products. Decreasing the initial concentration and inducing subsequent feeding shorten the total culture period (especially lag time) and also enhance the yield in some cases.

In case of auxotrophic strains, production yield is decreased due to excess nutrient supply. This happens because of the overgrowth of cells or feedback regulation by nutrients. It can be overcome by growing the auxotrophic strain in a controlled nutrient supply. Examples include L-tryptophan fermentation by an L-phenylalanine and L-tyrosine auxotrophic strain of *C. glutamicum* (Ikeda and Katsumata 1999).

In fed-batch technique, oxygen limitation can be prevented by lowering the substrate concentration. Glucose-limited fed-batch cultures are commonly used in industrial processes. Further productivity can be increased by drawing out some broth, being refilled by nutrient feeding. However, economic aspects should be well considered initially as prolonged culture leading to maximum yield is often uneconomical.

15.3.3 Continuous Production

Here, a constant culture volume is maintained by feeding fresh medium to fermenter at a specific rate, and same broth quantity with some microorganisms is continuously withdrawn from the fermenter. This is generally performed either by the chemostat method with nutritional limitations such as phosphate-limited and glucose-limited cultures or by the turbidostat where the feeding and removing rates are controlled so that the cell mass concentration in the culture is maintained. At steady state the concentration of each component becomes constant and cells grow in such conditions. In a continuous fermentation process, cultivation is started in a batch or fed-batch manner and is shifted to continuous culture when productivity per unit time becomes relatively high. This process reduces production costs and capital investments as there is no requirement of new fermenters and also increases overall yield and productivity. Most research done on continuous fermentation processes have been related to cell culture or production of alcohol and organic acids (Aeschlimann et al. 1990), and only a few studies were focussed on amino acid fermentation (Fujimura et al. 1984; Azuma et al. 1988; Hirao et al. 1989). The continuous cultures which are limited in carbon and phosphate are favourable as they allow a strong control of the process, reducing the risk of contamination (Hermann 2003). Continuous sterilizers are often used to sterilize the medium which when used with some salts and nutrient combinations project the risk of blockage.

Hirao et al. (1989) investigated the continuous fermentation of L-lysine by *C. glutamicum* B-6, an L-lysine-producing mutant. The strain showed stable L-lysine production for more than 300 h with the maximum values of L-lysine HCl concentration and volumetric productivity of 105 g l^{-1} and 5.6 g l^{-1} per hour, respectively. While strain B-6 had the ability to produce 100 g l^{-1} L-lysine HCl within 48 h in a fed-batch process, the productivity did not exceed 2.1 g l^{-1} per hour. It was concluded that the productivity of the continuous fermentation was more than 2.5-fold higher than that of the fed-batch culture. Despite the definite increase in productivity, the application of continuous systems to industrial fermentation is very limited due to the mentioned reasons:

- At industrial scale, it is tedious to maintain sterility of fresh media and air which are to be fed continuously. This causes difficulty in maintaining culture purity over a long term.
- In nutrition-limited chemostat culture, spontaneous mutations occur in microorganisms over long-time duration. Growth rate of such mutants is faster than their wild-type counterparts in the culture resulting in subsequent reduction in the productivity. This problem has been exemplified in the continuous fermentation of L-arginine by *Corynebacterium acetoacidophilum* (Azuma and Nakanishi 1988).

15.4 L-lysine-Producing Microorganisms

The most common bacterium used for L-lysine production via fermentation is *C. glutamicum.* (Ramakrishnan et al. 2013). *C. glutamicum* has been used to produce a wide range of L-lysine, and various alterations using metabolic engineering have been applied to enhance their L-lysine-producing performance. Genetically modified *C. glutamicum* is used to produce lysine or glutamic acid (Becker et al. 2011) with high yields (up to 50 % w w^{-22121}) (Aoki et al. 2005). The various microorganisms used for the production of L-lysine are given in Table 15.1 along with the culture conditions and titre values.

Table 15.1 Microorganisms used for the production of L-lysine

Strain	Culture condition	Titre gL^{-1}	Reference
C. glutamicum B-6	Molasses, 32 °C, 48 h	100	Hermann (2003)
C. glutamicum H-8241	Sucrose 10%	48	Nakano et al. (1993)
C. glutamicum	W/V, 32 °C, 72 h	60	Sassi et al. (1996)
C. glutamicum MH20–22B/pJC23	Glucose 18% W/V, 27 °C, 70 h	50	Eggeling et al. (1998)
B. lactofermentum AJ12592	Glucose 10% W/V, 32 °C	11.8	Yokomori et al. (1994)
B. lactofermentum AJ12937	Glucose 3.6% W/V, 31.5 °C, 48 h	120.5	Shiratsuchi et al. (1995)
B. lactofermentum AJ11082/pSSM30BS	Glucose, 31.5 °C, 58 h	95	Sugimoto et al. (1996)
B. lactofermentum AJ11082/pCL and pPwm	Sucrose, 31.5 °C, 35 h	45.5	Hayakawa et al. (1998)
B. lactofermentum AJ11082/pCABL and pORF1	Glucose 10% W/V, 31.5 °C, 72 h	48.8	Araki et al. (1998)
B. lactofermentum AJ3990/pHSG::THYB	Glucose 10% W/V, 31.5 °C, 72 h	14.5	Kojima and Totsuka (1995)
C. thermoaminogenes AJ12521	Glucose 3.6% W/V, 31.5 °C, 72 h	3.0	Murakami et al. (1992)
E. coli W3110*tyrA*/pCABD2	Glucose 10% W/V, 43 °C, 72 h	12.23	Kojima et al. (1999)
Bacillus methanolicus NOA2#13A52–8A66	Glucose 4%, 37 °C, 30 h Methanol, 50 °C, 60 h	47	Lee et al. (1996)

15.4.1 *Corynebacterium glutamicum*

C. glutamicum is Gram-positive aerobic non-pathogenic soil bacterium widely used in the amino acid production industry; *C. glutamicum* belongs to the order *Actinomycetales*, which also include *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Rhodococcus* and other related microorganisms. Production of more than 1.5 million tons of L-glutamic acid and more than 600,000 tons of L-lysine have been reported. It is expected that the demand for amino acid production will further increase in the coming times (Hermann 2003). To achieve the efficient production of amino acids like L-lysine, genetic modification has been carried out over a few decades (De Graaf et al. 2001). *C. glutamicum* is known to efficiently produce various organic acids and ethanol; the bacteria prefer glucose as carbon source (Eggeling and Reyes 2005) but can also utilize sucrose, fructose, ribose, mannose and maltose (Zahoor et al. 2012). Its optimal growth conditions are at a temperature of 30 °C to 35 °C and a pH of 7 (Eggeling and Reyes 2005).

15.4.2 Central Carbon Metabolism of *Corynebacterium glutamicum*

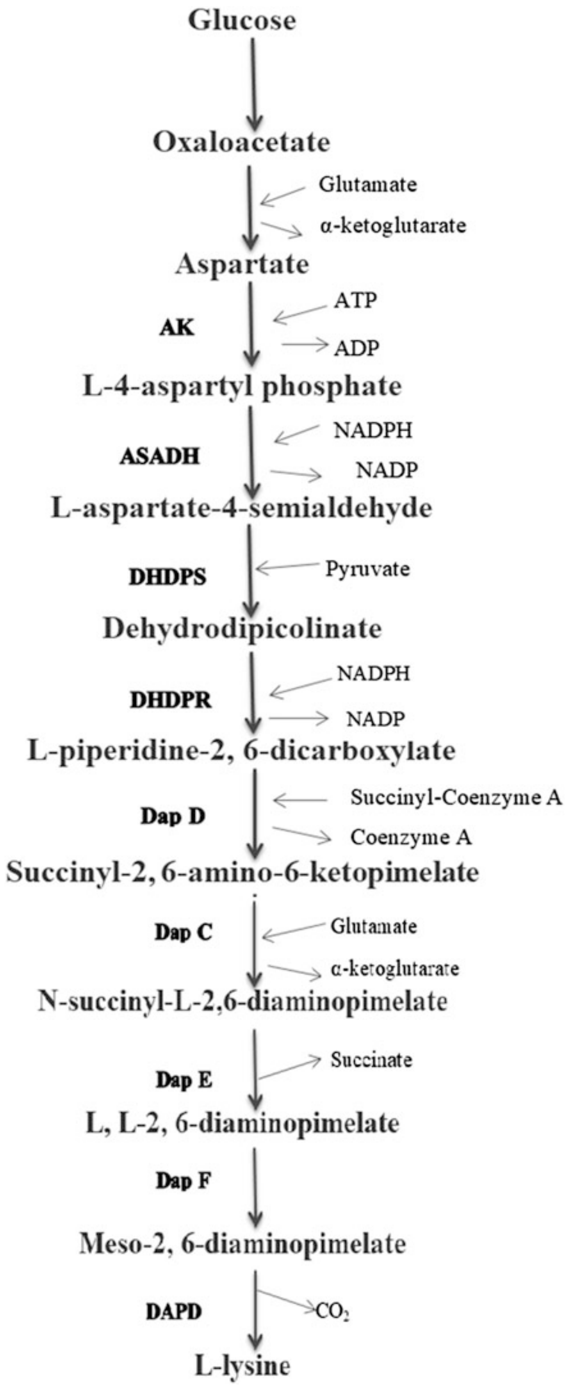
Embden-Meyerhof-Parnas pathway (glycolysis), the pentose phosphate pathway (PPP) and the tricarboxylic acid (TCA) cycle are the three main pathways identified (Ikeda and Nakagawa 2003; Watanabe et al. 2013). Enzymes such as 6-phosphogluconatedehydrogenase and isocitrate dehydrogenase are involved in the conversion of carbon between TCA and glycolysis (Watanabe et al. 2013).In glycolysis, glucose is converted into pyruvate and energy carriers such as ATP and NADH. The phosphoenolpyruvate (PEP)-pyruvate oxaloacetate node, PEP and pyruvate, end products of glycolysis, enter into the TCA cycle (Watanabe et al. 2013). During the catabolic phase, the degradation of sugar forms acetyl CoA which is oxidized through several steps in CO_2, simultaneously generating NADH (Bott 2007). Besides intermediates like succinyl-CoA, the TCA cycle produces two precursors of glutamate and aspartate family of amino acids, namely, 2-oxoglutarate and oxaloacetate. The PPP provides NADPH along with ribose-5-phosphate and erythrose-4-phosphate which are required for biosynthesis of building blocks (Stincone et al. 2015). In addition, vitamins and cell wall constituents essential for amino acid production are also produced.

Lysine is produced converting its precursor oxaloacetate to aspartate with the aid of the aspB gene product (Wittmann and Becker 2007). Aspartate kinase enzyme catalyses the phosphorylation of aspartate to L-4-aspartyl phosphate, which is in turn converted to L-aspartate-4-semialdehyde in the presence of aspartate semialdehyde dehydrogenase (ASADH). By reacting with pyruvate, L-aspartate-4-semialdehyde is further converted to dihydrodipicolinate in presence of dihydrodipicolinate synthase (DHDPS). Reducing agent NADPH and the enzyme dihydrodipicolinate reductase (DHDPR) result in the formation of L-piperidine-2,6-dicarboxylate. L-piperidine-2,6-dicarboxylate can be directly converted to meso-2,6-diaminopimelate adding an amino group, process called dehydrogenase variant, catalysed by diaminopimelate dehydrogenase (DAPDH) or by the succinyl variant where several reactions are involved. Finally, the enzyme diaminopimelate decarboxylase (DAPDC) catalyses the decarboxylation of meso-2,6-diaminopimelate to lysine (Fig. 15.2)

15.5 Substrates for L-lysine Production

In case of amino acid fermentation, the carbon source forms the major raw material as it is required for developing the structural frames of amino acids and also as energy source for microorganisms. Hence, the selection of a suitable carbon source is of primary importance. Cane molasses, beet molasses and starch hydrolysates (glucose) from corn and cassava are widely used for the industrial production of amino acids. Preference of the carbon source depends upon the location of the plants, costs and availability in concerned regions. For example, starch hydrolysate from corn, corn syrup, is the usual carbon source in the United States, while cane molasses is preferred in Europe and beet molasses are used in South America. In South Asia, tapioca hydrolysate is favourably used for amino acid fermentation. Inorganic

Fig. 15.2 Biosynthesis pathway of L-lysine in *Corynebacterium glutamicum*



nitrogen sources such as ammonia and ammonium sulphate are generally the preferred nitrogen sources. Nitrogen can be supplied both by ammonium salts present in the initial medium or by controlling pH with ammonia. Adequate concentrations of phosphate, magnesium, and other minerals and vitamins are also required for normal cell metabolism during fermentation. Inexpensive natural nutrients of plant and animal origin are industrially used as the sources of minerals and vitamins. When natural materials such as corn steep liquor are used in industrial processes, careful optimization of culture conditions like medium composition is required because the components in such natural materials vary in quality and quantity with different lots. Another crucial aspect to be considered for process stability is the achievement of a reproducible sterilization of media. The heating often brings about vague changes in the media due to chemical reactions like the Maillard reaction or decomposition of essential nutrients necessary for the growth of microorganisms. Thus, for an optimum scale-up with respect to sterilization conditions, it is important to ensure both sterility and the retention of the full nutritional value of the media by considering the contradictory effects of lethality and medium quality. From these viewpoints, continuous sterilization is in general easier for scale-up than batch sterilization.

15.6 Process Design for L-lysine Production

In a fermentation process, there is a necessity to control and monitor crucial parameters and process variables such as quality of inoculum, pH, feed rate, aeration intensity and process temperature (Scheper and Lammers 1994). The key step in a bioprocess is the inoculum preparation as it could specifically affect the productivity and yield (Ikeda and Nakagawa 2003). Therefore it is required to thoroughly test the stability and productivity of inoculum before its transfer to the main fermenter. Sterility maintenance is also an important task to be accomplished throughout the process. Integration of continuous sterilization systems into classical fermenters ensures aseptic conditions during the entire process (Junker et al. 2006). It has been demonstrated that the oxygen transfer rate (OTR) also influences the productivity based on the biochemical characteristics of the amino acids (Villadsen et al. 2011). Temperature control is also an important aspect. It has been reported that in the case of *C. glutamicum*, a high temperature of up to 41 °C increases the productivity of L-glutamic acid (Delaunay et al. 2002). Investigations are being done on some thermo-tolerant bacteria like *Bacillus methanolicus* for the production of L-lysine and L-glutamate at temperatures up to 50 °C (Brautaset et al. 2007). Additionally, a higher working temperature provides an advantage of less cooling water requirement.

Downstream Separation and Purification of L-lysine

Industrially, two methods are generally used for the recovery of amino acids which include either conventional chromatographic method or concentration-crystallization method. In the conventional chromatographic method, after the

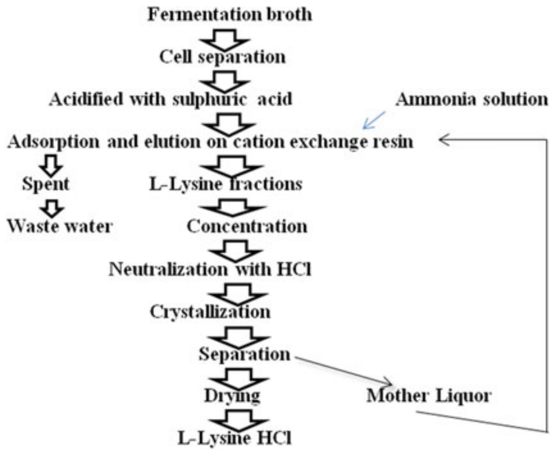


Fig. 15.3 Process flow diagram for recovery and purification of L-lysine

removal of cell mass by centrifugation or ultrafiltration, an ion exchange resin is used to purify a desired amino acid, followed by crystallization or spray drying. In concentration-crystallization method, cell mass is removed, and the filtrate is merely concentrated and crystallized.

The various steps involved in the recovery and purification of L-lysine HCl from fermentation broth is shown in Fig. 15.3. The chromatographic purification method has an advantage over concentration-crystallization method that it results in a higher quality of product but its drawback includes the generation of large volumes of waste liquor increasing the waste liquor treatment cost. Thus, the concentration-crystallization method is advantageous from the economic aspect. The selection of the recovery process depends on many factors like the grade of the product, the chemical characteristics of the amino acid itself, the composition of the fermentation broth, the raw materials used, the environmental regulation around the location of the plant and many others. The discharged mother liquors can be utilized as fertilizers due to the large amount of plant growth-promoting factors present in them. In Kyowa Hakko Kogyo Co., the mother liquor from amino acid fermentation is efficiently utilized for the manufacture of organic fertilizers aiding in resource recycling and environment protection. This closed cycle system process makes the cost of waste-liquor treatment tolerable.

15.7 Process Modelling and Analysis

Fermenter scale-up is another key factor in the amino acid fermentation industry (Takors 2012). Different structural framework and physical conditions in a large-scale fermenter may affect the process stability, reproducibility and yields leading to the formation of unwanted by-products ultimately affecting the final product quality (Takors 2012). Industrial bioprocesses are often affected by lower mixing efficiency combined with long mixing times that, with the high metabolic activity of microbial cells, results in the formation of local gradients into the bioreactors (Lara et al. 2006). In a conventional fermenter design, the supply of substrate and aeration from top and bottom, respectively, create an opposite trend in the concentration gradient of both along the reactor height. Due to the large size of industrial fermenters, these gradients become more marked resulting in larger oxygen and substrate depletion zones, longer mixing times due to large broth volume as well as stronger hydraulic pressure gradient influencing the oxygen transfer rate (Lara et al. 2006). Therefore, the microorganisms at the top of the fermenter are simultaneously exposed to a high glucose concentration along with oxygen limitations, while the one at the bottom are exposed to glucose restraints (Schmidt 2005). Therefore, high glucose concentration along with oxygen limitation in the reactor leads to acetate, ethanol, lactate, hydrogen, succinate, and format formation in high amounts (Castan and Enfors 2002). Formation of these acid products causes acidification of medium that combined with excess heat generation by agitation results in formation of stress zones where the microorganisms are unable to perform efficiently (Bylund et al. 1998).

15.8 Advancement in L-lysine Production

Recombinant DNA technology has made it possible to apply more rational approaches to strain improvement of *C. glutamicum* for *L-lysine production*. The terminal pathway leading to an amino acid production is the initial target for engineering. The production of L-lysine from modified strains of *C. glutamicum* along with their characteristics is given in Table 15.2.

The development of Omics technologies combined with computational analysis which enabled the target specific genes by knocking out or introducing them into the microorganisms (Chen et al. 2013). The central genes for metabolism directly impact the production of amino acids, and therefore, techniques like riboswitch and CRISPRi have enabled the development of optimized microorganisms leading to the enhanced production of amino acids and other high value chemicals (Wendisch et al. 2016). Cleto et al. (2016) used the CRISPRi interference technology with the deactivated Cas9 protein (dCas9) to determine the effect of target gene repression on

Table 15.2 Various modified strains of *C. glutamicum* used for the production of L-lysine

Strain	Relevant characteristics	Culture conditions (% w/v)	Titre (g L^{-1})	Reference
C. glutamicum MH20-22B/ pJC23	AEC (AKIS) and Leu-, *dapA* on plasmid	Glucose 10%	50	Ikeda and Nakagawa (2003)
C. glutamicum AGM5	*hom* and *lysC* mutations in the coding region	Glucose 50%	About 90	Ikeda et al. (2006)
C. glutamicum Lys-12	Replacement of *tkt* operon by the *sod* promoter	Glucose 10%	120	Becker et al. (2011)

L-lysine and the L-glutamate production. In their study they demonstrated that the application of CRISPRi/dCas9 mediated for pathway engineering in *C. glutamicum* strongly enhanced L-lysine and the L-glutamate production. *pgi*, *pck* and *pyk* genes coding for enzymes involved in the production of L-lysine and L-glutamate were the repression targets using sgRNA/dCas9. Their study proved that the *pgi* deletion causes an overproduction of NADPH through the pentose phosphate pathway with a consequent higher L-lysine yield. Moreover, the deletion of *pck* and *pyk* genes leads to an accumulation of L-glutamate by an enhanced flux of oxaloacetate in the TCA cycle, due to the absence of the conversion of oxaloacetate to phosphoenolpyruvate. They demonstrated that final L-lysine and L-glutamate yields obtained with the reduced expression of *pgi*, *pck* and *pyk* genes are comparable to the one obtained by gene deletion (Cleto et al. 2016).

15.9 Global Industrial Production

L-lysine is one of the nine essential amino acids which cannot be synthesized by the body, and so it must be supplied in sufficient quantities by the daily diet (Eggeling and Sahm 1999). The worldwide production volume of L-lysine is 850,000 tons/year. The global market for L-lysine has increased more than 20 times in the past 20 years. The estimates assume that the market is currently increasing by 10% $\pm$ 15% per year (Leuchtenberger et al. 2005). The isolation of high producing mutant strains and the development of processes have progressively increased the industrial L-lysine production during the last decades (Anastassiadis 2007). Studies about design, operation and sustainability have been reported (Taras and Woinaroschy 2012). Optimization, monitoring and control activities including process engineering are still open research issues for this by-product. Modelling and dynamic analysis of the process is important for carrying out such studies. Moreover, advancements in biotechnology and the (re)use of biomass have created a vast field for the production of L-lysine from biomass.

15.10 Conclusion

The application of different amino acids in food, feed nutrition or pharmaceutical industries is expected to increase steadily in coming years. L-lysine, an essential amino acid, forms a major part of these industries. L-lysine has a crucial role in providing health benefits from muscle building, maintenance of healthy blood vessels to assistance in calcium absorption. Recombinant DNA technology has made it possible to improve strains of microorganisms such as *Corynebacterium glutamicum*, increasing the levels of L-lysine production.

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16 Pomegranate Peel: Nutritional Values and Its Emerging Potential for Use in Food Systems

Reshma Saroj, Radha Kushwaha, Vinita Puranik, and Devinder Kaur

Abstract

Pomegranate (*Punica granatum* L.) belongs to the family *Puniceae* and is commonly known as pomegranate. In India pomegranate is commercially cultivated in the districts of Solapur, Sangli, Nashik, Ahmednagar, Pune, Dhule, Aurangabad, Satara, Bijapur, Belgaum, Bagalkot, etc. The peel of pomegranate is rich in antioxidants, which help in curing degenerative diseases such as obesity, diabetics, cardiovascular disease, cancer, etc. The therapeutic potential of pomegranate peel has been also reported. The rind of pomegranate is a rich source of hydrolyzable tannins of the ellagitannin group. From previous studies, it can be concluded that pomegranate peel is rich in proximate composition and antioxidant potential. Hence it is an antioxidant used for natural preservatives and also used in the production of dyes. Pomegranate peel helps in the treatment of intestinal worms, diarrhea, inflammation, infertility, cough, etc. Pomegranate peel extracts used for antibacterial activity were tested against *Staphylococcus aureus*, *Enterobacter aerogens*, *Salmonella typhi*, etc. Recent studies show that from pomegranate peel pectin can be extracted. The pomegranate peel can be available at very low cost expenditure from industries, which can be used as value addition in different food products such as cookies, pasta, biscuits, etc.

Keywords

Pomegranate peel · Antioxidant · Antibacterial activity · Degenerative diseases

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16.1 Introduction

Pomegranate (*Punica granatum* L.) is an ubiquitous name derived from the Latin words ponus and granatus, and it is a granular apple which is a delicious fruit consumed worldwide. Pomegranate (*Punica granatum* L.) from Punicaceae and Lythraceae family is one of the most world famous fruits. Pomegranate peel is an agro-industrial waste and has a high content of polyphenolic antioxidant compounds with proven health-benefiting and technological properties (Galaz et al. 2017). This is due to the high content of polyphenolic antioxidant compounds that help to mitigate various cancers and prevent type II diabetes and cardiovascular disease, among other health benefits (Akhtar et al. 2015; Ismail et al. 2012). Hooker is the widely accepted one by botanists around the world. Taxonomy – Bentham and Hooker Classification – Domain – Eukarya, Kingdom – Plantae, Sub-kingdom – Tracheobionta, Division – Magnoliophyta, Class – Magnoliopsida, Sub-class – Rosidae, Order – Myrtales, Family – Lythraceae, Genus – *Punica*, Species – *P. granatum*, Generic Group – Pomegranate.

The peel amounts are approximately 60% of the pomegranate fruit weight (Lansky and Newman 2007). Pomegranate peels are used in various cosmetics and act as functional food. In pomegranate processing, the skin of the fruit is considered a waste product and in the best case is used as animal feed without any added value. The pomegranate peel (PP) has a content of high polyphenolic compounds compared to the edible portion of the fruit (Fischer et al. 2011). Pomegranate peel has the highest antioxidant in comparison to pulp and seed extract. These compounds are mainly hydrolyzable tannins (ellagitannins), flavonoids (anthocyanins), and condensed tannins (proanthocyanidins). Hydrolyzable tannins are the pomegranate phenolic compounds considered most promising for their high antioxidant activity and are mainly found in the peel and internal membranes (Akhtar et al. 2015; Fischer et al. 2011). They help to maintain blood sugar level, reduce the risk of cancer, and treat diarrhea, dysentery, etc. Total phenolic, flavonoid, and proanthocyanidin content is high in the peel than the pulp of pomegranate fruits. Pomegranate peel contains the material with high antioxidant, antiviral, and anti-tumor properties and is said to be the excellent source of vitamin A, vitamin C, and vitamin E and also rich in folic acid. Chidambara Murthy et al. (2004) the pomegranete peel showed a wound healing property in human.

The peel of pomegranate is discarded and considered as the waste material which contains the wide variety of antioxidant component in comparison with other fruits. The antioxidant present in the pomegranate is used for lipid oxidation, which affects the quality of the food product. The leading cause of rancidity, indicating lipid oxidation, is secondary oxidation end products such as ketones, aldehydes, isofurans, malonaldehyde, etc. (Min and Ahn 2005; Saldamli 2007). Traditionally, pomegranate peels were used as anthelmintic and anti-tracheobronchitis and for healing stomatitis, diarrhea, vaginitis, wounds, ulcers, bruises, and against excessive bleeding (Ross 2003). Polyphenols are secondary metabolites that are found in the entire plant kingdom with about more than 8000 phenolic compounds identified (Gropper et al. 2009). Polyphenols have also different pharmacological properties such as antioxidants, anti-carcinogenic, anti-mutagenic, anti-inflammatory, and anti-microbial. In recent years, various medicinal values of pomegranate peel have been investigated such as abortifacient, anti-mutagenic, antiviral, antispasmodic, diuretic, hypoglycemic, hypothermic, analgesic, antiamoebic, antibacterial, anticonvulsant, antifungal, antimalarial, and antioxidant activities (Seeram et al. 2006). Pomegranate peel phytochemicals are the polyphenols that are predominant in the fruit and include flavonoids (flavonols, flavanols, anthocyanins), condensed tannins (proanthocyanidins), and hydrolyzable tannins (Seeram et al. 2006). Pomegranate peel tannins are highly susceptible to both enzymatic and non-enzymatic hydrolysis. Pomegranate peel contains the critical source of bioactive compounds such as ellagitannins and the punicalagin. Additional phytochemicals present in pomegranate peel include organic and phenolic acids, sterols and triterpenoids, and alkaloids (Fischer et al. 2011). Pomegranate peel is an excellent source of flavonoid content, dietary fiber, and antioxidant content and also used as cattle feed. The peel of pomegranate is high in tannin, polyphenols, and antioxidant which help in reducing the LDL level (bad cholesterol) without affecting the HDL level (good cholesterol) and also used in the production of dyes.

Neumerous studies has broadly recognized the therapeutic potential of pomegranate peel; intestinal worms, diarrhea, inflammation, infertility, and cough have been treated by using pomegranate peel extract (Bharani and Namasivayam 2016). The antioxidants present in pomegranete peel help in preventing degenerative disease like obesity, diabetics, heart disease, etc. Pomegranate peels are distinguished by an internal network of membranes encompassing almost 26–30% of total fruit weight and characterized by considerable amounts of phenolic compounds, including flavonoids such as anthocyanins and catechins and other complex flavonoids and hydrolyzable tannins like punicalagin, punicalin, pedunculagin, and ellagic and gallic acid. The compounds present are intense in pomegranate peel and juice, which depicts 85–95% of the antioxidant activity allied with the fruit (Afaq et al. 2005; Negi et al. 2003; Zahin et al. 2010). Pomegranate peels consist of pericarp, albedo (white fleshy membrane), membrane (translucent yellow material surrounding the pomegranate arils), and rind (outer peel or husk of a pomegranate); the pericarp is a good source of polyphenols such as anthocyanin, leucoanthocyanins, catechins, and flavonoids and contains about 30% tannins (Chalfoun-Mounayar et al. 2012). Another part of pomegranate fruit is seeds, which are rich origins of polyunsaturated fatty acids (PUFA) mostly linolenic (n−3) and linoleic (n−2) acids. The phenolic content is the main component present in the pomegranete peel that make it suitable for value addition (Viuda-Martos et al. 2011). According to the Mehder (2013) pomegranate peels and wheat flour have fiber, ash, carbohydrates, and protein 12.52, 6.02, 76.61, and 3.77 % and 0.92, 0.50, 12.25, and 85.55%, respectively. Another recent study demonstrated that CCl_4 caused liver damage in rats, whereas pre-treatment with a pomegranate peel extract enhanced the free radical scavenging activity of the hepatic enzymes catalase, SOD, and peroxidase and further resulted in 54% reduction of lipid peroxidation values compared to controls (Chidambara Murthy et al. 2002). Pomegranate fruit peel is an inedible part obtained during the processing of pomegranate juice. From recent studies, it was concluded that peel of pomegranate is a rich source of flavonoids, tannins, and other phenolic compounds (Li et al. 2006).

The recent studies were done on mice by giving them pomegranate juice and molasses, two by-products widely used by Lebanese consumers, to test their efficacy. The results obtained under our experimental conditions showed that pomegranate juice and, to a greater extent, pomegranate molasses induced weight loss in the animals. These findings were corroborated by a decrease in the TG (triglyceride) levels and lipid peroxidation but SOD (superoxide dismutase) activity increase in the heart, lungs, and liver. Meanwhile, the protective effects of the pomegranate molasses against ROS (reactive oxygen activity) generated by the electrolysis were histologically demonstrated (Chalfoun-Mounayar et al. 2012). Extraction of antioxidant from pomegranate peel and seed by using methanol, acetone, or water found that methanol gave an excellent antioxidant effect. Pomegranate peel tissue contains a large number of phenolic, anthocyanin, and flavonol compounds. Recently, the vital importance of pomegranate peels on functional foods has given, apart from the essential nutritional benefits they are providing physiological benefits and play a crucial role in preventing or slowing down the progress of chronic diseases. There has been a virtual increment of interest in the pomegranate application in medicinal purpose and for development of nutritious product because of its multi-functionality and its noteworthy profit for human beings as it has many groups of substances that are important in chronic disease risk reduction.

Pomegranate rind is rich in hydrolyzable tannins of the ellagitannin group. The rind extracts have recently attracted interest because of their significant use as natural food preservatives and in nutraceuticals (Negi et al. 2003). Various studies shows that the pomegranate fruit peel powder is composed of many essential amino acids like lysine, leucine, phenylalanine, tyrosine, and valine and threonine. On the other hand, the pomegranate fruit peel powder had less content of amino acids containing sulfur (methionine and cysteine) and isoleucine, which has an amino acid score of 95.7 and 93.2, respectively. Therefore, In Egypt the inexpensive pomegranate by-products were incorporated into the foodstuff especially which deficient in amino acids containing sulfur, aromatic amino acids, leucine, and isoleucine, has tremendous economic value and a functional standpoint in food technology and human nutrition (Rowayshed et al. 2013). Therefore, due to a significant amount of polyphenols present in peel, it can be used in valuable pharmaceutical and nutritional compounds; the current research is carried out to throw the light on its nutritional value indices and its proximate chemical composition (Mehder 2013). Its peel can be used as functional ingredient and is a good source of crude fibers which provide numerous health benefits such as their ability to decrease serum LDL cholesterol level, improve glucose tolerance and insulin response, prevent hypertension and gastrointestinal health diseases, reduce hyperlipidemia, and also help in the prevention of certain cancers such as colon cancer ((Lansky and Newman 2007; Viuda-Martos et al. 2010a, b). According to Fawole et al. (2012) peels of pomegranate contains high antioxidant capacity, and its extracts have been reported to possess a wide range of biological actions including anti-cancer activity, antimicrobial activity, anti-inflammatory and anti-diabetic activities, anti-diarrheal activity, apoptotic and anti-genotoxic properties, and anti-tyrosinase activity. The therapeutic properties of pomegranate peels are wide-ranging, and they are used in the treatment of many diseases like cancer, Alzheimer's disease, male infertility, arthritis cardiovascular diseases, and diabetes and as protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia and obesity (Jurenka 2008).

16.2 Phenolic Compounds

The peel of pomegranate constitutes about more than 40% of the fruit and is a valuable source of antioxidants (Nasr et al. 1996). Pomegranate marc is the excellent raw material for producing natural antioxidants, which can be further used for making fortified food and its extract used in pharmaceutical (Qu et al. 2009). Pomegranate peel contains polyphenolic compounds like flavonoids, anthocyanins, and tannins which are considered the leading group of antioxidant and phytochemical group with beautiful properties and having biological and free radical scavenging properties (Elfalleh et al. 2011). Polyphenols have beneficial functional benefits (Leja et al. 2003) having free radical scavenging capacity and antioxidant ability (Kahkonen et al. 2001).

Pomegranate peel exhibited high antioxidant activity in various in vitro models (Li et al. 2006). The peel of the pomegranate has been extensively used in folk medicine (Ahmad and Beg 2001). Pomegranate polyphenols are connected with some health benefits including a role as antioxidant, an antimicrobial agent, anti-inflammatory agent, anti-proliferative agent, lipase inhibitor, and an inhibitor for α glycosidase (Faria and Calhau 2011; Akhtar et al. 2015). Certainly, pomegranate peel antioxidant is being examined as natural ingredients used for food processing and preservation in food application (Tanveer et al. 2015). Chemical composition of pomegranate peels is shown in Table 16.1.

The polyphenol content of pomegranate peel also helps in reducing the cholesterol level. The intake of a phytochemical-rich food has been found to reduce the risk of chronic human diseases like certain types of cancers, inflammation, and cardiovascular and neurodegenerative diseases (Kong et al. 2003; Beretta et al. 2009). According to various researchers, its peel possesses wound healing properties which are mainly attributed to its polyphenol extracts. Recent studies shows, the pomegranate peel extract (PPE) rich in polyphenols could modulate the gut micro-biota in favor of bifidobacteria (Yan et al. 2013). Polyphenols including anthocyanins and

Table 16.1 Chemical composition of pomegranate peels (on the basis dry weight) (Zaki et al. 2015)

Varieties	Moisture (%)	Ash (%)	Crude oil (%)	Crude fiber (%)
Wardey	6.35	2.61	3.28	15.52
Manfalouty	7.71	2.59	2.22	12.03
LSD 0.05	0.02	0.02	0.01	0.01

ellagitannins prove the anti-inflammatory and antioxidant bio-activities in the fruit, which are found majorly in the peel and piths of the fruit (Basu and Penugonda 2009). Recent studies show that PPE (pomegranate peel extract) rich in polyphenols can modulate the gut microbiota in favor of bifidobacteria. The pre-biotic effect is accompanied by a lower expression of critical inflammatory expression in the colon and the visceral adipose tissue. The PPE (pomegranate peel extract) treatment changes the gut of micro-biota accompanied by an improvement of atherogenic markers such as LDL-cholesterol in HF (high fat) diet-induced obesity results suggest that PPE (pomegranate peel extract) can confer positive health impacts associated with gut microbiota modulation and maybe a natural alternative in the prevention of obesity and CVD (Neyrinck et al. 2013).

16.3 Flavonoids

From human consumption aspects, flavonoids are one of the major groups of phytochemicals with high antioxidant activity (Ardekani et al. 2011). The peel of pomegranate contains an excellent source of phenolic, flavonoid, and tannin content. Flavonoids are acknowledged as non-nutritive agents; its significant role is to prevent the degenerative diseases. Pomegranate peel is the vital source of anthocyanins 3-glucosides and 3, 5-O-diglucosides of cyanidin, delphinidin, ellagitannins, gallotannins, and pelargonidin. It also contains 1 g L^{-1} citric acid and 7 mg L^{-1} ascorbic acid (Gil et al. 2000) which dramatically affect various disorders including neurological and cardiovascular diseases and cancer (Halliwell 1999). Pomegranate peel has an extraordinary amount of flavonoid content in comparison to the pulp of pomegranate. Anthocyanin pigment is responsible for the red color of the pomegranate peel and juice. Kabul variety of pomegranate has a dark red peel, which indicated the presence of excess anthocyanins and other polyphenolic compounds (Elango et al. 2011). These water-soluble pigments are intensely colored and are responsible for nearly all of the pink, red, scarlet, mauve, blue, and violet colors found in the petals, leaves, and fruits (Khoshayand et al. 2012). Other phytochemicals reported in pomegranate include catechin, procyanidin, organic acid, phenolic acid, triglyceride, fatty acid, and some other compounds. The rind of pomegranate contains about 19% of tannic acid. In pomegranate peel, the hydrolyzable tannin includes derivatives ellagitannin, gallotannin, and ellagic acid. Pomegranate peel is rich in phenolic fractions which are responsible for the anti-cancer activity. The study indicates that the consumption of pomegranate peel extract containing anthocyanins (polyphenol content 1 g/kg diet) despite a significant increase in serum antioxidant capacity cannot protect the kidneys from hypercholesterolemia-induced damages during the treatment period (Sharifiyan et al. 2016). Extracts from pomegranate peel were found to contain highest total polyphenols and total flavonols and also exhibited superior antioxidant activity followed by apple peel, citrus peel, banana peel, and five other agricultural wastes (Sultana et al. 2008).

Also, various researchers showed that pomegranate peel has anti-cancer and cardiovascular preventing properties and chemo-preventive and adjuvant therapeutic effect on breast cancer (Kim et al. 2002). There are some researches done about the presence of tannins, alkaloids, glycosides, flavonoids, and phenolic compounds as antioxidant factors in juice, peel, pulp, and seed fractions of pomegranate (Halvorsen et al. 2002; Chidambara Murthy et al. 2002; Noda et al. 2002). The difference between white pomegranate peel and red pomegranate peel is that white peel contains less anthocyanin than red peel. The extract of peel includes a considerable amount of hyperoside and anthocyanin than the pulp. It is worthy noted that the antioxidant capacity of pomegranate peel extract was higher as compared to the extract of pulp. Pomegranate peel can also be collected in the vast quantity from the pomegranate processing industries or the waste products (Khanavi et al. 2013). Anthocyanin is a water-soluble pigment, which is found in an abundant amount in aril of pomegranate, has a characteristic flavor having mild astringency, and health benefit. Many pomegranate constituents are present in the juice of arils, red skin, and white membrane; the polyphenols observed are high in membrane fraction in comparison to seed extract. Antioxidant activity is higher in the juice extracted from whole fruits than the juice obtained from arils only. Peel of red pomegranate contains the best effect of scavenging ability of superoxide anion.

16.4 Tannin Compound

Tannin compound of pomegranate peel is an astringent, polyphenolic biomolecule that binds to and precipitates amino acids, proteins, alkaloids, and various other organic compounds. It required at least 12 hydroxyl groups and five phenyl groups to work as protein binders. Physical observation of detanninated and fresh pomegranate peel powder (Bhagva variety) is given in Table 16.2.

The fresh pomegranate peel powder contains good nutritional composition and high tannin content, but detanninated peel powder showed double benefits like separated hydrolyzable tannin/ellagitannin, and detanninated pomegranate peel powder contains a good amount of nutritional components with appropriate amount of tannin which can be suggested as novel cattle feed supplement (Kushwaha et al. 2013).

Approximately 30% of all anthocyanidins found in pomegranate are contained within the peel (Batta and Rangaswami 1973). The flavonoids and tannins such as

Table 16.2 Shown effect in color, odor, and the appearance of detanninated peel powder and fresh peel powder (Kushwaha et al. 2013)

Parameter	Detanninated peel powder	Fresh peel powder
Color	Light brown color	Dark brown color
Odor	Characteristic with the pleasant odor	Characteristic with tannin odor
Appearance	Light brown-colored granular powder	Dark brown-colored granular powder

gallic acid are isolated from an extract of *Punica granatum* peel. Gallic acid is an essential natural compound because it has antioxidant, anti-inflammatory, antifungal, and anti-tumor properties (Claudio et al. 2012).

16.5 Applications of Pomegranate Peel in Food Industries

Addition of antioxidant for making bakery or extruded product will improve their nutritional qualities as well as good eating quality (Paul and Bhattacharyya 2015). Pomegranate peel is used for fortified bread, and it was reported that phenolics and flavonoids compounds had a significant effect on inhibiting pancreatic lipase activity and concluded that the action of the mechanism is responsible for decreasing the serum triglyceride effect and transforming VLDL into LDL (Sayed-Ahmed 2014). It is rich in anthocyanin pigment which is red and helps in doubling the shelf life of tomatoes by delaying over-ripening and reducing susceptibility to grey mold (Bassolino et al. 2013; Zhang et al. 2013). While pomegranate peel extract and pomegranate bagasses might be used for the fortification of food commodities producion of a new functional product with a deserving health benefit, increases quality and longer shelf life (Charalampia and Koutelidakis 2017). Pomegranate peel is a good source of dietary fiber and has antioxidant properties, phytochemical properties, anti-cancer properties, etc. From above studies it was concluded that peel of pomegranate is used as for the value addition of products like a bakery or extruded product such as pasta, cookies, biscuits, cakes, etc. because it is rich in antioxidant which helps in increasing the health benefits.

16.6 Conclusions

Recent research showing their functional properties as well as their antioxidant properties of pomegranate peel added to our knowledge. Due to low-cost wastage, pomegranate peel is available from the industry which can be used as product formulation because the peel of pomegranate is rich in antioxidant which helps in curing degenerative diseases like obesity, diabetics, cardiovascular disease, cancer, etc. It contains various types of nutritional supplement, water-soluble vitamin, as well as bioactive compound such as polyphenols, tannins, flavonoids, anthocyanins, etc. It can be recycled as a value-added product or as a food supplement. Also, established use of the pomegranate peels is also alleviating the pollution problem in our surrounding caused by inferior disposal. The extract of pomegranate peel can lower the LDL level, which is bad cholesterol, without affecting the HDL level, which is good cholesterol. And it is non-caloric agents. Further studies have to be conducted to identify the active compound present in the extract and to elucidate the pathways regulated in lowering the fat level of the body.

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Part III

Food Technology and Environmental Biotechnology

Bio-fertilizer from *Trichoderma*: Boom for Agriculture Production and Management of Soil- and Root-Borne Plant Pathogens

17

Charles Oluwaseun Adetunji and Osikemekha Anthony Anani

Abstract

One of the greatest challenges that hamper sustainable and enhanced increase in food production is large numbers of root rot diseases that affect agricultural crops. Moreover, the application of chemical fertilizers has been documented with various negative hazards like high pesticide residues, bioaccumulation of toxic substance, and buildup of heavy metal in food crops. Therefore, there is a need to search for an alternative solution that will mitigate all the highlighted challenges. The application of *Trichoderma* spp. has been highlighted as a sustainable biotechnological tool that could supply the necessary nutrient and serves as a natural fungicide for effective management of root rot diseases. Therefore, this chapter provides recent techniques utilized for the utilization of numerous *Trichoderma* spp. for the management of root rot diseases and their eventual application as a bio-fertilizer. Moreover, this chapter illustrates the application of cheap agricultural wastes *such as* vegetable wastes, maize bran, cow dung, water hyacinth, molasses, sawdust, and poultry refuse for the mass production of *Trichoderma* spp. toward the development of several products like Tricho-compost, as well as Tricho-leachate which is a liquid product obtained from the solid-state fermentation of agricultural wastes using *Trichoderma* spp. Also, several illustrations were provided on the effectiveness of already commercialized *Trichoderma* spp. for their usefulness as a bio-fertilizer and management of several root-borne pathogens affecting an increase in agricultural production.

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Keywords

Trichoderma · Root rot diseases · Agricultural waste · Tricho-compost · Tricho-leachate · Bio-fertilizer

17.1 Introduction

Naturally, fungi represent one of the beneficial soil microflora that are present in the soil performing different ecological functions. A number of strains of the general *Trichoderma* that inhabit flora roots are symbiotic in nature (Ranveer et al. 2018). These strains can excite flora development and stimulate rapid growth of agricultural crops (Harman et al. 2004a, b). The application of these strains as a natural mediator (bio-fertilizer) in soil improvement can reduce the cost of acquiring expensive fertilizers and efficiency in agricultural approach. This will go a long way in alleviating all the various hazards that border around the utilization of chemical fertilizers (Adetunji et al. 2018, 2019). Several strains of *Trichoderma species* have been documented for their effectiveness in enhancing plant growth because they possess numerous plant growth-promoting characteristics. Examples of such *Trichoderma species* which have been commercialized as a bio-fertilizer with wilder application in agriculture includes *T. harzianum*, *T. viride*, *T. reesei*, *T. polysporum*, *T. koningii* (Ranveer et al. 2018). Moreover, some of these strains are utilized together with manures to give an improved effect as green nourishment for crop improvement. This shows that some of these strains pose stimulating effects on plant growth most especially strains of *T. harzianum* and *T. viride*. Also, application of *Trichoderma species* as a bio-fertilizer could serve as a permanent replacement to an old-fashioned way of using a combination of compost mixed with chemical fertilizer contain N.P.K (nitrogen, phosphorus, and potassium). Furthermore, *Trichoderma* species have been reported to play a crucial role in the bioavailability of trace nutrients like copper (Cu), zinc (Zn), iron (Fe), and sodium (Na) as well as the bioavailability of macronutrients such as phosphate which are not readily accessible to plant (Ranveer et al. 2018).

Apart from the supply of adequate nutrient to plant, *Trichoderma* species have been observed to secret some important metabolites which allow them to perform numerous ecological functions in the soil. Examples of such biological active metabolites which could led to an improvement of agricultural crops which are produced by *Trichoderma* species include hormones, endochitinase and proteolytic and chemical catalyze (enzymes), respectively (Benítez et al. 2004; Ranveer et al. 2018). Moreover, some strains of *Trichoderma* metabolites can alter the proteome and transcriptome composition of crops (Marra et al. 2006; Alfano et al. 2007; Shoresh and Harman 2008).

Simulated bio-fertilizer which is formulated using the strains of *Trichoderma* is well-known for their beneficial importance in the natural ecosystem. These strains take advantages of flora-microbe connections and improve the microorganisms in the soil. They also aid in mitigating greenhouse effects of gases such as CO_2 and

CH_4 that are precursors of worldwide atmospheric or global warming (Ranveer et al. 2018; CSIR-India; Mia and Shamsuddin 2010). *Trichoderma* species such as *T. harzianum* and *T. viride* are widely applied in agriculture a biopesticide and bio-fungicide. It is used for the treatment of fungal diseases caused by pathogens via combating with the systemic negative impact on leaves and seeds and improves soil nutrients. It produces enzymes that increase flora adaptation to stress as well as stimulates an increase in absorption rate of inorganic and organic nutrient to agricultural plants (Harman et al. 2004a, b; Gupta et al. 2014). This green action of *Trichoderma* protends them as a special biotechnological tool that could help to mitigate all the adverse effect of chemical fertilizers. The could also be referred to as a double-edged sword that could perform a dual role as effective bio-fertilizer and bio-fungicides against numerous disease-causing fungi including *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Verticillium*, *Alternaria*, *Phytophtrora* (Abu-Taleb et al. 2011). More so, its ability to improve soil quality makes it a preferred potent strain that can be used to improve the natural quality of crops yields when mixed with compost and surfactants for remediation, decontamination of contaminants and dilapidated agricultural soil.

This species of *Trichoderma* also play a major role in the management of polluted soil and plays a crucial role in the prevention of crops that are very venerable to the attack of pest and diseases like mushrooms (Biswas 2014). It executes its biological control activity by inhibiting pathogenic strains and later secretes enzymes that could inhibit these pathogenic microorganisms.

Trichoderma viride is a well-known bio-fungicide-fertilizer fungus. It can be used for the treatment and overpowering of seed and soil diseases caused by similar strains of the disease-causing organism. *T. viride* acts as a bio-fertilizer. When it's applied to the potential growing crop, it improves seed sprouting level if the application is done simultaneously. The mode of action involves the inhabitation of the seed superficial area and exterminates not only the disease-causing agent extant on the seed leaves but serves as a fortification against the disease organism (Ranveer et al. 2018). *Trichoderma viride* has been tested to be effective in the control of widespread of tomatoes diseases when used as a bio-fungicide-fertilizer (Dababat et al. 2006). This makes *T. viride* a widely potent strain used as a biological control agent used for effective management of plant disease-causing fungi like *Rhizoctonia*, *Pythium*, and *Armillaria.* Moreover, they play a significant role in the enhancement of seeds by exhibiting protection against seed pathogens like *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium* which are responsible for pre-harvest and postharvest poi crops like cotton, tomatoes, soybean, cowpea, sorghum, and mung bean, respectively (Ramakrishnan and Jey Arajan 1986; Alagarsamy and Sivaprakasam 1988; Singh et al. 1990; Mia and Shamsuddin 2010). Therefore, this chapter intends to provide comprehensive details about the application of *Trichoderma* spp. for the management of root-borne plant pathogen and their application as a bio-fertilizer.

17.2 Various Application of *Trichoderma* as a Bio-fertilizer and Bio-fungicide

One of the largest groups of microbes that play a very important function in the natural ecosystem is the general *Trichoderma*. The strain of organisms in this genus has a technical pattern at which it conquers and inhabits their specific ecological role. Many strains of *Trichoderma* have a way at which they impact absolutely on the development, growth, as well as the defense of flora crops against disease-causing organisms such as other microbes, bacterial and microbes acting as a biocontrol agent and as well as a bio-fertilizer.

Błaszczyk et al. (2014) reported a review on the biological protection of flora using *Trichoderma* strains and confirmed that are widely used as bio-fungicides and for decontamination of polluted areas. More so, they also confirmed that certain strains members of the genus *Trichoderma* are also used in various agricultural purposes in the enhancement of the growth and development of comestible and therapeutic mushrooms – a bio-fertilizer method for the cultivation of green mold. Zeilinger et al. (2016) reported from a review on the genomic of *Trichoderma* chemistry metabolites and confirmed that the genus has the potential to leave in any ecosystem and go through a variation of connections with similar strains or diverse fauna because of their mycotrophic way of life and the rhizosphere capability of many species. They do this by the possession of gene clusters and peptaibiotic formations. They also reported that *Trichoderma* strains are effectively used as bio-fungicides owing to their floral-protecting capabilities, and they are creative manufacturers of advanced metabolites which come alongside with an enhancement related to their genomes.

Weindling (1943) and Druzhinina et al. (2011) reported that *Trichoderma* strain (*T. virens*) has been used as biocontrol messengers because of its capability to produce a surplus of improved metabolites such as glitoxin. The mode of actions is irritation and execution of their fungal victims through a mutual hydrolytic organic catalyst that lyses the organism's cell wall. More they also reported that gliotoxin and endochitinase have been proven to have synergetic inhibition of *B. cinerea* reproductive structure sprouting (Lorito et al. 1994). This has been shown in the reduction of mycelial growth of plant pathogens like *F. oxysporum*, *B. cinerea*, and *R. solani*. In addition, besides being a biocontrol agent, numerous *Trichoderma* strains are linked with flora root systems where they interact and form a close symbiotic relationship. These strains aid in triggering local reactions in the flora roots by discharging catalytic materials like harzianolides, volatile organic compounds (VOCs) and peptaibols for the development and growth of flora (Harman et al. 2004a, b). In this case, *Trichoderma* act as a bio-fertilizer to plant-stimulating biological activities, development regulation, and inducing systemic defense by mimicking growth hormone auxin in plants (Vinale et al. 2008). This has been applied to the 6-PP management of tomato crops which was associated and complimented with the variations in the configuration of the tomato and elicited positive enhancers like gamma-aminobutyric, metabolome, and acetylcholine acidic materials (Mazzei et al. 2016). Similarly, in pea, canola tomato, maize, and peanut plants, it has been reported that oxylipins play intermediating role between flora and fungi acting as a coenzymes (cytochrome P450 epoxygenase and lipoxygenase) in spurring the growth and developmental activities of active and dividing cells in flora system (Vinale et al. 2008; Ding et al. 2012; Fischer and Keller 2016).

Rajesh et al. (2016) reported the importance of *Trichoderma* as fungus for agriculture as an eco-friendly elucidation to extensive control of biocontrol agents because of the challenges faced by modern farming agrochemical approaches to plant growth. Being a biocontrol and bio-decontamination agents, *Trichoderma* manufacture numerous types of an organic catalyst which interact with the mechanisms of microbes-fungi-bacteria in soil and plants through symbiotic relationship to establish a beneficial impact. Of recent, findings show that *Trichoderma* strains have morphological and well-designed genomic potentials that enable its applications as bio-fertilizer in agricultural production and recommended as green sustainable strains as against other agrochemicals fertilizers.

Topolovec-Pintarić (2019) confirmed the positive impact of the use of *Trichoderma* strains in agriculture. He reported that the strains in the general *Trichoderma* may possess beneficial biological control against plant diseases and growth boosters of plants (mycofungicide and bio-fertilizer) and their application in agriculture can increase plant development swiftly, serve as an alternative to high production expenses of some inorganic fertilizers, and reduce ecosystem impact. The perspective of *Trichoderma* as a bioagent have been put in conservational usage in commercial products, bio-fungicides and bio-fertilizers, which account about 60% of the current market-based income (Topolovec-Pintarić 2019). However, there are challenging factors as regarding the registration of *Trichoderma* as potential biological control agent, fungicides and fertilizers, patenting it and its efficiency as a green or eco-friendly bio-reagent for agricultural use.

Akladious and Abbas (2014) evaluated the application of *Trichoderma harzianum* T22 strain as a bio-fertilizer in order to enhance the development of maize plant. The isolate *T. harzianum* was inoculated in the soil, and the treatment was done by treating the seeds (maize) with varied metabolic concentrates containing 100, 200, and 300 μl of *T. harzianum* prior to the planting period. The findings from their study revealed that *T. harzianum* elicited plant hormone (phytohormones) activation, growth-promoting impact, chlorophyll-starch contents, increase in DNA materials (nucleic acids), high adaptation response to environmental stressors, and increase in the nitrate-protein level in *Zea mays* (maize plants), when used as a bio-fertilizer to boost soil nutrient loss. It was inferred that the application of *T. harzianum* improved the entire growth developmental pattern of *Zea mays*.

Zhang et al. (2018) evaluated the biocontrol activities of *Trichoderma* strains in the improvement of grassland biomass, transformed soil chemistry, and microbial community. The results of the biological controlled experiment showed that the use of *Trichoderma* strains as organic or bio-fertilizer showed a positive probable soil-microbial and community reactions. The findings of the grassland biomass showed an increase in response to the alteration, compost and inoculum: about 9000 kg ha^{-1} of *Trichoderma* bio-fertilizer, and it was significant ($p = 0.019$) when related with other evaluated organic or bio-fertilizer rates of 3000 kg ha^{-1}. The findings of the soil treated with *Trichoderma* bio-fertilizer revealed an increase in soil antifungal components which likely may suppress or inhibit some disease-causing fungi that will possibly somewhat be responsible for the better quality of the grassland dry or wet body mass. Moreover, the findings from the use of the NMDS (nonmetric multidimensional scaling) showed that the fungi groups and soil interaction differed by enriched regime or application of the bio-fertilizer.

In general, the bioactivities of the *Trichoderma* bio-fertilizer improved the virtual large quantity of a specific fungus (*Archaeorhizomyces*), but contrarily reduced the activities of another specific fungus (*Ophiosphaerella*). This was observed using an SEM (structural equation modelling). It was further observed that *Trichoderma* spp. can be used as a potent green bio-fertilizer for sustainable management of microbes in soils, soil quality, and the dry and wet mass of grassland flora.

Adan et al. (2015) tested the effectiveness of the formulation containing *Trichoderma* which was applied as a biopesticidal agent used in combating damping disease in eggplant seedlings. The various formulations (sawdust, grass pea bran, lentil bran, gram bran, wheat bran rice bran, black gram bran, and mustard oil cake) water and peat soils were mixed with T4, T5, and T7 *Trichoderma harzianum* [T5 (*Trichoderma* + water + peat soil + black gram bran), T7 (*Trichoderma* + water + peat soil + grass pea bran) and T4 (*Trichoderma* + water + peat soil + gram bran)] to test its effectiveness counter to *Sclerotium rolfsii* in eggplant seedlings. The results obtained revealed *Trichoderma* reproductive body was able to reduce the damping-off pathogens and excite rapid sprouting percentage in terms of seedling vitality, height, and new body mass of the studied seedling in comparison to the control. The maximum growth percentage (78.00%) was noticed in treatment T5 at day 16 after planting, while the minimum percentage (6.33%) before the advent of damping-off was observed in T5 in eggplant seedling at day 16. The lowest preemergence damping-off and tip over were observed in T5 in eggplant (2.33% and 1.33%). It can be concluded from the findings that *Trichoderma harzianum* did not only act as a biopesticide but as well as a bio-fertilizer, inducing the biological activities of the eggplant and inciting several plant hormones to bring about growth and development in the young growing seedlings.

Mahato et al. (2018) evaluated the effect of *Trichoderma viride* as bio-fertilizer on development and growth of wheat. The biological controlled experiment was made up of seven treatments made up of several mixtures excluding the control all in triplicates (T1, control; T2, soil + N.P.K; T3, soil-inoculated *Trichoderma*; T4, *Trichoderma* + FYM {farmyard manure}; T5, *Trichoderma* + ½ N.P.K; T6, *Trichoderma* + N.P.K; and T7, *Trichoderma* + N.P.K + FYM). The results revealed that *Trichoderma viride* spurred the root weight (1.5%), grain yield (36.5%), wheat's height (4.6%), panicle weight (9.1%), leaf length (0.3%), biomass yield (2.7%), biological yield (13.7%), and number of grains (3.8%) over the control, whereas panicle length (−8.4%), number of leaves (−8.4%), tiller number (−10.8%), root length (−17.4%), and panicle number (−6.7%) emphasized the opposite effect of *T. viride* on the studied plant. The findings of this studied can be concluded that *T. viride* exhibited dislike with inorganic manure. However, when *T. viride* and N.P.K were supplemented with farmyard manure, there was striking increase in the development and growth of the studied plant. Nevertheless, certain growth parameters were reduced by *Trichoderma viride*; however, it is still used as bio-fertilizer because it is eco-friendly, cheap, and highly sustainable.

17.3 Management of Root-Borne Plant Pathogen

Biological control has been as a significant part of managing plant pathogens most especially the soilborne fungi. *Trichoderma* spp. has been identified as a sustainable biotechnological solution that could be utilized for the disease management for plant pathogens over the years. Therefore, there is a need to explore all the effectiveness of the biological control of this native Trichoderma from different agroecological zones worldwide.

Manandhar et al. (2019) obtained 50 soil samples from which several species of *Trichoderma* were isolated from the rhizosphere of these important agricultural soils. The ten best strains of *Trichoderma* spp. were screened against some soilborne pathogens including *Sclerotinia sclerotiorum*, *Fusarium solani*, and *Rhizoctonia solani*, respectively, in an in vitro assay. The result obtained showed that all the selected *Trichoderma* sp. exhibit a significant mycelial inhibition growth in comparison with the control. It was observed that these *Trichoderma* sp. exhibited a various degree of inhibition against all the tested soilborne pathogens. It was noticed that isolate (T363) exhibited 80% inhibition against *S. sclerotiorum*, while T357 demonstrated more than 80% inhibition against an *F. solani.* The authors suggested that there is a need to validate the biological control activity of these *Trichoderma* isolates in pot and field experiments, in order to establish their biological control activity.

Bastakoti et al. (2017) isolated 5 *Trichoderma* species available from 26 various soil samples in order to assist their biocontrol activity against some soilborne pathogens including *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani*, *and Sclerotinia sclerotiorum*. The in vitro biological control effectiveness of these *Trichoderma* species was carried out using dual culture method**.** The result obtained showed that three out of the *Trichoderma* isolates exhibit 100% against *Sclerotium rolfsii* and 62%, 68%, and 23% against *Rhizoctonia solani*, *Fusarium solani*, and *Sclerotinia sclerotiorum*, respectively. Their study showed that *Trichoderma* species could be utilized as a biological control agent for the management of various types of soilborne fungal plant pathogens. It was further observed that more inhibition was observed against *Sclerotium rolfsii* when compared to other pathogens.

Ganesan et al. (2007) tested the effectiveness of two different strains containing *Trichoderma harzianum* (ITCC – 4572) and *Rhizobium* and validated their utilization for the biological control of stem rot disease of groundnut. The result obtained from their study shows that these biocontrol agents can be used for the management of stem rot disease of groundnut (*Arachis hypogaea L.*) caused by *Sclerotium rolfsii*. Moreover, it was observed that there was an increase in the biomass of groundnut plants. Their study confirms the biological control potential and the plant growth effectiveness of *Rhizobium* and *Trichoderma harzianum* (ITCC – 4572) used during this study.

Sharma et al. (2012) tested the biological control activity of *Trichoderma harzianum* (Th3) against the following root rot disease affecting the normal growth of groundnut including *Pythium aphanidermatum*, *Aspergillus niger*, *Rhizoctonia solani*, *Thielaviopsis basicola*, *and Sclerotium rolfsii.* The three varieties of groundnut used during this experiment were GG-10, GG-20, M-13, and local varieties. The experiment was performed to minimize the high rate of losses recorded by the farmers in the year 2009 and 2010 around 12 villages in the Jaipur district of Rajasthan, India. The field experiment was performed with the application of *Trichoderma harzianum* in form of liquid bio-formulation and powder. Trials were performed by the treatment of seed, soil, and foliage in addition with the powdered bio-formulation (Th3 SD, SA) which was applied at the rate of 5 g per kg seed/soil followed by the spraying with liquid formulation (Th3 FS) at 5 ml/l along by following standard integrated pest management. The result showed that the crop planted without biological control serving as the control exhibited a significant decrease in yield coupled with blackening symptoms from the roots to stem which later affected the vascular system as well as shredding at root-stem internodes which led to complete wilting and finally death of the whole plant. However, the plant treated with Th3 showed a reduction in the rate of crop blackening, and there were no observable diseases in the root vascular system. Moreover, the following results were obtained from the groundnut crops with the following values: 14.03% for lowest root rot incidence, 39.17 Q/ha for maximum yield of groundnut, colony forming unit of 38.5 x 10^6, and the minimum root rot incidence of 14.03% which were observed when the groundnut was treated with *Trichoderma* of strain Th3. On the whole, the farmers become interested to adopt *Trichoderma* as a biological control agent for the management of the root vascular system affecting their groundnut because there was an increase in their daily income. The author suggested that there should be an effective collaboration between farmers and researchers so as to facilitate the quick use of information that will help the groundnut growers in Rajasthan state, India.

Pastrana et al. (2016) tested the effectiveness of two different commercially available biological control agent that could be used for the management of *Fusarium solani* and *Macrophomina phaseolina* which were the major soilborne pathogens affecting the normal growth of strawberry. The three formulations tested were *Bacillus megaterium* and *B. laterosporus* (Fusbact®) and *Trichoderma asperellum* T18 strain (Prodigy®), respectively. These biological control microorganisms were tested in an in vitro condition under environment and field conditions. These biocontrol agents were applied using inoculation techniques via root-dipping and soil application, while their application were performed two times as pre- and post-pathogen inoculation, as preventive and curative treatments, respectively. The result obtained from the dual plate assay showed that *T. asperellum* and *Bacillus* spp. exhibited 36% against *phaseolina* and *F. solani* by inhibiting their radial growth. Moreover, it was observed that the application of *T. asperellum* using root-dipping techniques reduced the occurrence of charcoal rot in the growth chamber at the rate of 44%, while 65% rate of inhibition was observed under the field conditions. Also, there was a drastic reduction in the percentage of crown necrosis, the rate of disease progression, the percentage of crown necrosis, as well as a decrease in the rate of infection determined as ng of pathogen DNA g^{-1} plant when assayed using quantitative real-time PCR. Furthermore, it was observed that the root-dipping techniques decrease the rate of root rot and crown rot up to 81% under field conditions and 100% in a greenhouse, respectively. All the result obtained showed that the biological control agents exhibited the same level of result with the chemical fungicide used as the positive control.

Amin et al. (2010) evaluated the biological control activity of six isolates of *Trichoderma* spp. for their capability to prevent the growth of soilborne pathogens affecting the following causing web blight of beans caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani* and collar rot of tomato caused by *Sclerotium rolfsii. The biological control assay was performed using* dual culture techniques. The result obtained showed that strain Tv-2 of *Trichoderma viride* and *T. harzianum* (Th-1) showed 65.71 and 60.51% when compared to the control that had 71.41% inhibition against *Rhizoctonia solani*. Moreover, it was observed that all the *Trichoderma* spp. used during this experiment prevented the production of sclerotia from the test pathogens. *T. viride* (Tv-1) exhibited the best mycela growth inhibition against all the test pathogen with 67.91 and 66.21% against *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* respectively. Moreover, *T. viride* (Tv-1) caused drastic reduction in the sclerotial production with 83.75, 80.18, and 70.15 for *R. solani*, *S. rolfsii*, and *S. sclerotiorum*, respectively.

Sreedevi et al. (2011) determined the biological control activity of *Trichoderma harzianum* obtained from the rhizosphere of groundnut against the pathogen responsible for the root rot incidence in an in vivo *assay*. Moreover, the capability of the *T. harzianum to induce* systemic resistance in the groundnut plant was carried out against the *Macrophomina phaseolina*, while biochemical alteration in *T. harzianum*-treated plants, healthy plants, and *M. phaseolina*-inoculated plants were performed at various stages of infection. It was observed that the application of *T. harzianum* in the presence of *M. phaseolina* stimulates the introduction of defense enzymes such as polyphenol oxidase and peroxidase as well as defense compounds ortho-dihydric phenol and total phenol. It was observed that the level of polyphenol oxidase, ortho-dihydric phenols, total phenols, and peroxidase activity was enhanced at various stages of infection. Moreover, the application of *T. harzianum* together with the tested pathogens showed enhanced polyphenol oxidase and peroxidase activity by 95.5% and 28.2%, respectively, in roots at stage 2 compared to uninoculated plants. Also, an enhanced level of polyphenol oxidase and peroxidase was observed in the shoot and the root of the treated plants which confirms the process of systemic protection has been initiated in the groundnut after applying *T. harzianum*. Their study shows that *T. harzianum* could induce systemic resistance in groundnut plant and prevent root rot diseases caused by *Macrophomina phaseolina*.

Nahar et al. (2012) developed a Tricho-compost, a *Trichoderma*-built compost fertilizer which consists of spores of *Trichoderma harzianum* along with some raw material such as vegetable wastes, maize bran, cow dung, water hyacinth, molasses, sawdust, and poultry refuse. Moreover, the Tricho-leachate, obtained as a liquid by-product obtained from the solid-state fermentation was also assayed. The two products containing Tricho-compost and Tricho-leachate, respectively, were tested in seedbed nurseries and in the laboratory for their effectiveness as a biological control agent against soilborne pathogens that normally affect the growth rate of cabbage seedlings. It was observed that the application of Tricho-compost and Tricho-leachate decreased the seedling mortalities of cabbage caused by *Sclerotium rolfsii* by about 98%. Interestingly, it was observed that the *Trichoderma harzianum* that was re-isolated from the Tricho-leachate and the Tricho-compost demonstrate a high level of inhibition against *S. rolfsii* mycelium. It was reported that it took 5 days to record 59.7% inhibition of radial growth of *S. rolfsii* mycelium, while it will take 10 days for total destruction. The result obtained from the seedbed nurseries showed that application of Tricho-leachate and Tricho-compost enhanced the rate of seedling germination, decreased the rate of soilborne diseases incidence, and decreased the rate of infestation of root-knot nematodes.

The field trial carried out showed that Tricho-leachate and Tricho-compost decrease the seedling mortalities by 53.3–62.1% in Bogra and 40.9–64.5% in Gazipur. Moreover, the application of Tricho-leachate at a rate of 500 ml per sq. meter enhanced the plant weight up to 55.6% and decreased the rate of seedling mortality up to 84.0% in Gazipur. Also, the application of Tricho-leachate and Tricho-compost decreased the level of root-knot nematode infestation up to 80.7–91.0%. Their study shows that the application of Tricho-leachate and Tricho-compost is very effective and economical and enhanced adequate growth and massive production of healthy cabbage seedlings.

17.4 Future Direction and Conclusion

This chapter has provided several illustrations that show the practical application of *Trichoderma* spp. for the management of several root-borne pathogens affecting an increase in agricultural production and their eventual application as a bio-fertilizer. There is need to develop a strong collaboration between the farmers, researchers, policymakers, and rural extension workers because this will enable all the concern individual to know the best solution that could mitigate the problem hampering massive production of agricultural food most especially the issue of the root-borne pathogen. There is a need to explore and isolate several *Trichoderma* spp. from some uncultivated soil where chemical pesticides have not been applied before. There is a need to validate the modes of action through which the *Trichoderma* spp. executes its biological control activity and the mechanism involved in the release of nutrient which potentiates them as a sustainable and effective bio-fertilizer agent. Moreover, they could serve as a bioremediation tool for reviving soil that has been polluted with chemical fertilizers, substantiating them as the most urgent biotechnological tool for massive increase in agricultural production of food that could cater for ever-increasing population worldwide.

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Influence of Heavy Metal on Food Security: Recent Advances

18

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Abstract

Food safety has been identified as one of the highly placed sustainable goals because of its relevance to the sustainability and well-being of mankind. Over the years human being is faced with several adverse effects that normally result in uncountable impairment in their health. This might be linked to the high level of contamination and environmental pollution as a result of heavy metal due to various anthropogenic activities as well as several agricultural and environmental activities. It has been observed that heavy metal can affect human metabolomics which normally results in a high level of morbidity in several countries most especially in developing countries. Therefore, this review intends to discuss extensively the influence of heavy metal contamination on mankind health and their eventual safety. Special emphasis was also laid on the influence of heavy metal on food contamination as a result of environmental and agricultural

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activities through the application of pesticides which normally lead to a high level of adsorption and accumulation of these toxic metal elements. Also, this chapter also elucidates the modes of action through various molecular and physiological translocation leads to the movement of these toxic metals into food crops. On the whole, several sustainable preventive and management strategies were proposed on how sustainability could be maintained in soil-food subsystems.

Keywords

Heavy metal · Metabolomics · Health · Safety · Food contamination · Toxic metal

18.1 Introduction

The ability to access and afford food as well as its consistent availability is termed "food security." FAO and UN (2003) defined food security as the steady acceptability of and beneficial, stable, and adequate access to food at all times. FAO (1996) and Raj Patel (2013) termed it as the corporeal and commercial access to adequate, harmless, and nourishing food to meet the needs of human vitality and well-being. More so, it is a state of some degree of or undefined accessibility of nourishing, satisfactory non-toxic nutrition that is publicly acceptable (USDA 2013).

The Sustainable Development Goals (SDGs) are assemblages of seventeen (17) worldwide goals agreed by the UN (United Nations General Assembly) in the year 2015 for the year 2030. The second and third Sustainable Development Goals (SDGs) state the need in addressing the issues of hunger, good health, and well-being (food security) (United Nations 2015). In recent times, the issues of food insecurity (heavy metals found in food) have pervaded the SDGs actions on sustainable living for the present and future generations, resulting to ill-health and poor well-being especially in the developing and third world countries, who have limited knowledge of their impacts.

Heavy metals appear in the WHO and IPCS (International Programme on Chemical Safety) (2019) lists of chemical of public worry. Examples of these metals are Ti (thallium), Sb (antimony), Ag (silver), Se (selenium), Zn (zinc), Cu (copper), Ni (nickel), Co (cobalt), Cr (chromium), Mn (manganese), V (vanadium), and lead (Pb).

Heavy metal build-up in the environment can trigger several environmental and health conditions (Anani 2017; Anani and Olumukoro 2017, 2018a, b). The reason is because their ions persevere in the ecosystem owing to their residential potentials and non-degradable natural state and they bioaccumulate in food (plant and animal tissues) and thence transfer along the food chain as probable serious threat to humans (Anani 2017; Enuneku et al. 2018, 2019). Studies have proven that the level of heavy metal pollution in the environment has gone beyond standard thresholds and might pose negative threat to food security and life forms (Tak et al. 2013; Gaur et al. 2014; Dixit et al. 2015; Anani 2017).

The negative influence of heavy metals on food security has become a major discourse in the sphere of environmental toxicology. One of the major point sources of heavy metals is through farming activities and lithogenic origin (Anani 2017; Anani and Olumukoro 2017). Consumption of metals from food produced from polluted soil has been proven to surpass legislative acceptable limits and posed probable health risks (Kohrman and Chamberlain 2014; Kaiser et al. 2015; Xiao et al. 2017) and likely carcinogenicity. Additionally, the ingestion of polluted water and seafood has also been confirmed to have xenobiotic compounds like heavy metals (Jin et al. 2003; Enander et al. 2004; Peters et al. 2008; Anani and Olumukoro 2018a, b). These complexes have even also resulted to the death of land/aquatic fauna and flora, when the rate of accumulation is higher than their absorptive potentials. But when they are able to contain the metal concentrations (bioaccumulation), they are thus affected, and their fitness for consumption is in doubt!

This chapter therefore seeks to establish several recent advances to checkmate food insecurity because of the influence of heavy metal in them, ascertain the routes of heavy metals that pose risk to food security, evaluate the health risk on their consumption via food (flora and fauna), and proffer the future direction in addressing food insecurity.

18.2 Current Influences on Food Security Via Heavy Metal(s)

Ayangbenro and Babalola (2017) did a review on the impact of heavy metals on an environment using microbial bio-sorbent. The authors elucidate the need of using microbial bio-sorbents in the remediation of heavy metals from contaminated farmlands because of their eco-friendly potentials, non-toxic impact, and wide-range mechanisms for heavy metal appropriation abilities. In conclusion, they recommend microbial bio-sorption method in the removal of excess toxic metals from soil in order to compliment and beef up food security.

Omwenga (2013) tested the impact of heavy metal residue on freshwater tilapia. The results of the studied showed a significance difference ($P < 0.05$) in the mean of lead in the brain (31.31 ppm), liver (17.33 ppm), gonad (16.62 ppm), and muscle (3.78 ppm) correspondingly contrary to the threshold of 0.5 ppm. The mean Cd (cadmium) levels (7.25, 5.35, 3.35, and 1.66 ppm) in the brain, liver, gonad, and muscle were correspondingly contrary to the threshold of 0.05 ppm. The findings of the study revealed that the studied heavy metals were relatively high compared to the allowable limits in fish. The authors recommend monitoring of agriculture and industrial wastes into superficial aquatic bodies as well as appropriate locating of fishponds to reduce the possible ecological and health risk to the life forms therein and to safeguard their present and future usage.

Bawuro et al. (2018) evaluated and reported bioaccumulation of heavy metals on several body parts of different freshwater fish species. The metals (trace and heavy) evaluated were Zn, Pb, Cd, and Cu in the liver, gills, and flesh from the bottom (benthic) and surface (pelagic) habitual species of fish, sourced from Lake Geriyo for two hydrological seasons. The results of their study showed significant variations of heavy metal contents among the fish species as well as the organ parts. More so, the skin/flesh showed the minimum concentration of all the examined trace and heavy metals, while the major target organ, the liver, revealed high accumulation of Zn, Cu, and Pb. Then, Cd, a carcinogenic metal, showed a maximum concentration in the various examined fish gills. The findings of their studies confirmed that the examined fish species had close feeding habit and possess interspecific difference of metal uptake. These features relate to the ecological and biological factors governing the fishes: species-specific responses, differences in age, and geographical dispersal. The levels of metals in the fish skin were putative by the international standard limits for Cu, Zn, and Cd. On the contrary, the concentration obtained for Pb surpassed the recommended limits in *Clarias* and *Tilapia* during the wet season and *Heterotis* in both wet and dry seasons. The authors concluded by proposing stringent monitoring of wastes from source points into the aquatic environment in order to conserve the freshwater food.

Das et al. (2019) reported the impact of using slag fertilizer in agronomic activities to buffer soil acidity level, advance plant efficiency, lessen greenhouse gas releases, and alleviate heavy metals in contaminated soils. The authors opined and propose the use of slag-microorganism collaborations for the optimization of soil quality, crop output, and environmental abatement costs. This will address the problem using slag as an agricultural improvement only and avert any likely ecological and health risks that will affect food security in the future.

Olu et al. (2013) evaluated and tested the accumulation of heavy metal level in *Zea mays* grown in designated industrial regions in Ogun State, Nigeria, and its impacts on municipal food security. The results of their findings revealed that the *Zea mays* gotten from the industrial regions of Ogun State fluctuated and had the following contents: moisture (6.35–9.45%), crude (8.31–12.68%), protein (1.49–8.294%), ether extract (1.746–2.792%), and carbohydrate (71.32–78.896%) correspondingly. The results for the chemical analysis of the soil fluctuated and showed the following: moisture (0.65–1.90%), pH (6.63–7.89), and nitrogen (4.01–8.6%) correspondingly. The results obtained for the heavy metals in maize revealed Fe (28.5–59.5 mg/kg), Cu (2–10.7 mg/kg), Mg (248.3–321 mg/kg), Ni (1.8–4.775 mg/kg), Pb (62.5–150 mg/kg), and Ca (1.2–10.2 mg/kg) correspondingly, while the results obtained in the soil samples were Fe (28.9–59.6 mg/kg), Cu (4–12.2 mg/kg), Mg (297.3–350.5 mg/kg), Ni (4.55–9.28 mg/kg), Pb (83.3–177.5 mg/kg), Ca (7.6–21.8 mg/kg), and Co (0.075–0.46 mg/kg) correspondingly. The authors propose that higher level of some of the heavy metals in Zea mays may affect food security and call for public attention.

Hezbullah et al. (2016) did a review of heavy metal pollution/influence on food security. The authors reported food security in Bangladesh impacted by As (arsenic), Cd (cadmium), Cr (chromium), and Pb (lead). They opined that agricultural activities are the major precursors of the source impact of food security via heavy metals in fertilizers and pesticides. They propose the non-consumption of food that contained the above because of the potential health risks involve: organ dysfunctions and cancer.

Raymond and Okieimen (2011) review the risks involved via heavy metal contamination in soil and its attendant effects on food security. The authors reported the likely bio-hazards and preeminent existing remedial approaches for some heavy metals (Pb, Cr, As, Zn, Cd, Cu, Hg, and Ni) frequently found in polluted soils: immobilization, soil-washing, and phytoremediation. They opined that these techniques for the utilization and remediation of heavy metal pollution in soils for agricultural activities, will advance farming and food security for the present and future generations.

Raia et al. (2019) reported the influence of heavy metals in food crops, their health risk via consumption, fate, mechanisms, and possible management. They highlighted that food security is a global concern and needed high primacy for sustainable protection. Heavy metals and metalloids such as Hg, As, Pb, Cd, and Cr found in food can interrupt human metabolic activities if consumed/ingested in large quantity and result to high ill-health and eventually death. Finally, the authors propose the sustainable management of soil in order to prevent food insecurity and to promote availability of non-toxic food to the teeming population.

Zhang et al. (2015) evaluated the effectiveness and influence of soil heavy metal contamination on food security on Chinese farmland. The results of their study revealed that Cd (cadmium) had the maximum contaminated rate (7.75%); this was followed by the lowest contaminated level: Hg (mercury), Cu (copper), Ni (nickel), Zn (zinc), Pb (lead), and Cr (chromium) which had $<1\%$. The results of the sum percentage observed in the agricultural land in China were 10.18%, sourced from Cd, Hg, Cu, and Ni. The findings of their study showed that human activities such as fertilizer application, industry heat-up, irrigation, sewage, town expansion, and mining set off the high levels of the metals into the farmland. More so, these affected the amount of grain produced to approximately 13.86% of the farmland. The authors recommended agro-soil management and security in China to protect food from toxic metals and achieve food security according to the SDGs.

Ojuederie and Babalola (2017) review the utilization of microorganisms and floras in the biodegradation of pollutants with the aim of meeting food security. The authors evaluated the various routes of pollutants to human and culminated that hazardous materials come from human activities and from natural or lithogenic origin such as earthquakes and volcanic eruptions. They stated that HM (heavy metals) which are toxic are bioaccumulated in plants via contaminated soils and pose major threat to food security. They propose an eco-friendly approach in remediating the HM in the soil by the utilization of microorganisms (micro-remediation) and plants (phytoremediation) as against the conservative and corporeal methods which are cost-effective but not active in reducing the toxic level of the soil. In conclusion, they recommend the stringent observance to bio-safety guidelines in the practice of bio-technological approaches to confirm the security of our environment and food.

Fasahat (2015) reported the topical development in understanding Cd (cadmium) noxiousness and tolerance in *Oryza sativa*. The author stated that Cd is transferred to the soil via phosphate fertilizer, industrial actions, mining, burning of paint materials, and sewage output such as sludge. It is provided to environment, mostly through effluent from sewage sludge, mining, burning, industries, and fertilization with phosphate. That Cd can be assimilated by *Oryza sativa* via this process and can cause serious impact in its metabolic activities. He further stated and elucidated current trend on heavy metals and their collaboration with vital soil components in understanding the harmfulness in the flora.

Shifaw (2018) reviewed the influence of heavy metals on agro-soils in urban areas of China. The authors reported the utilization of several ecological risk indices in elucidating pollution of heavy metals in soils as well as their point sources. The author was able to evaluate the more predominant polluted soils sourced from urban regions from agricultural activities such as the use of pesticides in farming. Apart from this point source, other dominant sources are via hazardous residues from industrial output, sewage, and waste gas. The author pointed the efforts made to reduce these pollutants and the policies put in place to abate sudden episode or prevalent occurrences. In conclusion, the author recommends sustainable use of chemicals, ecological management, and treating and monitoring of wastes before discharge into the ecosystem. If strigent attention is not placed on this ecological pollutant (heavy metals), soil poisonousness and bioaccumulation will linger and threaten eco-sustainability of China's food security and developmental strides.

Hu et al. (2019), reported and evaluated in a review the soil adulteration with HM (heavy metals) and their influence on food security in China. The authors pointed out that recently, the concern for the levels of HMs in soils has portended negatively on food safety and is on the increase. This is closely linked with many anthropogenic functions such as improper waste disposal, sewage-sludge outputs on land, fertilizers and pesticides, mining, and improper effluent disposal. They stated the food and cash crops can accumulate HMs and thence transfer via the food chain and web to humans. They propose continuous implementation of extant environmental policies that will safeguard the soils in China and also protect food for the present and future generations. More so, some modern knowhow for degradation of soil pollutants – heavy metals – were also recommended.

Enuneku et al. (2018), tested and evaluated the health implication of ingesting heavy metals in various parts of free-range chicken in humans. The results of their study revealed no significant difference ($P > 0.05$ and $P > 0.01$) in the mean heavy metal in all tested organs and across the sites where the chickens were sampled respectively. The results of the health risk indices indicted that there are probable risks in consuming Ni in the muscles and liver respectively. Their study showed that Zn and Cu were > 1 and Ni was >1 and < 1 respectively. They are major precursors of heavy metal toxicity in the organ parts of the chicken if consumed together. They forecasted serious health risk in humans and recommend stringent management and reduction of wastes before discharge into the environment in order to prevent food insecurity.

Anani and Olomukoro (2018a) tested and evaluated the health risk in the consumption of heavy metals in freshwater decapods in a tropical river in Nigeria. The results of their study indicated that the metal concentration in the decapods was in this order Fe > Zn > Mn > Cu > Pb > Cd > Cr > Ni = V for prawn and Zn > Fe > Mn > Cu > Pb > Cd > Cr > Ni = V for shrimp. The results of the health risk showed high values of iron (Fe) and zinc (Zn) and manganese (Mn) far higher than the threshold limits (>1). The finding of their study showed the high impact of the metals in humans along the food chain if consumed in the freshwater decapods. All studied metals were higher than the international permissible limits in prawn and crab correspondingly. They proposed stringent reinforcement and acquiescence of extant environmental laws, in order to safeguard humans from ingesting aquatic decapods or food sourced from different polluted sources.

Enuneku et al. (2019) evaluated and tested the probable carcinogenic impact of Pb (lead) in several canned food in Nigeria. The results of their study revealed that there was a small rise in values of Pb (lead) above the threshold limits in the different canned foods. They recommend quality control and monitoring of canned foods by concerned regulatory bodies in order to safeguard the end consumers for ingesting or consuming Pb.

18.3 Heavy Metals and Its Nexus to Food Safety

It has been observed that the presence of heavy metals available in the leachate produced during the process of composting food waste might lead to the generation of various types of pollution hazards which might warrant numerous environmental costs whenever the concentration of the heavy metal goes beyond the stipulated discharging quality standards.

In view of the aforementioned, Chu et al. (2018) utilized a logistic function technique for the determination of the extent by which pollution hazards from heavy metal such as arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), and mercury (Hg) could lead to the formation of leachates during food waste composting obtained from food waste treatment plant located in northern Shanghai, China. The result obtained showed that Cd had the highest pollution hazard rate with 94.03%, which might be linked to the Cd-containing materials such as plastics that are in contact with food waste. Moreover, it was also observed that the cost estimation of managing the environment; in the prevention of leachate produced during food waste composting, is about US$ 0.52 per tonne after reducing pollution risks which were assessed at 94.48%. Their study shows that the amount of money utilized in the management of the heavy metals in leachate produced in Shanghai's food waste was very exorbitant.

Soil has been identified to play some essential function in the maintenance of food safety because it serves as a reservoir that harbors feed and food at the base of the food chains. The quality of soil can be assessed based on the level on the amount of heavy metal present in them, which consequently has a detrimental effect on the food chain.

In view of the aforementioned, Tóth et al. (2016) collected topsoil from 22,000 different locations, and the soil was analyzed using coherent analytical techniques for the evaluation of trace elements available in them. The result gave a general overview of several heavy metals such as As, Ni, Cd, Co, Cr, Zn, Pb, and Hg. The authors reported that the presence of Hg and Cd in the topsoil might be linked to several human activities which was perceived at a regional level. Also, it was detected that many out of the European agricultural lands were confirmed to be appropriate for food production while 6.24% or 137,000 km2 necessitates local evaluation and ultimate remediation action.

Zhang et al. (2015) review critical different 465 published papers on heavy metal pollution rates in which the ratio of the samples supersedes the Grade II limits for Chinese soils, the Soil Environmental Quality Standards (1995), in farmland soil throughout China. Their result showed that Cd exhibited the maximum pollution rate of 7.75%, followed by Hg, Cu, and Ni, and Zn, Pb, and Cr had the lowest pollution rates at lower than 1%. The high concentration of heavy metal in these areas might be linked to the presence of human activities: the application of fertilizers which releases some heavy metals in the soil, irrigation by sewage, mining and smelting, and urban development lead to the high level of soil pollution. Moreover, it was observed that 13.86% of grain production were greatly affected which might be linked to the presence of heavy metal pollution in farmland soil. Their study formed a baseline study through which necessary information will be utilized for the reduction, control, and eventual prevention of heavy metal accumulation in agricultural soil in affected areas in China.

Ali Mohamed and Al-Qahtani (2012) evaluate the level of heavy metal like Hg, Fe, Cd, Mn, Pb, Cu, and Zn available in different vegetables like legumes, roots, leaves, cereals, stems, and fruits, normally planted in four districts in the Kingdom of Saudi Arabia using atomic absorption spectrophotometer. The result obtained showed that the level of concentration of the heavy metals obtained from these vegetable exceeded the stipulated values recommended by Joint FAO/WHO Expert Committee on Food Additives. The highest value of heavy metal was detected on the leaf of parsley (543.2 and 0.048 μg/g for Fe and Hg, respectively), Jews mallow (94.12 and 33.22 μg/g for Mn and Zn, respectively), and spinach (4.13 μg/g for Cd), while the peas in legumes group sustained the maximum Zn content 71.77 μg/g, and finally cucumber had the highest Pb content 6.98 μg/g on dry matter basis. The high concentration of the heavy metal in various parts of the vegetables might be linked to the high concentrations of polluted air most especially the one located around the industrial areas, in the eastern and the middle districts. In conclusion, the authors suggested that marketing systems and atmospheric depositions play a crucially significant in promoting the amount of heavy metals in the vegetables possessing prospective consumers of locally made foodstuffs.

Rai et al. (2019), wrote a comprehensive review of the influence of some heavy metal such as Cr, Hg, Cd, As, and Pb on human health whenever some food crops are contaminated with such metals. The authors highlighted that these metals portend the capability to affect human metabolomics which could eventually lead to high rate of morbidity. They also suggested the possible modes of action through which these contaminants enter into the food crops, and they suggested several possible bioremediation techniques that could be used for effective mitigation of these heavy metals. Also, detailed and recent information on the worldwide geographical pattern of heavy metal was also highlighted in detail.

18.4 Conclusion and Future Recommendation

This chapter establishes through various scenarios that there is a nexus between food security and safety, environmental contamination, and human health. The level of heavy metal in the environment has been validated to increase through various daily anthropogenic activities and which varies from countries to countries. Moreover, special regulatory policy needs to be enforced in most of the developing countries that will monitor the application of pesticides to agricultural crops whenever sewage sludge, synthetic fertilizer, and industrial effluents are applied as fertilizers. They constitute the primary source of food contamination sources, most especially in developing countries where the irrigation water is not treated effectively. Moreover, there is a need to examine human health risks at a global scale using various epidemiological techniques. Also, sustainable remediation which is eco-friendly in nature needs to be adopted in various countries most especially in developing nations. There is a need to search for techniques that are precise and accurate for efficient mapping of soil in order to avert the movement of heavy metals into the food and soil. The application of beneficial microorganism has been discovered to be sustainable, economical, and effective bioremediation strategy for the remediation of contaminated soils. Furthermore, the application of eco-feasible technological innovations through the application of nanotechnologies as well as the sensitization of poor farmers could lead to improvement of GDP (gross domestic production) and drastic improvement in the economy of various countries.

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19 Utilisation and Management of Agriculture and Food Processing Waste

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Abstract

Agriculture and food industries are very close to each other. Food industries are usually divided into two forms: food manufacturing and food processing units. The food industries mostly depend on the agriculture sector. The agriculture sector fulfils the primary requirements of food industries by providing raw materials in the form of fruits, vegetables, grains, etc. Agriculture and food processing wastes are the end products of various food processing industries that have not been recycled or used for other purposes. They are the non-product flows of raw materials whose economic values are less than the cost of collection and recovery for reuse and therefore discarded as wastes. Recycling and eventual utilisation of food processing residues offer potential of turning these by-products to beneficial uses rather than discharging these to the environment which cause detrimental environmental effects. The food industry produces large volumes of wastes, both solids and liquid, resulting from the production, preparation and consumption of food. These wastes pose increasing disposal and potential severe pollution problems and represent a loss of valuable biomass and nutrients. Besides their pollution and hazard aspects, in many cases, food processing wastes might have a potential for conversion into useful products of higher value as by-product, or even as raw material for other industries, or for use as food or feed after biological treatment. The composition of wastes emerging from food processing factories is extremely varied and depends on both the nature of the product and the production technique employed. The present studies focused on how waste materials of agriculture and food processing industries can be utilised and manage appropriately.

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Keywords

Food · Waste materials · Agriculture · Food manufacturing · Packaging wastes

19.1 Introduction

The report which was submitted by the Council for Agricultural Science and Technology (CAST) describes the agricultural enterprise of the United States and identifies major streams of by-products and waste products associated with several sectors of the enterprise. The food production process begins with the biochemical reaction occurring in the leaves (green parts) of plants, or *photosynthesis*, where carbon dioxide is combined with water in the presence of natural sunlight to form organic matter and oxygen. The resulting products include grasses, hay, fruits, vegetables, trees and grains, e.g. wheat, barley and corn. These products are harvested, processed and packaged for consumption by humans for feed to animals producing, for example, milk, eggs, wool or meat, all of which in turn can be processed and packaged. Approximately 18% of private sector employment derives from the food enterprise system, which employs farmers, herders, processors, transportation workers, wholesalers, retailers and others. This industry employs more than 22 million Americans and annually generates $902.5 billion, or 14.2%, of the gross domestic product (GDP). A growing segment of the agricultural industry is directed to exports, which were projected to hit a high record of $45 billion in 1995. Agricultural and food products are crucial to the well-being of society. Food production and processing sectors generate solid and liquid residues, or wastes. As concerns about environmental pollution, and in some instances human health risks, mount, it becomes increasingly important to understand the nature and the extent of wastes generated from the production, processing and consumption of food and the manner of their reuse or disposal. The United States has both an ideal climate and superior soil resources for agricultural production. The world has just five large contiguous areas highly suitable for crop production, namely, in the United States, Asian Russia, India, China and Western Europe. These areas have adequate rainfall so that nonirrigated agriculture is sustained, primarily flat and served by large river systems.

Most agricultural production waste when measured in terms of total mass is organic and consists of straw, leaves, partly digested materials in the form of manure and residues left after the processing of harvested grains, fruits or vegetables. One can obtain an approximate idea of total mass by assuming that each acre (0.4247 hectare) of land used for agriculture produces about 11,000 pounds (lb) (500 kg) of dry matter consisting of cellulose, starch, sugars and proteins, all biodegradable materials. The 964 million acreage used for agriculture thus yield 10.6 trillion lb (4.82 × loL2k g), which, packed to the density of hay or 50 kg/m (mY), would need to be stacked 0.66 m high if it were distributed evenly over the land area of the state of Illinois. This amount of organic material must be disposed of each year. Much of this material is left on the land, but a large part is consumed by animals and then appears in the form of manure, which must be used or disposed. Waste materials or by-products generated on farms or at processing plants are almost exclusively organic. An excess of these materials improperly managed can – like an excess of inorganic material – cause environmental pollution. Such pollution can be defined as point source, e.g. pollution discharge through a pipe, for instance, from factories, food processing and manufacturing plants, or municipal wastewater treatment plants, or *nonpoint* source, or diffuse, e.g. pollution from agricultural, silvicultural, urban and construction runoff (US Environmental Protection Agency 1987). Production inputs required for crop production and utilisation include fertiliser, pesticide, herbicide, fuel and irrigation water. Waste occurs when any of these are spilled or used excessively; pollution can occur when they escape into the environment. Soil erosion from cultivated lands is another aspect of waste generation in the food production system. Diffuse runoff from both cropland and rangeland contributes to suspended solids, dissolved solids and biological oxygen demand in the nation's waterways. The composition of wastes emerging from food processing factories is extremely varied and depends on both the nature of the product and the production technique employed. For example, wastes from meat processing plants will contain a high fat and protein content, whilst waste from the canning industry will contain high concentrations of sugar and starches. Also, the waste may not only differ from site to site but also vary from one time of the year to another. Furthermore, the volume and concentration of the waste material will not be constant. This may cause some problems in managing a consistent working process due to fluctuations in the nature, composition and quantity of raw materials.

In general, wastes from the food processing industry have the following characteristics (Litchfield 1987):

1. Large amounts of organic materials such as proteins, carbohydrates and lipids
2. Varying amounts of suspended solids depending on the source
3. High biological oxygen demand (BOD) or chemical oxygen demand (COD)

The food processing industry also uses a large amount of water. A proportion of water used may leave as a part of the product, for example, in beer production, a large volume of it is discharged as effluent in to surface waters where the substances it contains may cause pollution. A large volume is discharged to surface waters, where the substances it contains may cause pollution. A variety of processing wastes results from the processing of grains, vegetables and fruits. The management of processing wastes requires the passage of great quantities of water through treatment facilities. Wastes produced in animal-based agricultural enterprises include production wastes in cattle feedlots, pastures and rangelands, dairy farms, poultry farms, swine farms and meat and poultry processing plants. Important waste components of the animal production facilities for cattle, dairy animals, poultry and swine are manure and dead animals. Manure can be used successfully on land as a fertiliser. Seafood production is also a large and diverse industry. Since disposal of seafood waste depends on species, plant location and processing method, use of waste products of seafood industry is complicated. Procedures for disposal of this industry, i.e. ocean dumping/landfilling, that are designed to minimise disposal problems are becoming increasingly difficult and costly as a result of environmental regulations.

Pesticides are substances used to prevent, destroy, repel or mitigate the damage of any insect, rodent, nematode, fungi or weed or any other form of life declared a pest and any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant. Pesticides can be either natural or synthetic: modern pesticide materials include synthetically produced organic chemicals, naturally occurring organic and inorganic chemicals, microbial agents (both naturally occurring organic and genetically engineered agents) and miscellaneous chemicals not commonly thought of as pest control agents, e.g. chlorine added to swimming pools for algae control or to household disinfectants.

19.2 Utilisation of Food Wastes Discharged from Different Industries

19.2.1 Dairy Industry

Dairy processing generates heavy loads of polluted effluents, which contain dilutions of whole milk, separated milk, whey and sanitisers used in the clean-in-place (CIP) system. Drippings from packaging process and accidental leaks as well as CIP water find their way into the sewer system. Yoghurt processing involves heating milk and addition of starter culture and sodium stearate to improve consistency followed by incubation. White cheese processing consists of mixing skimmed and dry milk, heating and addition of salts and rennet; the mixture is left for separation. In the past, the separated whey was being discharged to the sewerage without pretreatment, thereby increasing production losses and aggravating pollution problems. In today's milk processing plants, the whey is being recovered, and the dried whey is blended with basic food materials to produce less expensive foods such as fruit-based beverages, custards and bakery goods. Reverse osmosis has been applied on a limited scale to produce a protein supplement; the technical and economic aspects of full-scale application of this advanced treatment remain to be evaluated in depth.

Being a major by-product of cheese production, whey is defined as the serum or watery part of milk remaining after separation of the curd, those resulting from the coagulation of milk by acid or proteolytic enzymes. It is generated at a rate of about 9 kg for every 1 kg of cheese. Discharging of whey as waste creates severe pollution problems due to its high BOD (35–40 g/l). This high BOD is mainly due to the lactose which is present at concentrations between 4.5 and 5%. Although whey is utilised and converted to valuable products by the big factories, it is estimated that there are still some losses in the small factories. Some researchers have been done for utilisation of whey such as the production of single cell protein, concentrates and different food products such as alcoholic and non-alcoholic beverages produced from whey, addition of whey to bakery products and enrichment of milk by whey proteins. Whey is used in different ways and can be classified in three categories:

Table 19.1 Composition of various nutrients present in whey

Substances	Amounts
Lactose	4.9%
Salt	7.8%
Total carbohydrate	5%
Protein	0.86%
Oil	0.32%
Ash	0.31%
Riboflavin	0.10 mg/100 ml
Niacin	0.10 mg/100 ml
Fe	0.12 mg/100 ml
Na	14.5 mg/100 ml
K	30 mg/100 ml
Ca	13.5 mg/100 ml
Water	92%
pH	5.5

1. Fermentation: production of ethyl alcohol, lactic acids, and vitamins
2. Concentration: whey protein, dried whey and production of lactose
3. Pasteurisation: whey cream and pasteurised sweet whey

Membrane filtration techniques are used in milk processing and can be defined as the separation of two or more compounds from a fluid stream. Ultrafiltration (UF) is generally used for whey utilisation to separate the proteins from the permeate containing mostly lactose. Recovery of sugars, proteins and minerals in the waste effluents by crystallisation, evaporation and spray-drying are practised in many dairy plants. However, the increased cost has made many of these operations economically unattractive. Applications of UF and RO for concentrating the whey solids have gained an importance. Whey protein concentrate is an item of worldwide commerce due to its nutritious composition as shown in Table 19.1.

19.2.1.1 Production of Biomass (SCP)

It is one of the major implications of biotechnology to food waste utilisation. In general, the composition of food wastes is such that they are suitable as growth media for microorganisms. Biomass, or single cell protein (SCP), is the name given to the total dry matter of bacteria, yeast, moulds and higher fungi when used for animal feed or human food. The use in feed is related primarily to considerations of nutritional value and balance of the ingredients, whilst the level of utilisation in food is certainly limited by the concentration of nucleic acid in the final product. Regarding liquid wastes of an appropriate chemical composition, large quantities are produced in the food processing industry to be used as substrate for microbiological fermentations.

19.2.2 Fruits and Vegetables

Large amounts of fruit and vegetable processing wastes are produced from packing plants, canneries, etc., which may be disposed in several ways including immediate use for landfill or drying to a stable condition (10% moisture) in order to use an animal feed during out of season, or which, alternatively, may be processed biotechnologically in order to produce SCP. The first choice is not economical, and the second one is expensive due to drying cost. The industry continues to make progress in solving waste problem through recovery of by-products and waste materials such as peel, pulp or molasses by the employment of fermentation process. The protein content of fruit and vegetable processing wastes with an adequate level of fermentable carbohydrates can be increased to 20–30% by using solid substrate fermentation. The composition of some fruits and vegetables indicates that many have a significant proportion of fermentable sugars. Oranges, carrots, apple and peas have been successfully utilised as a substrate in the fermentation. Vinegar, citric acid and acetic acid are produced from the by-products of fruit and vegetable industry. For example, apricot is one of the main agricultural products in Turkey, and it produced annually around 60,000–80,000 tons of dried apricots. Around 180–200 tons of dried apricot wastes are formed in the factories per year, which is easily available. Citric acid is produced using *A. niger* in the fermenter using dried apricot wastes as substrate. Additionally, brine, which is used in the production of pickles and olives, is an important environmental problem because there is about 20% and 7% of salt in the effluents of olive and cucumber pickles, respectively (Cooney et al. 1980). Also, wastes from potato and wheat starch processing factories can be fermented to ethanol.

Commonly used methods of managing fruit and vegetable waste are as follows:

1. Return fruits and vegetables waste to the field on which it was grown through composting.
2. Store the culled fruit and vegetables in a pile or burned area for a limited time.
3. Feed fruit and vegetable waste to livestock.
4. Process fruit and vegetable waste to separate juice from pulp.
5. Dispose fruit and vegetable waste in landfill areas.

19.2.2.1 Disposal of Fruit and Vegetable Waste in Local Landfills

Disposal of culled fruit and vegetable waste in landfill area is a method that should be considered after all other options. From a sustainability standpoint, disposal of these culls in landfill is probably not the best option based on fees.

Advantages Once the fruit and vegetable culls are dumped, all responsibility is transferred to the landfill operator. Juice associated with fruit and vegetable decomposition could increase methane production in the landfill that would be beneficial if the landfill is collecting methane for energy production.

Disadvantages High cost of disposal. Juice associated with fruit and vegetable culls will add to leachate that has to be handled.

19.2.2.2 Citrus By-Products as a Fruit Juice

The method of separating the fruit and vegetable waste into juice and pulp is accomplished by using a press. Typical systems are screw presses that can effectively separate the juice from the pulp. After separation, each fraction has its benefits for different reasons and purposes. If the waste/culls are of good food quality, they can be used as juices in food applications based on available markets. The pulp can also potentially be used as a component of foods. For those culls that are not of human food quality, the separated pulp can be used as one component of compost or animal food. The pulp can also be used as a soil amendment or as one component of a composting process. The juice can also be used as a feedstock for ethanol production or anaerobic digestion processes. For either process, there should be a market for the final products, ethanol or methane. Advantages are low disposal cost and potential on-site processing. Moreover, the pulp can also be used in composting, and can be fed to animals. The fruit and vegetable juice will be easier to transport and apply to the receiving field/pasture, it can be a feedstock for ethanol or anaerobic digestion processes and is easier to store than whole. Due to the large amounts being processed into juice, a considerable by-product industry has evolved to utilise the residual peels, membranes, seeds and other compounds. Residues of citrus juice production are a source of dried pulp and molasses, fibber pectin, cold-pressed oils, essences, limonene, juice pulps and pulp wash, ethanol, seed oil, pectin, limonoids and flavonoids. Fibre pectins may easily be recovered from lime peels and are characterised by high fibre contents. The main flavonoids found in citrus species are hesperidin, narirutin, naringin and eriocitrin. Peel and other solid residues of lemon waste mainly contained hesperidin and eriocitrin, whilst the latter was predominant in liquid residues. Citrus seeds and peels were found to possess high antioxidant activity. Both in vitro and in vivo studies have recently demonstrated health-protecting effects of certain citrus flavonoids.

Citrus juice processing is one of the important food industries of the world, yielding an enormous quantity of processing residue. Juice recovery from citrus fruit is about 40–55%, with the processing residue consisting of peel and rag, pulp wash, seeds and citrus molasses. Citrus peel and rag are normally used either for pectin manufacture or they are dried and sold as cattle feed. These citrus by-products and wastes have already drawn attention not only as important sources of dietary fibber for human consumption but also as fermentable substrates for obtaining valuable chemicals and feedstock. Citrus by-products and wastes also contain large amount of colouring material in addition to their complex polysaccharide content. Hence, they are a potential source of natural clouding agents, which are in great demand in soft drinks industry. Commercial clouds presently used in the soft drinks industry are very expensive and may contain compounds restricting their use in certain countries. Little information is available on the composition, strength or stability of commercial clouds.

Several researchers (Sreenath et al. 1995) conducted studies to utilise citrus industry by-products and wastes as natural sources for the production of beverage clouding agents using fermentation techniques, pectinolytic treatment and alcohol extraction. They also evaluated the strength and stability of prepared clouds in model test beverage systems to determine their similarities to commercially available beverage cloud types.

19.2.3 Oil Industry

The by-products resulting from olive oil extraction (some examples are rice bran oil from husk of rice, etc.) are the vegetation water, also called black water or vegetable water, and the olive husk including skins and stones. Depending on the processing conditions, 50–110 kg of water results from 100 kg of olives. Most of the olive oil factories have no treatment systems, and the discharge of this dark brownish wastewater causes serious environmental problems with high BOD (90–100 g/L). Many studies have been done to find an alternative ways to use this by-product. Composition of olive oil black water is given in Table 19.2.

The husk can be reprocessed for the recovery of olive oil, or extracted with an organic solvent to yield husk oil. Dried husk is utilised as fuel or animal feed. A process for the separation of the vegetation water from the solids by evaporation has been recently described. The remaining solid fraction representing 98% of the organic load could be mixed with the husk and used as a fuel. Olive oil wastewaters are rich in antioxidant compounds, particularly in hydroxytyrosol derivatives. Hydroxytyrosol strongly inhibited LDL oxidation stimulated by 2,2′-azobis (2-aminopropane) hydrochloride. Further investigations point out that hydroxytyrosol and oleuropein are potent scavengers of superoxide radicals. Several researchers have studied production of single cell protein from the black water of olive. In addition to SCP, olive oil black water has been studied for production of natural antimicrobials (Demirel and Karapınar 2000). Naturally occurring phenolic compounds in black water such as phenolic glycoside, oleuropein and its hydrolysis products inhibit the growth of some fungi and a number of G(−) bacteria.

Table 19.2 Composition of olive oil black water

Substances	Amounts
Sugar	0.98%
Protein	0.77%
Oil	0.4%
Dry matter	6.2%
Ash	1.4%
Fe	0.3 mg/100 ml
Ca	7.5 mg/100 ml
K	112 mg/100 ml
Na	39.5 mg/100 ml
pH	4.5

Microbiological studies have shown that discharge of black water to soil inhibits the growth of aerobic spore-forming bacteria, such as *B. megaterium*. In addition, it is shown that oleuropein is able to prevent the germination of *B. cereus*. Some studies, such as inhibition of growth of *S. aureus* and production of its toxins and also inhibition of growth of *A. flavus* and aflatoxin production, gives good results from the point of using these phenolics as natural antimicrobials. Additionally, usage of sludge as an organic fertiliser has been studied (Anac et al. 1993).

The world's demand for energy grows rapidly, and therefore, the time has come to look for alternative sources of energy, such as renewable energy, to replace the rapidly depleting supply of fossil fuel. Producing energy from renewable oil wastes can contribute to avoiding the use of fossil fuel for this industry. The oil industry is one of the biggest industries all over the world. Using the fibber and shell from the processing wastes as an alternative fuel for electricity generation for this industry can do a lot of savings. In Malaysia, where many giant palm oil plantations and processing industries have been developed, researchers (Mahlia et al. 2001) have dealt with energy conversion from the fibber and shell of the industry wastes as an alternative energy source. They proved that the fibber and shell could be conventionally used as fuel for a steam boiler. The calculations have shown that oil wastes can generate more than enough energy to meet the energy demand of the palm oil mill. Another advantage of using the fibber and shell as a boiler fuel is that it helps to dispose these bulky materials which otherwise would contribute to environmental pollution. The ash from the combustion process is also found suitable for fertiliser for palm oil plantation.

19.2.4 Food Packaging Wastes

Of all the manufacturing industries, the food industry makes the largest demand on packaging, and finding ways to reduce this packaging quantity and its subsequent waste is a demanding task. In practice, optimised packaging criteria for the least environmental impact are often difficult to define without extensive dialogue. The needs of the consumer, the requirements of the supermarket, and the optimisation available to the producer or manufacturer may variously conflict with one another. In order for waste minimisation to provide continuing environmental improvement in the key areas of putrescible wastage and packaging, improved consensus and dialogue is required between purchasers, including both consumers and major supermarkets, and those that produce and manufacture (Gustavsson et al. 2011). Food producers and manufacturer must give greater priority to reducing raw material wastage as far as practically possible and identifying the process options that result in a lower waste burden.

19.2.5 Food Waste Composting

Composting is the natural process of decomposition and recycling of organic material into a humus rich soil amendment known as *compost*. For any business or institution producing food waste, this organic material can be easily decomposed into high-quality compost. Fruits, vegetables, dairy products, grains, bread, unbleached paper napkins, coffee filters, eggshells, meats and newspaper can be composted. If it can be eaten or grown in a field or garden, it can be composted. Items that cannot be composted include plastics, grease, glass and metals including plastic utensils, condiment packages, plastic wrap, plastic bags, foil and silverware, drinking straws, bottles, polystyrene or chemicals. Items such as red meat, bones and small amounts of paper are acceptable, but they take longer to decompose. Food waste has unique properties as a raw compost agent. Because it has a high moisture content and low physical structure, it is important to mix fresh food waste with a bulking agent that will absorb some of the excess moisture as well as add structure to the mix. Bulking agents with a high C/N ratios, such as sawdust and yard waste, are good choices. Pre-consumer food waste is the easiest to compost. It is simply the preparatory food refuse and diminished quality bulk, raw material food that is never seen by the consumer. This food waste is generally already separated from the rest of the waste stream generated; thus, no change is needed to keep contaminants out of the future compost.

Food waste that is not composted generally goes directly to a landfill. Composting provides a way in which solid wastes, water quality and agricultural concerns can be joined. An increasing number of communities, businesses, institutions and individuals are expected to turn to composting to divert materials from landfills and to lower waste management costs. Although waste stream managers view composting primarily as a means to divert materials from disposal facilities, the environmental benefits, including reduction in water pollution, and the economic benefits to farmers, gardeners and landscapers can be substantial. Composting culled fruits and vegetables is one option that can reduce the volume of culls as well as other "waste" materials in a community, if the land and equipment are available. Culls used in the compost process would either be transferred in a truck to the composting facility or mechanically transported if the compost facility is on-site. The culls would be mixed in proper ratios with other organic materials as recommended by composting professionals to produce compost suitable for reincorporation into fields or for selling. Georgia has a set of composting guidelines and regulations that should be referenced to ensure proper environmental protection and to obtain information on final management of the compost product. The pros and cons of a composting system are:

Pros
- Low disposal cost.
- Potential on-site composting.
- Potential low transportation cost to disposal site.
- Fruit and vegetable culls will generally decompose in weeks.

- Associated fruit and vegetable juice will be one source of needed water in the compost pile.
- The final product can potentially be sold for profit.
- The product can be returned to the growing field to provide stable nutrients and organic matter for the next crop.

Cons
- Filler material for composting fruit and vegetable culls can be a high cost, etc.

The food industry today has become highly diversified, with manufacturing ranging from small, traditional, family-run activities that are highly labour-intensive to large, capital-intensive and highly mechanised industrial processes. Many food industries depend almost entirely on local agriculture or fishing. In the past, this meant seasonal production and hiring of seasonal workers. Improvements in food processing and preservation technologies have taken some of the pressure off workers to process food quickly to prevent spoilage. This has resulted in a decrease in seasonal employment fluctuations. However, certain industries still have seasonal activities, such as fresh fruit and vegetable processing and increases in production of baked goods, chocolate and so forth for holiday seasons. Seasonal workers are often women and foreign workers. The world's food product output has been increasing.

19.2.5.1 Different Composting Methods

Composting can be done by different methods, these are:

(i) **Passive composting** or **piling:** This is simply stacking the materials and letting them decompose naturally. This method is simple and low cost but is very slow and may result in objectionable doors.
(ii) **Aerated static piles:** In aerated static piles, air is introduced to the stacked pile via perforated pipes and blowers. This method requires no labour to turn compost, but it is weather-sensitive and can have unreliable pathogen reduction due to imperfect mixing.
(iii) **Windrows:** It is long, narrow piles that are turned when required based on temperature and oxygen requirements. This method produces a uniform product and can be remotely located. However, turning the compost can be labour-intensive or require expensive equipment. Windrows are typically used for large volumes, which can require a lot of space. In addition, windrows can have door problems and have leachate concerns if exposed to rainfall.
(iv) **Bins:** In bins, using wire mesh or wooden frames allows good air circulation, is inexpensive and requires little labour. Three chamber bins allow for faster compost production utilising varying stages of decomposition. Bin composting is typically used for small amounts of food waste.
(v) **Vermicomposting:** This type of composting uses worms to consume the food waste and utilises its castings as high-quality compost. This is usually done in containers, bins or greenhouses (Kim 2014). Typically 1 pound of worms can

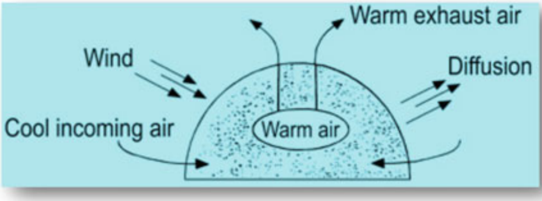

Fig. 19.1 Passive composting (Risse 2003)

Fig. 19.2 Aerated static pilling methods (Risse 2003)

eat 4 pounds of waste per week. Worm castings bring a premium price, but the investment in worm stocking may be high depending on the size of the operation. If too much waste is added, anaerobic conditions may occur. In addition, worms cannot process meat products (Figs. 19.1 and 19.2).

19.2.5.2 Monitoring Food Waste Compost

For getting better compost, proper monitoring of food waste composting is very essential. In this regard, appropriate nutrient or carbon to nitrogen ratio (C/N) is important for bacteria to process organic material into compost. The optimum ratio to begin composting is 30:1. If the ratio increases, decomposition is slowed, and then the ratio decreases foul doors and nitrogen loss can occur. Food waste is typically 15:1, fruit waste 35:1, leaves 60:1, bark 100:1 and sawdust 500:1. For example, a process using 1 part leaves and 1 part food waste by volume would achieve close to a 30:1 ratio. A moisture content of 60 per cent is optimal for microorganisms to break down the compost. Moisture contents above 70% create anaerobic conditions, slow down the process and can create foul doors. Moisture below 50% also reduces the decomposition process. The moisture content of fresh food waste is 80–90%, sawdust is 25% and yard waste is 70%. Compost with a proper moisture content will form a clump and will slightly wet your hand when squeezed. If the clump drips water, it is too wet and may require additional aeration or more bulking agent. If the compost falls through your fingers, it is too dry and may need water additions or more food waste.

Aeration or oxygen is essential for optimum microorganism populations to effectively break down the composting material. Turning, mixing and the use of blowers, fans, aeration tubes, aeration holes or raising the compost off the ground can do this. Particle size can affect the rate of decomposition of compost. The smaller the particles, the more aeration the compost receives, and microorganisms can break down smaller pieces faster. Shredding, chipping, chopping or cutting composted materials before they enter the compost pile can accomplish this. pH levels from 6.0 to 7.8 are considered high-quality compost. Proper C/N ratios should create optimum pH levels. Starting with a fairly neutral pH will ensure high levels of microorganisms for efficient decomposition. Temperature of the compost is important whilst biological activity takes place in the decomposition process. Low outside temperature would slow down the process, whilst warmer conditions would speed up the process. Mesophilic bacteria function between 50 and 113 °F to begin the composting process. Thermophilic bacteria take over and grow between 113 °F and 158 °F. These high temperatures are what destroy weed seeds and pathogens in the compost. Some composting manures can reach temperatures of 200 °F. However, temperatures above 158 °F may burn the compost or create conditions suitable for spontaneous combustion.

19.3 By-Products of Agriculture as Sources of Functional Compounds

There is a rapidly growing interest in the role of agricultural plant secondary metabolites in food and their potential effects on human health. Furthermore, consumers are increasingly aware of diet-related health problems, therefore demanding natural ingredients, which are expected to be safe and health promoting. By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of their favourable technological or nutritional properties (Helkar et al. 2016).

19.3.1 Apple

Production of pectin is considered the most reasonable way of utilising apple pomace both from an economical and from an ecological point of view. In comparison to citrus pectins, apple pectins are characterised by superior gelling properties. However, the slightly brown kind of apple pectins caused by enzymatic browning (polyphenolase enzyme) may lead to limitations with respect to their use in very light-coloured foods. Apple seeds are also very harmful for our health; if we eat 200 apple seeds or 20 seeds with its core, it will produce much amount of cyanide.

(Apple seeds contains amygdalin, a substance which releases cyanide when it contacts with human digestive enzyme. Amygdalin contains cyanide and sugar which when ingested by our body is converted into hydrogen cyanide (HCN), which is highly toxic). Apple pomace has been shown to be a good source of polyphenols, which are predominantly localised in the peels and are extracted into the juice to a minor extent. Major compounds isolated and identified include catechins, hydroxycinnamates, phloretin glycosides and quercetin glycosides. Since some phenolic constituents have been demonstrated to exhibit strong antioxidant activity in vitro, commercial exploitation of apple pomace for the recovery of these compounds seems promising. Inhibitory effects of apple polyphenols and related compounds on carcinogenicity of streptococci suggest their possible application in dentifrices.

19.3.2 Grapes

Apart from oranges, grapes are the world's largest fruit crop with more than 60 million tons produced annually. About 80% of the total crop is used in winemaking (red grape is mainly used in making red wine from its peel and due to this it is costly), and pomace represents approximately 20% of the weight of grapes processed. From these data, it can be calculated that grape pomace amounts to more than 9 million tons per year. Its composition varies considerably, depending on grape variety and technology of winemaking. A great range of products such as ethanol, tartrates, citric acid, grapeseed oil, hydrocolloids and dietary fibre are recovered from grape pomace. Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes are the principal phenolic constituents of grape pomace. Anthocyanins have been considered the most valuable components, and methods for their extraction have been reported. Catechin, epicatechin, epicatechin gallate and epigallocatechin were the major constitutive units of grape skin tannins. Since grape and red wine phenolics have been demonstrated to inhibit the oxidation of human low-density lipoproteins, a large number of investigations on the recovery of phenolic compounds from grape pomace have been initiated. From a nutritional point of view, these phenolics are highly valuable since they are absorbed to a large extent. The antioxidant activity of grape pomace has led to the development of a new concept of antioxidant dietary fibber.

19.3.3 Peach and Apricot

Bitter apricot seeds are by-products of the apricot processing industry. Apart from the use of apricot seed oil in cosmetics, peeled seeds serve as a raw material for the production of persipan, this requires debittering by hydrolysis of amygdalin (excess intake of amygdalin is toxic). Pomace of wild apricot proved to be a rich source of proteins but also contained low levels of amygdalin. Besides apricot seeds, peach seeds may also be used for the production of persipan. Recently, the recovery of pectin from fresh peach pomace has been described. Quality evaluation revealed that peach pectin is highly methoxylated and has favourable gelling properties. Whilst storage of dry powdered pomace led to significantly higher pectin yields, quality parameters of the pectins deteriorated.

19.3.4 Mango

Mango is one of the most important tropical fruits. Mango and mango products experience worldwide popularity and gained increasing relevance also in the European market. The major wastes of mango processing are peels and stones, amounting to 35–60% of the total fruit weight. Mango seed kernel fat is a promising source of edible oil and has attracted attention since its fatty acid and triglyceride profile is similar to that of cocoa butter. Therefore, legislation has recently allowed mango seed kernel fat to be used as a cocoa butter equivalent. Mango seed kernels may also be used as a source of natural antioxidants. The antioxidant principles were characterised as phenolic compounds and phospholipids. The phenolics were assumed to be mainly gallic and ellagic acids and gallates. Ethanolic extracts of mango seed kernels display a broad antimicrobial spectrum and are more effective against Gram (+ve) than against Gram (−ve) bacteria. A standardised method for the recovery of good quality mango peel pectin with a degree of esterification of about 75% has recently been developed. Mango peels are also reported to be a good source of dietary fibber containing high amounts of extractable polyphenolics.

19.3.5 Pineapple

The world's annual pineapple production is about 13×10^6 tons. Pineapple juice is largely consumed around the world, mostly as a canning industry by-product, in the form of single strength, reconstituted or concentrated and in the blend composition to obtain new flavours in beverages and other products. The pulpy waste material resulting from juice production still contains substantial amounts of sucrose, starch and hemicellulose and may therefore be used for ethanol production. In contrast to papain from papaya, the proteolytic enzyme bromelain may also be recovered from the mature fruit. Enzymatic browning of fresh and dried apple rings is inhibited by pineapple juice. The antioxidant principles have been structurally elucidated, and methods for their recovery from pineapple juice and from pineapple processing plant waste streams have been described.

19.3.6 Banana

Banana represents one of the most important fruit crops, with a global annual production of more than 50 million tons. Worldwide production of cooking bananas amounts to nearly 30 million tons per year. Peels constitute up to 30% of the ripe fruit. About 1000 banana plants are estimated to yield 20–25 tons of pseudo-stems providing about 5% edible starch. Attempts at utilisation of banana waste include the biotechnological production of protein, ethanol, α-amylase, hemicellulases and cellulases. Very recently, anthocyanin pigments in banana bracts were evaluated for their potential application as natural food colorants. It was concluded that the bracts proved to be a good and abundant source of anthocyanins of attractive appearance, as well as being a useful tool in anthocyanin identification since all six most common anthocyanidins (delphinidin, cyanidin, pelargonidin, peonidin, petunidin and malvidin) are present. Most of the carotenoids found in banana peels were demonstrated to be xanthophylls esterified with myristate and to a lesser extent with laurate, palmitate or caprate.

19.3.7 Guava

Guava is a rich source of relatively low methoxylated pectins (50%), amounting to more than 10% of the dry weight. Since wastes constitute only 10–15% of the fruit, the use of guava for pectin production is limited. The seeds, usually discarded during processing of juice and pulp, contain about 5–13% of oil rich in essential fatty acids. The results of very recent investigations indicate that peel and pulp of guava fruits could be used as a source of antioxidant dietary fibber.

19.3.8 Papaya

Papain, a proteolytic enzyme used as a meat tenderiser and as a stabilising agent in the brewing industry, is recovered from the latex of papaya fruit. Furthermore, papaya fruits may also be used for the production of pectin. Owing to their spicy flavour, which is caused by glucosinolate degradation, the seeds are sometimes used as a substitute and even as an adulterant for pepper. The seed oil is low in polyunsaturated fatty acids, but defatted papaya seed meal contains high amounts of crude protein (40%) and crude fibber (50%).

19.3.9 Passion Fruit

The waste resulting from passion fruit processing consists of more than 75% of the raw material. The rind constitutes 90% of the waste and is a source of pectin (20% of the dry weight). Passion fruit seed oil is rich in linoleic acid (65%).

19.3.10 Kiwifruit

Kiwifruit waste results from rejected kiwifruits, which comprise up to 30% of the total kiwifruit crop, and from kiwifruit pomace after juice production. A comprehensive review of the components and potential uses of kiwifruit waste has recently been given, inferring that only little work has so far been conducted on finding uses for kiwifruit pomace. The total dietary fibber content of kiwifruit pomace amounts to approximately 25% on a dry weight basis. Phenolic acids, flavanol monomers, dimers and oligomers and flavonol glycosides have recently been characterised in kiwifruit pulp.

19.3.11 Tomato

Tomato juice is the most important vegetable juice with respect to per capita consumption, followed by carrot juice. About 3–7% of the raw material is lost as waste during tomato juice pressing. Tomato pomace consists of the dried and crushed skins and seeds of the fruit. The seeds account for approximately 10% of the fruit and 60% of the total waste, respectively, and are a source of protein (35%) and fat (25%). Tomato seed oil has attracted interest since it is rich in unsaturated fatty acids, especially in linoleic acid. Recently, the optimisation of degumming, bleaching and steam deodorisation was reported. Sensory evaluation of products made with tomato seed and sunflower oil revealed no significant differences. Lycopene is the principal carotenoid causing the characteristic red hue of tomatoes. Most of the lycopene is associated with the water-insoluble fraction and the skin. Therefore, skin extracts are especially rich in lycopene. A large quantity of carotenoids is lost as waste in tomato processing. Supercritical carbon dioxide extraction of lycopene and β-carotene from tomato paste waste result recoveries was up to 50%. Enzymatic treatment of tomato marc enhances lycopene extractability. Recently, saccharification to obtain biomass from tomato pomace has also been reported.

19.3.12 Carrot

Carrot juices and blends thereof are among the most popular non-alcoholic beverages. Steady increase of carrot juice consumption has been reported from various countries (consumption increases due to its carotenoid content and is the best source of minerals). Despite considerable improvements in processing techniques including the use of depolymerising enzymes, mash heating and decanter technology, a major part of valuable compounds such as carotenes, uronic acids and neutral sugars is still retained in the pomace which is usually disposed as feed or as fertiliser. Juice yield is reported to be only 60–70%, and up to 80% of carotene may be lost with the pomace. The total carotene content of pomace may be up to 2 g per kg dry matter, depending on processing conditions.

Various attempts were made at utilising carrot pomace in food such as bread, cake, dressing and pickles and for the production of functional drinks, and carrot and pineapple blended juice are nutritional heroes; they store a goldmine of nutritive. The carrot is a herbaceous plant containing about 87% of water and is rich in minerals, salt and vitamins. The high level of beta carotene is very important and gives carrots their distinctive orange colour. In recent years, a steady increase of carrot juice consumption has been reported in many countries (Schieber et al. 2001). However, consumer acceptance of such products still needs to be demonstrated, especially since sensory quality may be adversely affected. Pigments of spray-dried carrot pulp waste proved to be prone to degradation during storage, depending on storage time and temperature. It was suggested that the stability of carotenoid powder could be greatly enhanced by employing appropriate packaging methods and storage conditions. Freeze-dried powder shows higher pigment stability during storage than the spray-dried product.

19.3.13 Onion

The amount of onion waste produced annually in the European Union is estimated at approximately 450,000 tons. The major by-products resulting from industrial peeling of onion bulbs are brown skin, the outer two fleshy leaves and the top and bottom bulbs. Owing to their strong characteristic aroma and their susceptibility to phytopathogens, onion wastes are not suitable as fodder. However, they are a source of flavour components and fibber compounds and particularly rich in quercetin glycosides. The major flavonoids of mature onion bulbs are quercetin 3, 4′-*O*-diglucoside and quercetin 4′-*O*-monoglucoside, accounting for more than 85% of the total flavonoids. Since quercetin from onions is rapidly absorbed and slowly eliminated, it could contribute significantly to antioxidant defence.

19.3.14 Red Beet

More than 200,000 tons of red beets are produced in Western Europe annually, most of which (90%) is consumed as vegetable. The remainder is processed into juice, colouring foodstuff and food colorant, the latter commonly known as beetroot red. Though still rich in betalains, the pomace from the juice industry accounting for 15–30% of the raw material is disposed as feed or manure. The collared portion of the beetroot ranges from 0.4% to 2.0% of the dry matter. Beets are ranked among the ten most potent vegetables with respect to antioxidant capacity. A more recent investigation showed that total phenolics decreased in the order peel (50%), crown (37%) and flesh (13%). Epidermal and subepidermal tissues, i.e. the peel, also carried the main portion of betalains with up to 54%, being lower in crown (32%) and flesh (14%). Whereas the collared fraction consisted of betacyanins and betaxanthins, the phenolic portion of the peel contained tryptophan, *p*-coumaric and ferulic acids, as well as cyclodopa glucoside derivatives. Therefore, the exploitation of peel and pomace for phenolics and betalains is a real need. Betacyanins were demonstrated to be strong antioxidants in various model systems, and their positive charge may increase their affinity to biological membranes, which are the preferred targets of oxidation. Literature data imply a low rate of betalain absorption, and a critical concentration for the bioactivity of these compounds in human plasma has yet to be established. High content in folic acid amounting to 15.8 μg/g dry matter is another nutritional feature of beets. Folic acid is one of the ten essential vitamins in human diet, and its value has been recognised in recent years by an important increment in governmentally recommended allowances from 400 μg to 800 μg for pregnant women in the United States.

19.3.15 Potato

Whilst consumption of potatoes has decreased, processed products such as French fries, chips and puree have experienced a growing popularity. Peels are the major waste of potato processing. Losses caused by peeling range from 15% to 40%, their amount depending on the procedure applied, i.e. steam, abrasion or lye peeling. Aqueous peel extracts were shown to be a source of phenolic acids, especially of chlorogenic, gallic, protocatechuic and caffeic acids. The antioxidant activity of freeze-dried water extracts of potato peels was comparable to that of butylated hydroxyanisole. The extracts displayed species-dependent antibacterial but no mutagenic activity.

19.4 Future Prospects of Agricultural and Food Processing Products

The utilisation of by-products of food processing as a source of functional compounds and their application in food is a promising field, which needs interdisciplinary research of food technologists, food chemists, nutritionists and toxicologists. In the near future, we are challenged to respond to the following research needs:

1. Food processing technology should be optimised in order to minimise the amounts of waste arising.
2. Methods for complete utilisation of by-products resulting from food processing on a large scale and at affordable levels should be developed. Active participation of the food and allied industries with respect to sustainable production and waste management is required.
3. Natural and anthropogenic toxins such as solanin, patulin, ochratoxin, dioxins and polycyclic aromatic hydrocarbons need to be excluded by efficient quality control systems. Minimisation of potentially hazardous constituents in products, e.g. solanin and amygdalin, and optimisation of valuable compounds such as carotenoids and betalains may also be achieved by plant breeding.
4. There is a need for specific analytical methods for the characterisation and quantification of organic micronutrients and other functional compounds.
5. The bioactivity, bioavailability and toxicology of phytochemicals need to be carefully assessed by in vitro and in vivo studies.

Furthermore, investigations on stability and interactions of phytochemicals with other food ingredients during processing and storage need to be initiated.

19.5 Conclusion

Demographic pressure, irregular distribution of agricultural resources and the need to insure preservation of food products to facilitate their better distribution explain the rapid technical evolution in the food industries. In practice, to satisfy population requirements, there is a need not only for a sufficient quantity of foodstuffs, which presupposes an increase of production, but also strict control of cleanliness to obtain the quality essential to maintain the health of the community. Only transformation of techniques justified by production volumes in a stable production environment will eliminate manual handling hazards. In spite of the extreme diversity of the food industries, the preparation processes can be divided into handling and storage of raw materials, extraction, processing, preservation and packaging. Certainly, functional foods represent an important, innovative and rapidly growing part of the overall food market. However, their design, i.e. their complex matrix and their composition of bioactive principles, requires careful assessment of potential risks which might arise from isolated compounds recovered from by-products.

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20 Nexus Between Climate Change and Food Innovation Technology: Recent Advances

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Abstract

Agriculture is a significant sector of the economy of any country. The influence of climate change on food production as it relates to agriculture varies with respect to space and time. The impacts are diverse and highly ambiguous. Innovation technology in agriculture is a significant response for effective and equitable adaptation and mitigation, and we must have to reconsider how to encourage innovation technology to address the diverse and ambiguous impacts of climate change so as to improve food production. Therefore, we have to look towards climate-smart agricultural activities via innovation technology. For climate-smart agriculture, we will require more resilience in agricultural activities and also more proficiency of resource use for both adaptation and mitigation. Undoubtedly, climate change has strong connection with agriculture. This nexus is stronger in developing countries because their means of livelihood depends mostly on agricultural activities, and these activities generally depend on climatic condition. Hence, in this chapter, we will briefly review the recent advances in agriculture

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with respect to the nexus between climate change and food innovation technology.

Keywords

Agriculture · Climate change · Environmental pollutions · Food security · Technology

20.1 Introduction

It has been stated that the world population will rise tremendously in the year 2050. Globally, there are various countries that are currently facing difficulty in achieving food security and safety due to climate change, anthropogenic activities coupled with various environmental pollutions and many more. Therefore, there is the need to increase the production and distribution of high quality and sufficient quantity of food supply to the ever-increasing population globally (Keating and Carberry 2010). Also, his food must be safe, reliable and affordable for all various sectors of the society (Ingram et al. 2011; Clay 2011; Foley et al. 2011; Godfray et al. 2010; Beddington et al. 2012).

It has been highlighted that climate change is one of the most impending issues in the achievement of food production, food security and its safety (Pielke et al. 2007). Some typical examples of environmental changes that have a great influence to the future food security include the availability of freshwater, the nitrogen and phosphorus cycles, changes in biodiversity, oceanic acidification, and rapid changes in biodiversity (Rockstrom et al. 2009).

Climate change has been identified as one of the most challenging issues affecting the achievement of food security as well as a detrimental effect on livelihoods of low-income persons and populations, which have reduced amount of capability for adaptation and depend on extremely climate-sensitive activities such as agriculture (Nwankwo and Ukhurebor 2019; Nwankwo et al. 2020). It has been observed that short-term enhancement in the rate of climate variability has a greater influence as compared to the longer-term alteration in mean values, and the suitable emphasis of adaptation is climate risk supervision (Hansen et al. 2007).

Food chain events are the engineering and dissemination of inputs (seed, animal feed, fertilizers, pest control); agricultural invention (crops, livestock, fisheries, wild foods); primary and secondary handling, packaging, storage, conveyance and dissemination; marketing and retail; catering; domestic food management; and waste management. Most of the human intervention that plays an active role in the food systems poses numerous roles which includes securing food (for nutrition, pleasure and social functions, environmental stewardship, and livelihoods (Ericksen 2008; Ingram 2011).

It has been alleged that the present available procedures of securing and substaining food do not have the capability to guarantee nutritious and adequate nutritious food that could help to prevent extensive chronic malnutrition, and it has been estimated that 178 million children are malnourished, especially in developing countries such as Africa as well as in some developed countries. The intervention of government and some other initiatives to minimize poverty which includes social welfare schemes and job creation will go a long way towards the achievement of food security that has been subjected to acute and chronic disruptions to food obtainability, access and exploitation. Moreover, the influence of climate change on livelihoods has been recognized as a significant factor that determines maximum crop production which has a significant effect on the success of attaining food security (del Ninno et al. 2007; Ziervoge and Ericksen 2010; Zaman 2011).

Various anthropogenic activities which have been observed to create a significant tension on the already limited resources and environmental services available on the planet coupled with the ever-increasing population have a drastic impact on the climate worldwide. Therefore, there is the need for various scientists from different disciplines to use multidisciplinary approaches that need to be put together to create suitable innovative strategies in the areas of agricultural and native practices that could help the ever-increasing population worldwide in mitigating all various highlighted challenges and in coping and adapting with the already exigency of climate change. These state-of-the-art paths will indisputably be attained through the enlargement and vulgarization of suitable technology and services from the worktable of investigation that can convert worldwide susceptibility to applicable resilience, acceptable variations and operative/efficient coping motivational forces (Manyong et al. (1996).

This comprehensive review intends to highlight some critical nexus between climate change and some latest scientific innovations. Also, innovation technology in agriculture and food security was also highlighted.

20.2 Climate Change and Innovation Technology

Presently, climate change has become one of the most serious environmental menace that unpleasantly affects not just agricultural activities but other aspects of human endeavours globally (Muluneh et al. 2015; Yohannes 2016; Muluneh et al. 2017; Ukhurebor and Abiodun 2018). Climate change refers to any change in climate over time as a result of natural variability and human activities. According to the Intergovernmental Panel on Climate Change (IPCC) reports, it has to do with a change of climate which is ascribed directly or indirectly to human activities that modifies the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods (IPCC 2007a, b, c).

Climate change is believed to be mainly caused by greenhouse gas (GHG) accumulation in the atmosphere, which results in increased greenhouse consequence. Apparently, it is now universally recognized that the main cause of climate change (via global warming) is the emission of GHGs especially carbon dioxide from the heavy universal exploration and exploitation of coal and petroleum products in power plants, transportation, housing, industries and agriculture.

Proper understanding of climate change entails studying of the weather/climatic conditions. Climate has always been a major force of nature that influences mankind in a very authoritative manner right from the creation onwards. It has always been a universal concern that plays a major role in our everyday lives. For the past decades, meteorological issues have been one of the most scientifically challenging issues because of the constant dynamic nature of weather, and its continuous measurements and analysis would potentially be of assistance (Ahrens 2009; Devaraju et al. 2015; Ukhurebor and Azi 2019; Ukhurebor and Umukoro 2018; Ukhurebor et al. 2019; Salack et al. 2018; WMO 2008; Ukhurebor and Nwankwo 2020).

Climate change progression and impacts are mostly subject to human activities, and it is believed that the continuous measurements and analysis would assist in its mitigation. The severity of climate change impacts increases over time. The demand for accurate, specialized meteorological prognostications from various economic sectors has led to the continued growth of meteorological industries, particularly private meteorological prognostication services in most developed countries.

The progress of meteorological knowledge over the past years has been dependent mainly on the state of contemporary innovation technology (Wallace and Hobbs 2006). Hence, the growth of innovation technology as an application of science has helped in atmospheric science especially meteorological studies in the following ways:

- The development of instruments for measuring and monitoring various meteorological and thermodynamics variables
- Improved methods of communication for gathering meteorological data and transmitting prognostications accordingly
- The use of balloons, aircrafts, rockets and satellites for probing meteorological conditions of the atmosphere (both the lower and upper parts) and relaying such data to the Earth
- The utilization of high-speed computers for monitoring, processing and analysing the vast quantities of meteorological data acquired (Ukhurebor et al. 2017)

Meteorological measurements are required either jointly or independently and locally or globally for input to numerical meteorological models for hydrological, agricultural, environmental and geophysical purposes as indicators for climate change (Ukhurebor and Abiodun 2018).

The application of technology to agriculture can simply go wrong, eventually hurting the farmers and other beneficiaries it was intended to assist. But when cautiously considered, it can also nurture confidence, openness, reliability and inclusivity beneficial to all (Yohannes 2016).

Climate change occurs rapidly that technology alone just cannot solve its impacts, because it takes time to develop new technologies. To stay ahead of the curve, we need to identify climate impacts now and align our current research activities to breed varieties that will be able to cope with these changes. The need for innovation technology is becoming necessary to generate measurements and analysis which are critical in the precision of environmental hazards that have affected agriculture and other aspects of human endeavours. Innovation technology is developing our understanding of the threatening environmental hazards and also giving us innovative ways to their mitigation and management.

Presently, through innovation technology, there have been new approaches for measuring and reducing emissions of methane which is a powerful GHS that has generated more than 80 times the near-term warming power of carbon dioxide. It is reported that man-made methane emissions are responsible for about 25% of all the global warming presently experienced (Yohannes 2016). Climate adaptation projects entail activities that can sustain the hazardous effects of climate change from floods, drought, heat waves and desert encroachment. Hence, adaptation activities such as climate-smart agricultural practices, sustainable water and waste management and recycling, flood defence, climate insurances, and predictive climate conditions innovation technologies need to be put in place.

20.3 The Role of Innovation Technology in Agriculture and Food Security

Climate change has a greater effect on the severity and the rate of frequency of stresses such as drought, high saline intrusion, tropical storms, high temperature, the occurrence and greatness of climate-sensitive pests and diseases of both crops and livestock (FAO 2008). There are several creativities to promote innovate novel breeds and varieties and population that are resistant to biotic and abiotic stressors which are currently encountered globally (Tester and Langridge 2010; Chapman et al. 2012; Fisher et al. 2015). The application of agricultural innovative tools such as enhancement of crop diversities and crop-climate models could be utilized for the enhancement and improvement of crop germplasm modified to future climates (Ramirez-Villegas et al. 2015; Challinor et al. 2018). The application of enhancing germplasm, facts on complementary invention inputs, more proficient and improved resourced extension facilities and the establishment of climate information and rural financial services are all crucial (Tambo and Abdoulaye 2012).

20.4 Agriculture and Food Innovation Technology

It has been observed that the prevention of climate change will be very critical towards the prevention of food and livelihood systems and the eventual enhancement in provision of adequate and sustainable food for the ever-increasing population.

Historically, it has been reported that preservation of land varying from land alteration from forest to pasture or cropland and crop and livestock invention practices has been discovered as a major liberation of greenhouse gas emissions. Moreover, the identification of appropriate food system portends the significant potential to prevent the merging issue of climate change most especially at the end of the food chain. Furthermore, most of these preventive strategies will also serve as a crucial adaptation strategy for the attainment of commercial agriculture.

Therefore, in view of the aforementioned, there is a need to adopt the best practices that could help in the mitigation of all impending challenges to the problem of food security and also enhance the increase in the adaptation strategies for effective commercial agriculture sector, as well as improving the sustainability of susceptible livelihood systems, steadiness of global food markets and source of new employment prospects in the commercial agriculture sector and increasing the sustainability of vulnerable livelihood systems. Finally, there is a need to adopt the application of biopesticides in place of synthetic pesticides that will mitigate all the adverse effect related to the synthetic pesticides like pesticide residues, environmental impacts and impairment of human health (Adetunji et al. 2017a, b, c, d, 2019; Adetunji 2015; Adetunji and Oloke 2013a, b).

20.5 Connection Between Climate Change and Agriculture

Even if it is believed that agriculture plays a major role in the economy of any nation, it is vastly dependent upon climate for the production of food and fibre that are necessary for the sustenance of human life. Expectedly, agriculture is supposed to be an economic activity that is expected to be vulnerable to climate change. It involves natural processes that regularly involve fixed extents of nutrients, temperatures, rainfall and other situations (Vuren et al. 2009).

According to reports from some research studies, agriculture contributes about 40% of anthropogenic GHG emissions, and most of these agricultural GHG emissions take place in developing countries, and this is expected to rise up to 80% by 2050 (Thornton and Lipper 2013; Gebreegziabher et al. 2014).

With the current increase in world population, it is obvious that there will be a continuous demand for food for survival, and as such the amount of GHG emissions from the agricultural activities would certainly increase, and this increase is projected to continue sequentially. Several studies have shown that agriculture generates both direct and indirect emissions of GHGs. The direct emissions are alleged to come from the use of fertilizers and animal manure in agricultural land, whereas the indirect emissions come from runoff and leaching of fertilizers, emission from land-use changes and use of fossil fuels for mechanization, transport and agrochemical and fertilizer production (Keating and Carberry 2010). The most substantial indirect emissions of GHGs are variations in natural vegetation and traditional land use, including deforestation and soil degradation (Thornton and Lipper 2013).

According to the data from the World Bank (2008), climate change affects agriculture in several ways, which include:

- Variations in mean temperatures, rainfall and climate extremes with a significant impact on soil erosion (floods, drought, etc.)
- Variations in pests and diseases
- Variations in atmospheric carbon dioxide
- Variations in the nutritional quality of some foods
- Variations in growing season
- Variations in sea level

Several research reports show that agricultural yields have a strong correlation with variations in temperatures and with the duration of heat or cold waves and diverge based on crop maturity stages during extreme weather events (Hoffmann 2013; Ackerman and Stanton 2013).

Adapted precipitation patterns will improve water shortage and related drought stress for crops and modify irrigation water supplies. They also decrease the predictability for farmers' planning. Incidentally, variation in temperature and moisture levels could result in a change in the absorption rate of fertilizers and other minerals, which regulate agricultural productivity. Generally, it is reported that the upsurge in temperature together with the decrease in rainfall reduces agricultural productivity if both are beyond the threshold that is appropriate for their production (Hoffmann 2013). Also, Ignaciuk and Mason-D'Croz (2014) in their work reported that climate change presently reduces the yield of most agricultural produce like maize, rice, wheat, potatoes and vegetables, and this reduction will continue globally even beyond 2050.

In the area of livestock management, it is reported that climate change also affects livestock production both by affecting the quantity and quality of feed and by affecting the occurrence and severity of extreme climate events (Hoffmann 2013). Even with the limited literature that deals with climate change impacts on livestock, within our disposal, it is obvious that livestock management could be predominantly vulnerable to the effects of climate change.

Climate change and agriculture are interconnected processes. They both take place globally, and their connection is predominantly significant as the disparity between world population and world food production rises. According to some predictions, climate change, that is, changes in temperature, rainfall and severe weather events, is likely to reduce agricultural produce in several developing countries of the world, especially sub-Saharan Africa and some parts of Asia (Parvatha 2014; Yohannes 2016). The impacts could, in the next coming decades or so, cause millions of people especially those in developing countries to encounter scarcity of water and food with adverse effect on their health (Ogunniran 2018; Ukhurebor and Abiodun 2018).

The influence and consequences of climate change for agriculture tend to be more severe for countries with higher initial temperatures, areas with marginal or already degraded lands and lower levels of development with little adaptation capacity (Parvatha 2014).

The connection between climate change and agriculture is a two-way connection, and this connection has great consequence, particularly in developing countries due to their great dependence on agricultural activities as a major means of livelihood and their deficiency in infrastructure for adaptation (Yohannes 2016; Ukhurebor and Abiodun 2018).

Agricultural activities are affected by climate change affects due to their direct dependence on climatic factors. In high-latitude areas with low temperature, increased temperature due to climate change could allow for longer growing season. Agriculture affects climate through emissions of greenhouse from use of fossil fuels, tillage practices, fertilized agricultural soils and livestock manure in large proportion. Conversely, agriculture could be a solution for climate change by the widespread adoption of mitigation and adaptation actions. This happens with the help of best management practices such as organic farming, agroforestry practice, manure management, etc.

20.6 Conclusion

The recent change in the climate is becoming a universal issue particularly as a result of its influence on global warming and other environmental hazards (Odjugo 2010; Salack et al. 2018; Ukhurebor and Abiodun 2018). Its impacts have continuously threatened human existence to a large extent and have influenced the livelihood of most people globally. According to Ukhurebor and Abiodun (2018), the effects of climate change have a great impact on the development and achievement of the Millennium Development Goals (MDGs), and this has significantly made the achievement of the MDGs more tedious in most developing countries. The United States Agency for International Development (USAID 2007) reported the impacts of climate change are becoming higher, and the resulting effects are more felt in less privileged developing countries than the developed nations of the world. Global warming which is now a serious menace to human existence is caused majorly by the varaition in climate variables, resulting mostly from human activities.

Several recent studies have predicted that if urgent action is not taken to combat the impacts of climate change, the impacts could, in the next coming decades or so, cause millions of people especially those in developing countries to encounter scarcity of water and food with adverse effect on their health (Ogunniran 2018; Ukhurebor and Abiodun 2018). Food is essential for human survival. Hence, anything that would affect agriculture vis-à -vis food production should not be handled with levity. The chapter has further reviewed and revealed that there is a nexus between climate change and food innovation technology as it relates to agriculture. It is therefore imperative to urgently take the necessary measures and adaptation options for its mitigation and management via technology innovation.

Agricultural innovation technologies can play a fundamental role in addressing these ultimate challenges. While most technologies have climate consequences, some are of specific significance to agriculture and climate change, specifically in developing countries. The tremendous rate at which climate is changing also requires an immediate and fundamental shift in managing policies for agriculture.

*The rate at which climate change occurs makes it somehow difficult to believe that technology just cannot keep up, as it takes time to develop new technologies. To stay ahead of the curve, we need to identify climate impacts now and align with recent research activities to breed varieties that will be able to cope with these changes.*Reversing climate change may be technically achievable, but the political will to make the hard and necessary decisions in most of the developing world remains tenuous. Adopting innovative agricultural technologies with the potential to increase productivity while curtailing climate change is a critical step. Otherwise, we are figuratively farming ourselves out of food and demolishing our world in the process.

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21 Antibiotic Residues in Food: A Global Concern for Human Health

Avinash Singh, Sonali K. Kalra, Santosh K. Singh, and Amit Prasad

Abstract

Presence of antimicrobial resistance bacteria (ARBs) and antimicrobial resistance genes (ARGs) in food is a potential risk to the public health. Food animals are considered as key reservoirs of ARBs. Food can be contaminated with ARBs, ARGs and antibiotic residues in several ways, i.e. (1) by use of antibiotics during agriculture production, (2) presence of resistance genes in bacteria that are purposely added during the food processing and (3) through cross-contamination with ARBs during food processing. Contaminated food products without undergoing prior processing or preservation can be consumed, which poses a potential risk for transfer of antimicrobial resistance to humans. In this chapter, we are evaluating the food safety concerns related to the presence of antibiotic-resistant bacteria and residues in food, their impact on human health, their detection methods and few recommendations given by international and national agencies.

Keywords

Antimicrobial resistance bacteria (ARBs) · Antimicrobial resistance genes (ARGs) · Antibiotics · Food processing · Antibiotic residues

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21.1 Introduction

Antimicrobial resistance is an emerging public health hazard with severe socio-economic outcomes especially in the developing world. Recently, Centers for Disease Control and Prevention (CDC), Atlanta, USA, published a data on annual deaths due to antibiotic resistance (AR) in European Union, the USA, Thailand and India with 25,000, 38,000+ and 23,000 deaths per year and 58,000 offspring's death in a year, respectively (CDC 2018). Another report presented high crude infectious disease mortality rate in India, i.e. 416.75 per 100,000 persons and considered misuse of antibiotics along with suboptimal dosing, environmental contamination, etc. as the major drivers for incidence of AR (Laxminarayan and Chaudhury 2016). Sub-optimal rapid diagnostic tools are also one of the major risk factors for development of antimicrobial resistance (AMR) (Laxminarayan and Chaudhury 2016). In most of the developing countries, other factors including easy access to the antibiotics and their increased use in the animal husbandry sector without any professional supervision has immensely resulted in an increased emergence of AMR. Van Boeckel et al. (2015) reported 63,151 (±1560) tons of global antimicrobials consumption in food animal production in the year 2010 and projected it to rise by 67%, up to 105,596 (±3605) tons, by the year 2030. They also observed that China, the USA, Brazil, India and Germany had largest shares of global antimicrobial consumption in food animal production (Van Boeckel et al. 2015).

AMR is not just a health problem but an economic burden too. The overall cost, both in terms of mortality rate and fiscal depletion, is too great to be ignored and the whole world must come together on a common consensus to prevent the spread of AMR. A study evaluated the economic and health outcomes of AMR and observed medical costs ranging from $18,588 to $29,069 per patient (Roberts et al. 2009). In the recent past, an economist Jim O'Neill have estimated that about ten million people will die annually due to AMR infections, costing about 2–3.5% of gross domestic product (GDP) by 2050. These GDP losses are annual and compound over time and hence would result in a cumulative loss ranging between $2.1 and $124.5 trillion (O'Neill 2014).

21.2 Health Hazards of Occurrence of Antibiotic Resistance Bacteria (ARBs) in Food and Food Products

The increasing occurrence of ARBs poses a severe risk to human and animal health worldwide (Levy and Marshall 2004). Various studies have demonstrated the ability of ARBs to spread among humans and animals in the ecosystem, thus leading to major health-related concerns (Hu et al. 2017; Forsberg et al. 2012). In the current scenario, there has been an increased occurrence of different antibiotic-resistant genes (ARGs) like New Delhi metallo-β-lactamase (NDM-1), mobilized colistin resistance gene (mcr-1) and extended spectrum beta-lactamase (ESBL) in the multi-drug resistance bacteria (Kumarasamy et al. 2010; Liu et al. 2016). This along with enhanced use of antibiotics in humans and food animals and their products has amplified the problem of antibiotic resistance in the world. It has been estimated that the use of antibiotics in food animal production is more than the human antibiotic consumption, which is likely to increase in the same trend in coming years worldwide (Van Boeckel et al. 2017). Several antibiotics, which are used for growth promotion in animals, are more or less similar to antibiotics used in human therapeutics (Hammerum et al. 2010). This can lead to human health hazard owing to the risk of transfer of ARBs and ARGs from food animals to humans (Hammerum et al. 2010) (Fig. 21.1). ARB from animal origin can transfer to humans via food products as well (Price et al. 2005; Graham et al. 2009; Smith et al. 2013). The most detrimental effects of ARBs and ARGs transferred from food animals and other food products to humans are that the pathogens become resistant to antibiotics commonly used in treatment of human infections (Chen et al. 2019). This can lead to the mismanagement of infected patients. In 2001, antibiotic-resistant *Salmonella* strains were isolated from retail ground meat (White et al. 2001; Butaye et al. 2003). Authors claimed that the administration of antibiotics to dairy cattle and other livestock must have been transferred to humans through food web. Later, other resistant bacteria like *Campylobacter* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA) were reported to be transferred to humans via various food carriers (Bengtsson and Greko 2014). Another study on resistance in *Escherichia coli* strongly suggested that a large proportion of resistant *E. coli* isolates that cause bloodstream infections in humans might be derived from various food sources (Vieira et al. 2011). Researchers who worked in this field for more than two decades concluded that there is a strong link between the overuse of antibiotics in food-producing animals and the reduction in the efficacy of antibiotics against infections in humans (Chen et al. 2019).

Recent studies have focused on the occurrence of antibiotic residues in food products. These residues present in food can lead to health hazards via food chain and food web (Chen et al. 2019). Long term exposure to antibiotic residues in food can also lead to carcinogenicity, reproductive effects, teratogenicity and obesity (Ajslev et al. 2011; Cox and Blaser 2015; Bedford 2000; Sarmah et al. 2006; Al-Waili et al. 2012). Apart from this, antibiotic residues can interfere with commensal gut microflora, cause allergic reactions in human body or even interfere with the action of prescribed drugs among them. This would eventually lead to other human health hazards (Chen et al. 2019). Very recently, various policies and regulations have been formulated in different countries to combat health hazards due to antibiotic residues in food products. Ministry of Health and Family Welfare, New Delhi, India, passed a notification [S.O. 2607(E).] on 19 July 2019, that prohibiting the manufacturing, sale and distribution of colistin and its formulations for food-producing animals, poultry, aqua farming and animal feed supplements. However, more policies are needed to be establish a mutual cooperation among countries to ensure food safety and inhibit human health hazards due to occurrence of antibiotic residues in the food products.

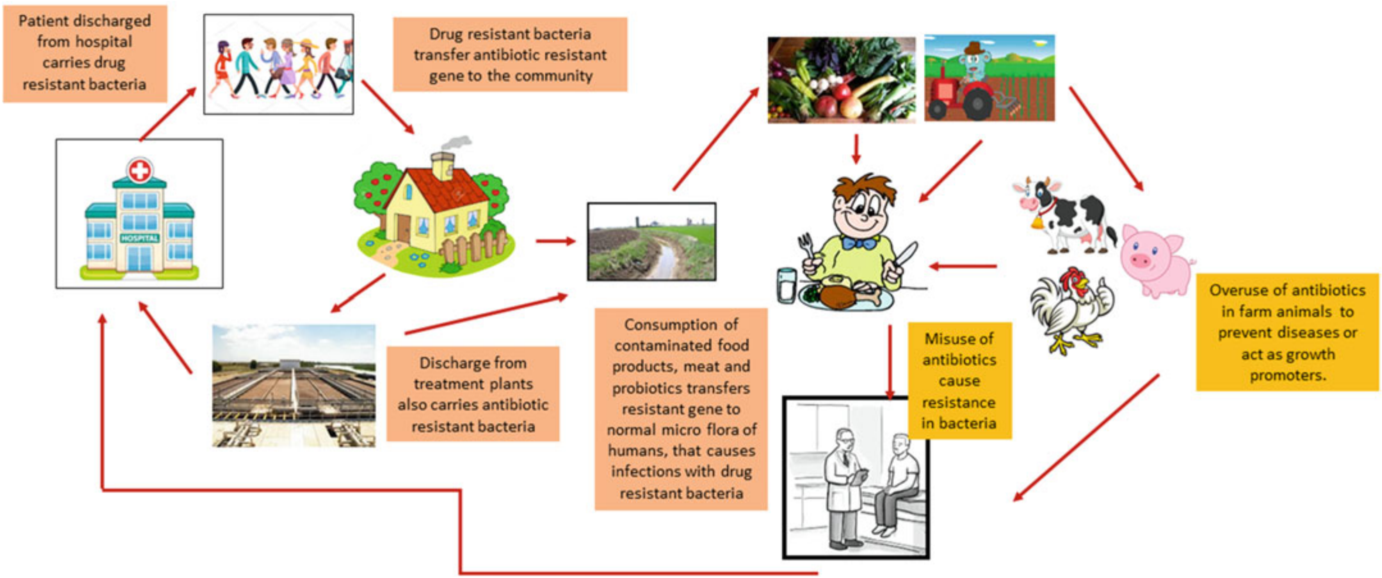

Fig. 21.1 Circulation of antibiotic resistant bacteria among human and animals via environmental factors and food products

21.3 International and National Agencies Governing Antibiotic Residues in Food and Dairy Products

European Union by means of the Council Regulation (EEC) No. 470/2009 and Council Directive No. 96/23/EC has laid down a standard guideline to limit the residue of veterinary medicines in food of animal origin like meat, fish and eggs and to monitor certain substances and residues in live animals and their products. In the USA the food microbial residue report is carried out by the Food Safety and Inspection Service (FSIS) of US Department of Agriculture (USDA). The Food Safety and Standards Authority of India (FSSAI) has set the tolerance limit for antibiotics only to fishery products in the domestic food market.

21.4 Screening Methods to Detect ARBs and Antibiotic Residues in the Food and Dairy Products

Currently, various screening methods are available to characterize (phenotypic and genotypic) AMRs present in the bacterial isolates. Major methods are described here briefly.

21.4.1 Plating Method for Detection of Antibiotic Resistance Bacteria in Food

The conventional approach of screening AMRs involves plating samples on non-selective or antibiotic-selective agar plates, purifying bacterial colonies and using disc diffusion, broth dilution, gradient strip and other similar methods to determine the minimum inhibitory concentration (MIC) for resistance to a panel of antibiotics. The MIC values are assessed against clinical breakpoints (e.g. those available from the Clinical and Laboratory Standards Institute, European Committee on Antimicrobial Susceptibility Testing or epidemiological cutoff values) to determine if a bacterial isolate is sensitive or resistant to different antibiotics. Although this method is very informative, yet it is time-consuming, cumbersome by taking several days to perform the full panel of MICs on isolates, following purification.

21.4.2 Automated Bacterial Characterization System

Automated bacterial characterization systems, such as Vitek 2 (bioMerieux, USA) or MicroScan (Siemens Healthcare Diagnostics Ltd., UK), which also perform antimicrobial sensitivity testing, are high throughput techniques and increasingly being used in large hospitals to characterize the resistance phenotype of bacteria to different antimicrobial compounds available. These automated methods are relatively rapid taking approximately 12 h following initial isolation of bacteria. However, the installation of this set up is costly.

21.4.3 Molecular Methods

Polymerase chain reaction (PCR) has been the most commonly used nucleic acid amplification technique for the detection of antimicrobial genes and their genetic support (Arthur et al. 1990). Conventional PCRs, defined as separate amplification and post-PCR detection assays, have been described for most resistant determinants (Fluit et al. 2001). The laborious post-PCR work and problems with carry-over contamination have been largely removed by the advent of real-time PCR. Real-time or quantitative PCR showcase the ability to monitor the amplified product during amplification. Real-time PCR techniques have permitted the development of routine diagnostic applications for the microbiology laboratory (Espy et al. 2006). Several reports have described the use of these techniques for the detection of resistance causing determinants and surveillance of antimicrobial-resistant bacteria (Fang and Hedin 2003; Francois et al. 2003; Woodford et al. 2002). The ability to monitor the accumulating amplicon in real time is based on labelled primers, oligonucleotide probes and/or fluorescing amplicons producing a detectable quantitative signal related to the amount and specificity of the amplicon. Several improvements have been introduced. Reduced amplicon size, shorter cycling times and removal of separate post-PCR detection systems have allowed automation, reduced the detection time, and minimized the risk for carry-over contamination. Technical aspects in the recent developments of real-time PCR methods and their use in diagnostic microbiology have recently been elegantly reviewed (Espy et al. 2006). Other significant technical developments include multiplex PCR assays, which use more than one primer set for simultaneous detection of several antimicrobial resistance genes (Vakulenko et al. 2003).

21.4.4 Pulsed-Field Gel Electrophoresis (PFGE)

PFGE technique is extensively used to detect the clonality between the bacterial strains. Following are the steps involved in PFGE technique:

1. PFGE-based separation of DNA fragments is performed in a CHEF DRII electrophoresis gel analyser.
2. 1.2% pulsed-field certified agarose gel (Sigma, India) is prepared in 0.5X TBE.
3. Electrophoresis is performed at 80 °C for 17 h at 6 V cm^{-1} at 1200 with switching times of 1–20 s.
4. Small-digested DNA of *Lactobacillus gasseri* ATCC 33323 is used as a standard.
5. For each run, the standard should be placed in the first, middle and last lane to allow alignment of the gel in the subsequent analysis of gel images.
6. Thereafter, the gel is stained with ethidium bromide and photographed on a UV transilluminator.
7. Gel Compare software version 6.6 (Applied Maths BVBA, Sint-Martens-Latem, Belgium) is used for the data analysis and phylogenetic tree formation.

21.4.5 Microbial Inhibition Method

This is the most widely used method for the detection of antibiotic residues in animal originated foodstuffs. It is very cost-effective method as it covers large number of different antibiotics in a single test run only. Briefly, this method is mostly preformed on Mueller Hinton agar (MHA) or nutrient agar media with specific test bacteria (*Micrococcus luteus*, *Bacillus stearothermophilus*, etc.). After addition of milk or meat, the plate is incubated at appropriate temperature for growth and germination of bacteria. In the absence of antibiotic residues, the growth of bacteria can be detected visually either by the change of opacity of the agar medium or by the colour change of the pH indicator (Jayalakshmi et al. 2017).

21.4.6 Swab Method

A novel screening method for the detection of antibiotic residues in chicken meat and poultry was reported by Johnston, Reamer, Harris, Fugate and Schwab (1981). This method involved inserting a cotton swab into meat or poultry tissue to absorb tissue fluid, which is kept in a test plate containing antibiotic medium No. 5 (BBL) and a seed layer of *Bacillus subtilis* ATCC 6,633 spores. This test shows similar sensitivity to conventional methods in screening of antibiotics like chlortetracycline, oxytetracycline, tetracycline, erythromycin, neomycin, penicillin, streptomycin and tylosin (Johnston et al. 1981).

21.4.7 Biosensors

Biosensor is a latest technique used for screening of antibiotic residues in milk. These sensors have shown successful detection of β-lactams (β-Ls), tetracycline, streptogramin and macrolide antibiotics at nanogram per millilitre concentrations in milk and serum (Weber et al. 2005; Toldra and Reig 2006). Microbial biosensor is mostly used for the detection of quinolones (Qs) and tetracyclines (TCs). However, they were not sensitive towards the detection of macrolides, β-Ls, aminoglycosides and sulphonamides (Kivirand et al. 2015). Any biosensor system comprises of two main components, namely, a transducing device and a recognition element. The advantage of biosensors includes the ability to detect nonpolar molecules, which are not receptive to most of the available devices, high specificity and quick and real-time application in the industry. However, the major drawback involves the contamination of biosensor, and it cannot be heat sterilized (Huet et al. 2010).

21.4.8 Enzyme-Linked Immune Sorbent Assay (ELISA)

It is a widely used method for the detection of antibiotic residue in all kinds of tissue samples (Tao et al. 2012). ELISA-based techniques have the advantage of high sensitivity, broad specificity and ability to handle large number of small volume samples in short time. However, this test is expensive and detection is not real time.

21.4.9 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography-Mass Spectrometry (LC-MS) coupling is another effective and sensitive system for the detection of antibiotic residue. Various methods of LC-MS include electro-spray ionization source, direct injection methods and mobile phase. Mass spectrometry works on the principle of mass-to-charge ratio (Martins et al. 2014).

21.4.10 High-Performance Liquid Chromatography (HPLC)

HPLC is one of the most powerful tools in analytical chemistry (Senyuva et al. 2000). HPLC usage is increasing day by day in the field of residue analysis because it involves a variety of mobile phases, the extensive library of column packings and the variation in modes of operation. Gavilán et al. (2016) evaluated the presence of four tetracyclines (chlortetracycline, doxycycline, oxytetracycline and tetracycline) in non-medicated feed at µg/kg level using HPLC-MS/MS method. Oxytetracycline was the most frequently detected tetracycline in the study.

21.5 Recommendations

Antimicrobial resistance poses a serious global challenge. ARGs can be easily carried to other nations via exportation of resistant pathogens through travel and trade. The global nature of resistance calls for a global response, not only in the geographic sense, i.e. across national boundaries, but also across the whole range of sectors involved with emphasis on the following points:

- The need for antibiotics in food animals should reduced by improving animal health through bio-security measures, disease prevention, good hygiene and management practices.
- Antibiotics should given to food animals only when prescribed by a veterinarian.
- Antibiotics should used only therapeutically, and should based on the results of resistance surveillance as well as clinical experience.
- Use of antibiotics as growth promoters should eliminated.
- Rather than broad spectrum antibiotics narrow-spectrum antibiotics should be the first choice when antibiotic therapy is justified.
- Veterinarians' professional societies should establish guidelines on the appropriate usage of antibiotics for different classes of food animals.

21.6 Conclusion

Antibiotic resistance is a global hindrance towards healthy human life and should considered as a micro terrorist worldwide. A strict vigilance by drug regulatory bodies is the need of hour to safeguard against powerful antibiotics being sold over the counter and to faze out the use of antimicrobial growth promoters in livestock. Strict implementation of policies, regular revision of strategies, routine surveillance and monitoring of appropriate use of antibiotics in human and animal health sectors and release of funds for development of newer antibiotics are urgently needed to combat the rise of AMR. The European Food Safety Authority recommends that bacterial strains harbouring transferable antibiotic resistance genes should not be used in animal feeds and fermented and probiotic food for human use. In addition, Indian Council of Medical Research-Department of Biotechnology (ICMR-DBT), India, also published a guideline and recommended to perform antibiotic susceptibility testing before the use of probiotics starter culture. A close monitoring on such type of resistance is needed to ensure the health issues because their direct consumption may alter the human microflora and can transfer the different drug resistance genes to the commensal bacteria of the human body. In addition to this, the use of antibiotics as feed additives at subtherapeutic dose should be strictly prohibited. For therapeutic purpose, it must be used as per advised by veterinarian. The menace being created by ARBs and ARGs against human health can be curbed by focusing on a common consensus at global and intra-sectoral level.

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Effects of Toxicant from Pesticides on Food Security: Current Developments

22

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Abstract

Pesticides are used for the management and control of agricultural pest, weeds, fungi and insects which are responsible for the reduction of the increase in agricultural and food production. Thereby, the application of pesticides will lead to an increase in feeding the ever-increasing population globally. Furthermore, the application of pesticides has been highlighted to be a greater source of toxicity to human beings which eventually has both chronic and acute health impacts. Also, it has been discovered that most of these pesticides could persist in water and soil, while some of them could form a pesticide residue on the agricultural residues. This chapter intends to elaborate on the trade-offs between

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food security, food safety and pesticides. Special emphasis on pesticide authorization, monitoring, pesticide residues utilization and bioremediation strategies for the bioremediation of heavily contaminated environment was highlighted. Also, recent and current innovations for the management and regulation of pesticide residues which are challenges in various sectors like food, agricultural and environmental were stressed. We also suggested some recommendations and proffered some research directions that need to be carried out in the near future using sustainable technologies for the regulation of different types of pesticides episodes.

Keywords

Pesticides · Toxicant · Food security · Food safety · Bioremediation

22.1 Introduction

Food is an integral part of human existence. It provides the basic nutrients needed for the metabolic functioning of the cells, tissues, organ and organ systems in life forms when consumed in the right proportion. To ensure the continuation of nutrients in the human body, there must be easy availability, stability, accessibility and utilization of food, which are the major bedrocks for food security for the present and future generations (FAO 2006). Everyone has the right to food which is the basic satisfaction of all other accepted human rights (FAO 2009).

The demand for food is growing at a geometric rate as the human population increases. Of recent, the United Nations (UN) (2001) and Carvalho (2006) in line with the UN population policy estimated the human population yearly growth rate to be 1.2%, approximately 9 billion by the year 2050. This sharp increase of the human population was also estimated and forecasted to come from the emerging nations (sub-Saharan African) about 95% of it (UN 2001, 2005). Consequent of this, to meet the growing teeming population, pesticides are used to protect, enhance with fertilizers and speed up the rate of food production. This has led to the ardent and uncontrollable use of it in farming activities without considering the adverse effects to the ecological systems, biodiversity and its surroundings at large (Karlsson et al. 2000; Jovanic 2006; Saltmarsh 2010; Nnamonu and Onekutu 2015; Richards et al. 2017; Annabella et al. 2018).

Pesticides are organic or inorganic chemicals used for different purposes (agricultural, industrial and domestic) to kill, control and eradicate plants (weeds), pests (insects, worms, molluscs and rodents) and microorganisms (Randall et al. 2014). The most widely use of the class of pesticides is herbicides, which consist of about 80% of its usage (Food print 2019). The advances of newfangled pesticides have elicited unwelcome side effects to both agricultural produce, livestock and the ecosystem (Carvalho 2006). The purpose of this chapter is to report and evaluate current, recent and modern developments of special effects of toxicants from pesticides as they portend food security, their health and ecological risks, impacts as well as proposed future directions on how to safeguard food from pesticides invasion.

22.2 Pesticides vs Food Security the Way Forward

The accelerating use of pesticides in agriculture and industrial activities has become a public discourse in recent times because of their possible health hazards and environmental noxiousness. Agriculture activities require two major chemicals: fertilizers and pesticides. The former aid in boosting plant growth and development, while the latter aid in controlling weeds and pests (US EPA 2017). Pesticides can be applied as a gas, liquid and solid, in the form of powder to the soil, roots and leaves of plants. The systemic action of any pesticides depends on its mode of actions on the cell physiological functions and the ecological factors affecting the soil structure. Pesticide residues in soil and plant are serious problems that influence food security.

William et al. (2019) assessed the health impact and non-safety of pesticides in food to humans. They project the degree, effect and dire exposure of pesticide exposure to food and the concern for innate consumption by the consumers without knowing what health risk it portends. However, the authors gave more insights on some dietary exposure limits and complicated intrinsic nature of their regulations. They further propose succinct contemporary ways to regulate pesticide usage in the ecosystem in order to forestall an eco-friendly environment through ecosystem governance.

Annabella et al. (2018) reported the negative role of pesticides on food security in Africa. The authors opined that the major challenges facing food security in Africa are the issues of economic mismanagement and lack of sentience which heightens the total cost of intrusions in related pesticide inputs in the safeguarding and the management of food. They recounted the health hazards that follow pesticide usage and propose a more eco-friendly and sustainable biological substitutes which can aid in the reduction of some antagonistic consequence of lingering chemicals in the soils and agricultural crops and make them more attractive and appealing without noxiousness to the end users.

Lesa and Wageh (2019) review and evaluated the global problem facing food and some chemicals found in the environment that affect its consumption. The authors laid emphasis on persistent organic pollutants (POPs), such as pesticides that are mainly sourced from anthropogenic activities like farming and how they can contaminate our water, food, livestock and results to several ill maligns in humans. They propose environmental monitoring, remediation and the re-enactment of environmental policies that will aid in the abatement of these pollutants in the environment in other to make food secure for the global populace.

Angelo (2017) reported the impact of agro-industries and climate change as impairment to global food security. The author laid emphasis on the essence of "Green Revolution" to combat food insecurity from pesticide influence. More so, the author stressed the negative relationship between worldwide climate change and pesticides in the developing and most susceptible nations that the impacts they bring are severe human health and ecological problems such as; water contamination, poisonous air contamination, the farm-labourer poisonings and natal weaknesses in children of farm-labourers, the world-wide pollinator calamity, the loss of life forms and bionetwork services. In conclusion, the authors propose green revolution, green ecology and agro-ecology as remediation for climate change and pesticides impacts on food security, which can serve as climate managed agronomy with little carbon frugality.

Zolin et al. (2017) reported the influence of pesticides on food security in China and EU (European Union). The authors recounted the impacts from agricultural practices on natural resource stating their evidences on the relationship with food security and safety in China and European Union. The authors exposed the pressure and common arguments that have happen before now which have increased the hazards for the end users and personnel who work with pesticides, diet humiliation and problems in the joint recognition of food worth warranty schemes, which call for viable manufacture approaches.

Mie et al. (2017) did a comprehensive review on the health risk of organic food and agriculture food on humans. The authors compared organic with conservative food production with reference to factors vital to human vigour and argue their probable influence as they relate to the European Union standard practices. More so, the authors were able to state the health impacts of organic food ingestion that they might reduce risk of hypersensitive illnesses, overweight and plump. However, this might not be 100% surety, because they might also have some residual complexes. They were able to demystify that the use of pesticides in organic agriculture is regulated, while remains of pesticides in conservative root vegetable and fruits establish the chief basis or pathway to humans. In conclusion, the authors proposed an integrated pest (IPM) and a green management as sustainable tools in agriculture.

Popp et al. (2013) did a review of food security and pesticides influence on their productivity. They recounted the increase rate of the human population and its likely impacts on food. That is because of this trend, the urge to produce food had led to the rapid use of pesticides to protect agro crops against invaders, without considering the likely impact in the long run. The authors stated that about 70% of emerging countries have been forecasted to increase the demand for food consequent of change in their feeding behaviours and the propensity to demand high-quality food. On this note, the authors proposed sustainable agro-production (organic and recombinant approaches) at higher levels or large scale, because pesticides are widely used in commercial agriculture unlike the substance practices. These will enable the wide-ranging community safeguard their food in order to prevent external complications related to the ecology and human health risks of pesticide influence.

Carvalho (2017) reported the relationship between pesticides, the environment with food security. The author opined that agricultural chemicals have double-boosted the production of food crops for about 100 years now, which have eked in the feeding of the rapid ever-growing teeming population. The author stated the impacts of the arduous usage of pesticides as well as fertilizers to protect and boost crop production, that residues of pesticides in soils, land-dwelling (terrestrial) and water bionetworks can be lethal to both fauna and flora. This is as a result of their persistent and non-biodegradable nature in the ecosystem. In the long run, this will reduce the quality of food and impede food security which is one of the fundamental rights to humans. The author stressed on substitute like tested green alternatives to the intensive usage of plant and animal protective chemicals such as biotechnologies, bio-surfactants, bio-sorption, bio-pesticides, bio-herbicides, bio-fungicides, etc., in order to safeguard the health of the consumers and the general public at large. In conclusion, the author proposes a better or improve technological research for the advances in food and environmental security and production. The need to develop special interest on GMOs (genetic modified organisms) is not optional in the quest for food security!

Özkara et al. (2016) reported in a book on the contamination of the environment with pesticides in relation to health risk. The authors stressed that pesticide usage for the protection of crops and livestock incursions are inevitable for the quest for economic and rapid production to meet the growing population. However, this rapidly evolving technological outbreak has exposed several types of xenobiotic substances that are carcinogenic and even noxious to human health and the environment. These possibly will pose impending risks to food security, the ecosystem and all life forms. The authors proposed a well sustainable way of intensive farming in order to safeguard our food, the environment and human health.

Matthew et al. (2017) evaluated and reported the issues of food security, pesticides and human health impact. The authors stressed the need to increase food production because of the rapidly growing population. However, to meet up with this demand, the use of crop booster (fertilizers) and protectors (pesticides) is needed to step up agro-production. In conclusion, the authors stressed the role pesticides have to play in food production and the possible ecological and health risks they also play in food insecurity. They proposed that environmental health policy makers should provide tenable ways to carefully meet the numerous tasks tangled with safeguarding our food in order to meet the current and future growing population needs.

Carvalho (2006) reported and evaluated the issues of the use of pesticides in agriculture and the influences on food security and safety. The author opined that the rationale of using pesticides was aimed at protecting and enhancing crops from pest invasion. However, pests have developed a more resistance gene to varied chemicals. Several new and modified pesticides have been produced with vigour potency to eradicate stubborn pests. These new chemical complexes have been used to safeguard crops resulting in various side special effects. The author proposed the use of GMOs that have a high vigour of combating pest that are highly resistant to the conventional chemical approaches and have been tested to have non-toxic impact to soil and agro-crops, eco-friendly and environmentally sustainable. In conclusion, the author stated some general efforts that will boost the manufacture of food, improve food value and safety and also aid in the monitoring of pesticide residues in our environment.

William et al. (2019) reviewed the recent regulation of pesticides in food and evaluated the safety for human health. The authors stressed the toxicology and the impact of food-pesticide exposures to human health and the complex nature of their monitoring and regulations. The authors examined the past, modern and current viewpoint on pesticides, steps taken to diminish their hazards in food to the end consumers. They also evaluated the application of the most widely pesticides (herbicides) – glyphosate by farmers on agro soils and its health risk exposures to humans. In conclusion, the authors were able to propose that public health professionals and researchers should help in propagating the need of sustainable approaches to agriculture in order to safeguard what we consume.

Zikankuba et al. (2019) review the malpractice involved in the regulations of pesticide and its implications to environmental and food security. The authors stressed the positive impacts of pesticides in agricultural purposes. However, their negative impacts cannot be quantified. Pesticides residues and metabolites have left untold impacts on non-target flora and fauna in the ecosystem. In conjunction, the usage of pesticides in non-agro activities such as spraying of cockroaches and mosquitoes have resulted to health symptoms, allergies, chronic and acute toxicities, neurotoxicity, cancer and even death in most causes. Consequent of these, the WHO, FAO and Codex Alimentarius Commission have set standard maximum limits for residues of pesticides in food, in order to consider their safety to human health. Globally, the most reported pesticides in food are chlorpyrifos and dichloro-diphenyl-trichloroethane. However, the strides of these regulatory bodies have been looked down upon by illegal bodies who have set-loose phoney pesticides in the flea market, and these have really affected food safety and proper regulations of toxic pesticides in the agro-industries. In conclusion, the authors recommend proper safeguarding of our food and sustainable green agricultural procedures.

Nakano et al. (2016) evaluated and reported 66 (seventy-six) different pesticides residues in citrus species (N = 57) in Brazil. The results of the mean range percentage recovery after quantification was 72–115 and the SD (standard deviation) was 1–11. In terms of mass quantifications, the range or limits of detection in the five replicate examined were 0.005–0.4 mg/kg and 0.01–0.8 mg/kg, correspondingly. Their findings of their study revealed that the average percentage of pesticides found in the 66 samples was 42.1% of the mean range of 0.06–2.9 mg/kg out of which 3.5% were contaminated (clofentezine and bifenthrin), 12.3% were unauthorized pesticides beyond the MRL (maximum residue level)—myclobutanil, fenitrothion, parathion, azinphos-ethyl and profenofos. More so, the probable risk estimated for humans were adults (0.04–6.6%) and children (0.1–26.5%) correspondingly. The authors recommended regular monitoring of pesticides in order to detect irregular residues and promote food safety as well as forecast any possible health risk prior to consumption.

Eze and Echezona (2012) reported the safety, security and the control of agricultural pest with different approaches. The authors stated the need of using IPM (conventional methods—synthetic pesticides, bio-pesticides—plant extracts, cultural methods), in the controlling such of plant pests. They stressed the current resistance of some species of pests to pesticides and the related possible health risks on the ecosystem. They also stressed that organic pesticides, bio-pesticides, would have been the best substitute methods, but their shelf live or expiration date and target actions on organisms have not yet been known. They concluded that no single pest control method has been proven to be effective in controlling food insecurity and propose the use of the integrated pest management system as a positive alternative.

Bolor et al. (2018) assessed the health risk of organochlorine pesticides remains on vegetables to humans in Kumasi, Ghana. They opined that organochlorine pesticides had been forbidden globally owing to their high noxiousness. Nevertheless, the majority of farmers especially in the emerging nations still use them illegally till date, because of the high vigour in exterminating plant pests. 15 samples in total were examined and analysed. The results of their studied revealed a high methoxychlor concentration (184.10 ± 12.11 μg/kg) and low beta-hexachlorocyclohexane (beta-HCH) (0.20 ± 0.00 μg/kg) in cabbage recorded from Ayigya. The results from the health risk indices showed potential significant health risk to the adults but more in children. It was observed in their results that the soil samples contained pesticides in the following order: methoxychlor > dichlorodiphenyltrichloroethane (DDT) > aldrin > OCPs of range, <0.01 to 49.00, <0.01 to 165.81 and < 0.01 to 174.91 μg/kg correspondingly. The following pesticides (p,p′-DDT, DDT isomers, HCH and β-HCH) were detected in samples of water examined. The potential carcinogenic risk value (>10–6) was observed from the risk indices of the groundwater which were above the standard limits. Additionally, the non-carcinogenic assessment revealed that aldrin surpassed the safe limits (>1). In conclusion, the authors proposed serious carcinogenic and non-carcinogenic health risk if the vegetables are ingested from the contaminated sources (soil and water). And call for health regulatory bodies concern on the probable impacts and food safety.

22.3 Conclusions

This chapter has presented the current scenario whenever pesticides are applied as an operative and proficient means to control pests in food production. Also, some environmental and protection standards of numerous pesticides, as well as the health impacts of these pesticides, were critically reviewed. The role of various federal regulatory agencies, public interest, farmers, food producers cannot be overemphasized in food safety and security. Moreover, adequate knowledge about the pesticides, food safety, human health and food production were also highlighted. There is a need to impairment policy that will regulate the application of pesticides and other issues boarding around pesticides residues on various agriculture produce in order to minimize the uncontrolled released of pesticides which are responsible for health and environmental hazards.

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Chromium Pollution: Impact on Plants and its Mitigation

23

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Abstract

Chromium (Cr) is a potential carcinogenic heavy metal. In plant physiology it does not have any essential metabolic function, but some plants show compatibility with this heavy metal and accumulate it in their living tissues without any injury. This chapter is going to review a plausible link among Cr speciation, bioavailability, phytouptake, phytotoxicity and detoxification based on data especially published from 2011 to 2017. On the basis of biogeochemical characteristics, Cr has various chemical forms where Cr III and Cr VI are mostly found in soil and plants. Accumulation of Cr by plant species depends on many factors such as its chemical form, plant type, physico-chemical properties of soil, etc. Plants do not have any specific transporter for Cr uptake; rather, it enters in plant tissues through specific and non-specific channels of essential ions. Cr accumulates in underground plant part and shows limited translocation to above-ground plant parts. Cr provokes deleterious effects to several morphological (plant growth and development), physiological (photosynthesis, nutrient uptake) and biochemical processes (enhancement in ROS and alteration in antioxidant activities), etc. in plants. Some plant species tolerate heavy metals toxicity through their internal defence mechanisms such as complexation by organic ligands, compartmentalization into the vacuole, scavenging ROS by altering antioxidant enzymatic activities, etc. Cr consumption in limited amount is beneficial for health, but in excess it can cause risk to human and can cause severe

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clinical conditions. Therefore, to escape from harmful effects, it is necessary to monitor biogeochemical behaviour of Cr in soil plant system.

Keywords

Phytotoxicity · Phytoremediation · Cr hyperaccumulators · Bioavailability · Heavy metals

23.1 Introduction

Chromium (Cr) is the 7th most abundant element and 21st most abundant heavy metal with atomic number 24, molecular weight 511 and density 719 g/cm^{3}of the Earth's crust (Shahid et al. 2017). Chromium is 1 of the 18 core hazardous air pollutants and has been ranked 7th among the top 20 hazardous substances by the Agency for Toxic Substances and Disease Registry (Oh et al. 2007). This heavy metal is ranked 5th among the heavy metals in the Comprehensive Environmental Response, Compensation and Liability Act (Ma et al. 2007). The National Toxicology Program and International Agency for Research on Cancer (IARC) announced it as number one carcinogenic. Therefore, this heavy metal prerequisites detailed and in-depth monitoring along with understanding, in the environment, especially in soil-plant system.

Chromium has a complex electronic and valence shell chemistry and is potent to convert from one oxidation state to another. Chromium has −2 to +6 oxidation state where Cr VI (hexavalent chromate) and Cr III (trivalent chromite) forms are most stable and common in the environment (Ashraf et al. 2017). Both these forms of Cr are different in many aspects (sorption, bioavailability in soil, absorption and translocation to areal parts and toxicity inside plant and animals) and so regulated separately by the Environmental Protection Agency (Choppala et al. 2016). Cr is an essential trace element for human and animal health as required for lipid and sugar metabolism. However, it is not required by the plants (Prasad 2013; Eskin 2016; Shanker et al. 2005).

Cr is considered as environmental pollutant due to high level of it in the water and soil originating from numerous natural and anthropogenic activities (Ashraf et al. 2017; Shahid et al. 2017). Chromium eventually accumulates in crops from contaminated soils and impart health risks to human and animals, through food chain contamination (Ahmed et al. 2016) Plant type, rate and type of root secretions, root surface area, transpiration by plants and texture, pH, cation exchange capacity of soil, etc. are some factors that control Cr translocation from soil to plant (Santos and Rodriguez 2012). In majority of plant species, Cr is poorly translocated from aerial plant part to underground plant parts (Jaison and Muthukumar 2017), whereas some plant species such as *Spartina argentinensis*, *Dyera costulata*, *Amaranthus dubius*, etc. can accumulate Cr in their tissue and considered as Cr hyperaccumulators (de Oliveira et al. 2016)

Plant species that grow in Cr-contaminated soil accumulate it into their plant tissue due to which plant biology get change, i.e. physiology, biochemical and metabolism of plant (Reale et al. 2016). Chromium toxicity is well reported to reduce plant growth, cause chlorosis in leaves, damage root cells, reduce pigment content, disturb water relation and mineral nutrition and alter enzymatic activities (Farooq et al. 2016; Shahid et al. 2017). High levels of Cr in plants also induce changes in the physiology and morphology of plants due to enhanced generation of reactive oxygen species (Islam et al. 2014). Numerous studies reported that Cr toxicity suppress the functioning and regulation of various proteins and high level of reactive oxygen species cause alteration of DNA and RNA, inhibition of enzyme, lipid peroxidation, protein oxidation, etc. (Dotaniya et al. 2014). In order to manage high level of ROS produced under biotic and abiotic stresses, plants have developed numerous complex adaptive strategies, including chelation by organic molecules followed by sequestration within vacuoles. Plants possess a secondary mechanism of producing antioxidant enzymes to scavenge the enhanced levels of Cr-mediated ROS (Yadav et al. 2010; Shahid et al. 2017) Therefore, it is important to take knowledge about biogeochemistry of Cr in soil-plant environment and the impacts that high levels of Cr will sustain on the ecosystem.

This review provides a plausible link among Cr mobility/bioavailability in soil, soil-plant transfer, toxicity and mitigation in plants. This review presents the following sections: (i) introduction, (ii) behaviour of Cr in soil, (iii) soil plant transfer of Cr, (iv) Cr toxicity to plants, (v) Cr mitigation in plants and (vi) conclusions.

23.2 Chromium in the Environment

23.2.1 Chromium in Nature and Its Permissible Limit in the Environment

Chromium was discovered by Louis-Nicholas in 1797 as a constituent of the mineral crocoite ($PbCrO_4$) and is used as powerful colouring agent (chromium is derived from Greek word 'chroma' which means colour). Chromium is found in nature in soil, rocks, water, volcanic dust and gases and always found co-precipitated with manganese, aluminium and/or iron oxides and hydroxides, which are generally absorbed on soil particles and complexed with soil organic compounds (Hsu et al. 2015; Quantin et al. 2008). In serpentine or ultramafic rocks, it is found as chromite ($FeCr_2O_4$) or as a constituent of vauquelinite ($CuPb_2CrO_4$), tarapacaite ($K_2Cr_2O_4$), crocoites ($PbCrO_4$), etc. (Shahid et al. 2017) (Table 23.1).

The protection of environment and human health set an acceptable level of Cr in soil as 64 $mgkg^{-1}$ (CCME 2015). Ding et al. (2014) mentioned that total Cr concentration in agriculture soils of different countries as Poland is 150 ppm, Czech Republic 100–200 ppm, Austria 100 ppm, Canada 64 ppm and Serbia 100 ppm. The threshold limits for Cr (III) in sea, fresh and irrigated water are 50, 8 and 5μgL^{-1}, respectively, whereas for Cr (VI) maximum allowable limit in drinking water is recommended as 50μgL^{-1}USEPA, and ATSDR independently has been set 100 μgL^{-1} as maximum contamination level of Cr in drinking water (Shahid et al. 2017).

Table 23.1 Natural and average Cr concentration in different sources and references

S. No.	Source	Range ppm	References
1	Sedimentary and igneous rocks	5–120 ppm	Kabata-Pendias (2010)
2	Ultramafic	1600–3400 ppm	
3	Mafic	170–200 ppm	
4	Limestone	5–16 ppm	
5	Earth's curst	01–03 ppm	Shahid et al. (2017)
6	Freshwater	01–117 mg L^{-1}	Nriagu (1988)
7	Sea water	02–50 mg L^{-1}	
8	Urban air	00015–003 μgm^{-3}	
9	Remote air	59×10^{-6}–$129 \times 10^{-3} \mu gm^{-3}$	
10	Indian soil	5-3000 ppm (highest level in the world)	Shanker et al. (2005)
11	Sweden	22 ppm (lowest level in the world)	Eriksson (2001)

23.2.2 Chromium Uses and Its Sources

Industrial and agricultural activities are main cause of production of Cr every year worldwide. Chromium mine production is continued to increase since 2000 and reached above 27,000 thousand metric tons and is still in hike. China and South Africa are leading stainless steel producers and so responsible for Cr pollution in environment, whereas Kazakhstan along with South Africa is considered as geological resource (95%) of Cr in world.

Significant concentration of Cr is released into the environment by anthropogenic activities such as leather tanning (Rani et al. 2016) along with metallurgy, alloying, electroplating, used as agent to inhibit corrosion in cooling towers in industries, wood preservation, textile dyes and mordents, production of paints, pulp paper production, etc. (Chen et al. 2016). Among all the industries, chrome tanning industry is one of the most potent, carcinogenic and toxic industry. It is remunerative and used in many part of the world for good quality products (leather). But direct discharge of their untreated, heavy metal-loaded effluent (specially Cr VI) into the environment is a matter of concern (Rani et al. 2016) which contribute towards Cr pollution of fertile soil. Dust from the rocks and volcanic activities are also responsible to release of Cr in the environment. It is estimated that the natural source emit about 43,000 tons/year of Cr worldwide (Lilli et al. 2015) (Table 23.2).

23.3 Bioavailability and Speciation of Cr in Soil

To know about ecological hazard by Cr, it is necessary to understand chemical speciation of Cr in contaminated soil and sediments. Chromium mainly exists in various valence states (0-VI), but Cr III and Cr VI are more stable and predominantly found in the natural environment (Elzinga and Cirmo 2010). Cr III occurs as cations

Table 23.2 Chromium-contaminated sites around the globe

S No	Cr (ppm)	Study site	Source	Fold higher than MAL	References
1	44,615	Ranipat, Tamil Nadu, India	Tamil Nadu Chromates and Chemicals Limited factory	179	Kanchiandham et al. (2015)
2	5406	Faridabad, India	Industrial area	22	Pathak et al. (2015)
3	1509	Agriculture soil, India	Mining area	6	Li et al. (2014)
4	1501	Agriculture soil, Vietnam	Mining area	6	Li et al. (2014)
5	856	Mouriki-Thiva area, Greece	Parent material (carbonate rocks, limestone, etc.)	34	Antibachi et al. (2012)
6	850	Moron borough, Argentina	Industrial and urban area	34	Gil-Cardeza et al. (2014)
7	692	Shenyang, China	Fertilizer plant site	28	Li et al. (2012)
8	321	Baghjar Chromite Mine, Iran	Baghjar Chromite Mine	13	Solgi and Parmah (2015)
9	75,000 tons	Slags and sludge near Mexico City	Industrial an urban waste dumping site	–	Ballesteros et al. (2016)

Maximum allowable level-250 ppm (Gil-Cardeza et al. 2014)

and Cr VI as oxyanions such as hydrochromate ($HCrO^{-}{}_{4}$), dichromate ($Cr_2O^{-2}{}_{7}$) and chromate ($CrO^{2-}{}_{4}$) ions (Shadreck 2013) The transition states, CII, IV and V, are usually produced during oxidation and reduction process of Cr III to Cr VI (Santos and Rodriguez 2012). Cr VI is highly mobile and reactive in soil as compared to Cr III and so extremely toxic to living organisms with mutagenic teratogenic potential; thereby, Cr VI is more potent health hazard (Rani et al. 2016; Prado et al. 2016). Inactivity of Cr III is due to precipitation at neutral pH. Both Cr III and Cr VI can co-exist in natural environment by reduction/oxidation process that is thermodynamically spontaneous (Ding et al. 2016) and can take place simultaneously. Cr conversion in soil depends upon chemical reactions (hydrolysis, reduction and precipitation) that occur in soil, and these dynamic reactions are affected by change in redox potential, soil pH, organic matter, biological and microbial conditions, competing cations and metal levels (Shahid et al. 2017).

23.3.1 Redox Potential

Soil has tendency to donate or accept electrons, and a measure of oxidation (at low Eh) and reduction (at High Eh) status of soil is known as redox potential. Reduction process increases soil pH by consuming protons, while oxidation process decreases soil pH by producing protons (Frohne et al. 2015). Cr speciation is found highly sensitive to soil redox potential. Reduced soil conditions cause conversion of toxic Cr VI into less toxic Cr III, as well as immobility and precipitation of Cr III (Xiao et al. 2015). Generally, Cr VI predominates in oxygen-rich environment at a neutral to alkaline pH. Cr VI has +138 V redox potential in acidic solution which is very high and representing its strong oxidizing potential (Shadreck 2013).

23.3.2 Soil pH

Soil pH control geochemical behaviour of heavy metals in soil solid and solution phase. It governs the sorption/desorption processes and chemical speciation of Cr and other heavy metals in soil (Ashraf et al. 2017; Dwivedi et al. 2014). Cr III has low solubility only at pH $<$ 55 above that it gets precipitated and show stability, whereas Cr VI show mobility in acidic as well as in basic pH (Kabata-Pendias 2010). The desorption of Cr III from soil solid into solution is most significant at low pH, while there is enhancement in adsorption of Cr VI on soil particles as pH decrease (Dias-Ferreira et al. 2015). Addition of amendments (lime) increased the Cr III sorption, but there was a slight decrease in Cr VI sorption. That happens because of the increase in pH of soil by releasing hydroxyl ions which also increases surface negative charge in soil, where Cr III precipitates to hydroxyl ions. An increase in soil pH decreases positive charges, hence decreases the sorption of Cr VI (Shahid et al. 2017).

23.3.3 Organic Matter

For mobility, bioavailability and sorption/desorption of Cr in soil, organic matter is a key component because OM acts as carrier for Cr and other heavy metals in soil via binding (Quenea et al. 2009). Factors such as ratio of soluble and stable organic carbon and concentration of micro- and macronutrients in OM are responsible for control of Cr adsorption and desorption in soil (Taghipour and Jalali 2016). Soil OM has potential to reduce Cr VI to Cr III which somewhere depends upon pH and redox potential, etc. High level of OM in soil helps in proliferation of the microorganisms which alert redox potential by creating reduced conditions, i.e. reduction of Cr VI to Cr III using various organic amendments (plant biomass, seaweeds, compost etc.) is followed by the process used in remediation and soil reclamation technique. Increased sorption of cationic Cr III by OM is attributed to the increase in cation exchange capacity. However, the increased sorption of Cr (VI) is facilitated by reduction mechanism or by stimulation of microbial growth by OM which ultimately reduces Cr VI to Cr III (Ashraf et al. 2017).

23.3.4 Microorganisms

Microorganisms are found everywhere in environment. In soil rhizospheric zone is the richest portion of soil rich in microbial number and activities that is vital to sustain soil fertility and plant growth and development. Numerous types of microorganisms that are found in soil help in reduction of hazardous Cr to nonhazardous one under aerobic and anaerobic conditions (Maqbool et al. 2015). Many algae, bacteria and yeast help in microbial reduction of Cr VI to Cr III.

Several bacterial strains such as *Sporosarcina saromensis*, *Pseudomonas putida*, *Pannonibacter phragmitetus*, *Microbacterium* species, *Arthrobacter* species, *Bacillus* species, *Shewanella* sp. etc. have potential to reduce hexavalent chromium to trivalent chromium. Microbial-induced reduction of Cr VI generally follows either one or a combination of the three processes. These processes include (i) use of soluble chromate reductase that use NADPH or $NADH^+$ as cofactors under aerobic conditions, (ii) use of Cr VI as an electron acceptor under anaerobiosis for some bacteria (*Desulfotomaculum reducens*) and (iii) use of some compounds such as glutathione, nucleotides, amino acids, vitamins, sugars and organic acids present in intra- or intercellular to reduce Cr VI (Shahid et al. 2017).

Microbial-induced Cr VI conversion to Cr III helps in reduction of its toxic effect, which can be attributed to the precipitation of various Cr III forms. In such way, Cr is stabilized in soil with minimum transfer to crop. That's why this technique is used in bioremediation for Cr contamination site (Wu et al. 2016). Evidenced species are *Bacillus* sp., *Pseudomonas fluorescens*, *Sporosarcina saromensis*, and *Leucobacter chromiireducens* (Joutey et al. 2016; Rani et al. 2016). At 30^0 C temperature and neutral pH, microbes reduce Cr at efficient rate, but it sometime varies according to strain and soil and Cr contamination status (Joutey et al. 2016).

23.4 Soil-Plant Transfer of Cr

Cr is a two-edge sword on one side; it is an essential element for human metabolism, and on other side it's carcinogenic. Therefore, its optimal amount in human nutrition has become crucial in risk assessment and remedial studies. Cr is taken up by plants by carrier specific for the absorption of essential ions for plant metabolism. Cr species decide the way of entry in plants, i.e. Cr III uptake in plants is a passive mechanism, whereas Cr VI uptake is active one (via phosphate or sulphate transporter, due to structural resemblance with Cr). The interaction of Cr VI with sulphate is supported by the fact that Cr VI and sulphate exposure to plant induces similar starvation effects in plants, which attributes a competition for uptake as well as in subsequent assimilation pathway (de Oliveira et al. 2016). Cr VI also interferes with uptake of some essential nutrients such as K, Fe, Mn, Mg, Ca and P due to their ionic resemblance (Gradea-Torresdey et al. 2004). Cr VI has high soil-plant transfer index due to its high solubility and adsorption by cells than Cr III (Han et al. 2004).

23.4.1 Chromium Sequestration in Plant Roots

Cr shows least mobility in plant root as compared to other heavy metals, and it may be due to formation of insoluble compounds inside it, a natural Cr toxicity tolerance mechanism of plant. Caldelas et al. (2012) advocated above statements in plant *Iris pseudacorus* and showed that highest concentration of Cr is found in vacuoles, cell walls of roots and the cytoplasm and intercellular spaces of the rhizome. Although Cr transfer from root to shoot is limited because translocation depends on its chemical form inside the tissue, the reduction in translocation could be due to sequestering of Cr in root tissues of plant where Cr VI got converted into Cr III, a less mobile form of Cr (Kabata-Pendias 2010).

23.4.2 Chromium Translocation to Plant Shoot

The pathway to Cr VI translocation towards shoots is an active mechanism involving phosphate and sulphate transporters. Chromium uses iron and sulphur channels for upward translocation in plants. As sulphur and iron accumulating plant species show high Cr accumulation capability in their shoot parts, example of such plant species are Brassica rapa, Spinacia oleracea, etc.

Pootakham et al. (2011) identified six sulphate transporters in green algae *Chlamydomonas reinhardtii*, and these transporters may also be involved in Cr transport inside plants. Marieschi et al. (2015) recommended that under S starvation, the activation of sulphur uptake/assimilation pathways can provoke direct significance related to Cr detoxification/tolerance by enhancing sulphate accumulation and availability to the cells for the production of sulphur containing Cr detoxification molecules by chelation using phytochelates and compartmentalization in vacuoles.

23.4.3 Chromium Hyperaccumulator Plants

In phytoremediation, hyperaccumulators are used to extract metals from contaminated sites (Khalid et al. 2016). Hyperaccumulators can accumulate high concentration of heavy metal in shoot with bioaccumulation and translocation factor > 1, along with fast growth and high biomass production and high metal tolerance. More than 400 plant species, belonging to 45 families, have been identified globally which can accumulate heavy metals in them. Chromium hyperaccumulators can accumulate$>$1000 mg Cr Kg^{-1} DW, in shoot (Redondo-Gomez et al. 2011). *Spartina argentinensis*, *Dyera costulata*, *Pluchea indica*, *Amaranthus dubius*, *Convolvulus arvensis*, *Prosopis laevigata*, *Pteris vittata*, *Leersia hexandra*, etc. are some Cr hyperaccumulator plant species (Shahid et al. 2017).

Chromium hyperaccumulators can tolerate Cr through chelation by biotransformation with reductants, high-affinity ligands and compartmentalization in the plant tissues Hyperaccumulation of Cr and other heavy metals are determined by many factors such as by (i) enhanced mobilization of metals in rhizosphere, (ii) increased absorption and translocation to shoot tissues via enhanced xylem loading and (iii) chelation and detoxification of metals within plant cells. There is major role of root secretion in Cr solubilization and phytoaccumulation and of metal transporter families in enhancement of metal absorption, xylem loading and transportation to shoot tissues. Metals are generally complexed by organic ligands followed by their sequestration into vacuoles. These ligands include amino acids, organic acids, peptides and polypeptides (Shahid et al. 2017).

23.5 Toxic Effect of Chromium in Plants

Cr is well known for its toxic effect on plant growth and development by alerting biochemstry and physiology of plants such as seed germination, root length, shoot height, leaf development, etc.

23.5.1 Seed Germination

Phaseolus vulgaris, *Triticum aestivum*, *Hibiscus esculentus*, *Avena sativa*, *Echinochloa colona*, *Medicago sativa*, *Eruca sativa*, *Saccharum officinarum*, etc. are some plant species that show toxicity effect in variable concentrations during seed germination (Shahid et al. 2017). Seed germination is the first physiological effect of Cr on plants. Cr induces toxicity to seed germination and may occur due to suppression in the activity of amylase under Cr stress. Amylase hydrolysis of starch is vital for sugar supply required for embryo development. Chromium toxicity reduces amylase activity thereby inhibiting seed germination (Rani et al. 2016).

23.5.2 Plant Root and Shoot

Cr-toxicity shows decrease in root, stem growth and their development in some selected plant species such as *Phaseolus vulgaris*, *Avena sativa*, *Caesalpinia pulcherrima*, *Eruca sativa*, *Solanum nigrum*, *Parthenium hysterophorus*, *Allium cepa*, *Triticum* sp., and *Phaseolus vulgaris* and other 32 species while exposed to different Cr-concentration (Sharma et al. 2016; Kamran et al. 2016; UdDin et al. 2015; Kumari et al. 2016; Karthik et al. 2016; Lukina et al. 2016). Chromium also alert secondary root number and lateral root development in *Zea mays*, and it may be due to root cell division. Cr interferes in uptake of water and nutrient uptake by root which consequently delayed cell cycle and so in stem growth and development (Mallick et al. 2010).

Leaf growth parameters act as appropriate bioindicators of heavy metals toxicity. Plant species exposed to Cr show reduced leaf growth ,wilting and , chlorotic symptoms as compared to control. Continuous and long-term Cr application caused old leaves to become necrotic, permanently wilted, dry, shed and decrease in leaf area which can consequently decrease cell division and number of cells in the leaves. Dube et al. (2003) reported chromium toxicity effects such as reduced size and number of leaves in watermelon plants and turned them yellow and wilted to loss of turgor hung down from petioles. Chromium stress in leaves negatively influences photosynthesis in terms of electron transport, CO_2 fixation, enzyme activities and photophosphorylation, a, b and total chlorophyll and carotenoids in *Taxithelium nepalense*, *Ocimum tenuiflorum*, *Phaseolus vulgaris*, *Hibiscus esculentus*, *Chenopodium quinoa*, *Phaseolus vulgaris* and *Lemna minor* (Sharma et al. 2016; Sahid et al. 2016). Cr VI and Cr III stress may inhibit pigment biosynthesis by degrading δ-aminolaevulinic acid dehydratase, key enzyme in chlorophyll biosynthesis, and by interfering with Fe and Mg for uptake and transport to leaves (Dey and Mondal 2016). Redriguez et al. (2012) reported that Cr can induce toxicity to photosynthesis through its interference with the enzyme RuBisCO, stomatal conductance, transpiration rate and substomatal CO_2 concentration.

23.5.3 Nutrient Uptake

Mg, N,P, K, Mn, Fe, Cu and Zn essential nutrients got alerted by Cr interference in many plant species such as *Oryza sativa*, *Cocos nucifera*, *Brassica oleracea*, *Amaranthus viridis*, etc. (Shahid et al. 2017). Hence, the competitive binding of Cr to common carriers can decrease the uptake of many essential nutrients that could be the reason of decrease in the activity of plasma membrane H + ATPase. Exposure to high levels of Cr may displace essential nutrients from physiological binding sites and synergistic interactions between Cr and essential nutrient such as Cu, Mn, Ca and Mg.

23.5.4 Plant Biomass

Chromium exposure decreases the plant growth, development and yield due to less water and nutrient uptake, and also it leads to decrease in cell division, cell division rate, imbalance in nutrient uptake, translocation, inefficiency of plants for selective and which may results the oxidative stress or damage to sensitive plants tissues such as mitochondria, pigment contents, DNA, RNA etc. (Sahid et al. 2016).

23.5.5 Genotoxicity

Mechanism of genotoxicity of Cr in plants is not properly understood; rather only consequences are visible such as Cr toxicity in plants which causes chromosomal abnormalities, impairing of cell division, arrest of cell cycle, repression of antioxidative enzymes and induction of micronuclei formation (Truta et al. 2014). Redriguez et al. (2011) came to know that in *Pisum sativum*, Cr stress induces alteration in cell cycle in roots at G2/M phase along with polyploidization at both 2C and 4C levels in leaves. Similarly Kumari et al. (2016) also reported that exposure to Cr containing tannery effluent enhanced mitotic index and micronuclei formation in root tips of *Allium cepa*. In *Vicia faba* root tips, Cr toxicity and micronuclei frequency showed linear relationship ($r^2 = 077$) when treated with sewage sludge (Louban et al. 2016). In addition to direct interaction with DNA, Cr can damage DNA indirectly via ROS production.

23.6 Chromium-Induced Oxidative Stress and Lipid Peroxidation

Primary response of plant (alteration in morphological and physiological processes) to heavy metal stress is overproduction of reactive oxygen species in their tissues, and Cr does the same. The ROS includes hydrogen peroxide (H_2O_2), singlet oxygen (½ O_2), superoxide anion (O_2^-), hydroxyl (HO), alkyl (RO), peroxyl (RO_2) and organic hydroperoxide (ROOH). ROS are natural scavengers and produces naturally as by product in aerobic reactions occurs in mitochondria, peroxisome and chloroplast (Shahid et al. 2017). Chromium-induced increased production of ROS has been reported in various plants such as *Brassica campestris* and *napus*, *Zea mays*, *Oryza sativa*, *Gossypium hirsutum*, *Ocimum tenuiflorum*, *Corchorus olitorius*, *Arabidopsis thaliana*, *Chenopodium quinoa*, etc. (Shahid et al. 2017). It is reported that Cr can induce overproduction of ROS by various ways: directly through the Fenton and Haber-Weiss reaction and indirectly by reducing the activities of various antioxidant enzymes. It is reported that during radical reduction of Cr VI, Cr IV and Cr V intermediates are produced which are involved in ROS generation. Both the intermediate products Cr IV and Cr V are catalytically active and are capable to produce ROS such as hydroxyl ion (Strlic et al. 2003).

Lipid peroxidation and malondialdehyde are generally considered as bio-indicator of oxidative stress in plants. Lipid peroxidation is the most deleterious influence caused by heavy metals and Cr-induced ROS because it causes loss of functionality and integrity of the membrane. Malondialdehyde is a decomposition product of lipid peroxidation (Pourrut et al. 2013) Plant species such as *Hordeum vulgare*, *Brassica campestris*, *Catharanthus roseus*, *Oryza sativa*, *Amaranthus viridis*, *Zea mays*, *Gossypium hirsutum*, *Phaseolus vulgaris*, *Corchorus olitorius*, *Brassica napus*, *Pisum sativum* and *Chenopodium quinoa* showed overproduction of ROS and lipid peroxidation when exposed to Cr contamination (Shahid et al. 2017).

23.7 Defence System Against Cr Stress in Plant

To escape from Cr stress or detoxification and to enhance metal tolerance, plant has adopted defence strategies. Various strategies have been developed by plants against Cr toxicity including chelation of Cr with ligands (by phytochelatins), activation of antioxidant enzymes, compartmentalization of Cr in the vacuoles, reduction of Cr VI to Cr III, etc. (Daud et al. 2014; Prado et al. 2016).

23.7.1 Phytochelatins

Phytochelatins (PCs) are heavy metals chelating cysteine-rich polypeptides with the general structure (c-Glu-Cys)n-Gly. PCs play role in homeostasis and detoxification of toxic metals including Cr. Phytochelatins are produced by phytochelatin synthase (PC-synthase) which uses GSH as a substrate (Sahid et al. 2016). During detoxification of Cr in plants, PCs first reduce Cr VI to Cr III and then transfer PC-Cr complexes to vacuoles of roots. Important role of PCs in detoxification of Cr has been reported for various plant species such as *Brassica juncea*, *Vigna radiata*, *Helianthus annuus*, *Xanthoria parietina*, *Raphanus sativus*, *Zea mays*, *Solanum lycopersicum*, *Oryza sativa*, etc. (Sahid et al. 2016).

In addition to PC, metallothioneins (MTs), cysteine-rich low molecular weight proteins, found on the Golgi apparatus membrane and are products of mRNA translation, also detoxify Cr in plants. Shanker et al. (2004) reported enhanced expression of MT3 genes under Cr stress in the tolerant variety compared to the sensitive one, thereby suggesting high transcription rates of MTs. They proposed that enhanced production of ROS under Cr stress triggered signals to induce MT mRNA transcription where MT-Cr ions bind and rendering Cr nontoxic.

23.7.2 Antioxidant Enzymes

Cr stress induce ROS production in plants, and so plants have evolved well-developed ROS scavenging enzymatic mechanism comprising of superoxide dismutase (SOD), catalase (CAT), glutathione, guaiacol peroxidase (POD) and ascorbate peroxidase (APX) (Maiti et al. 2012). Superoxide dismutase is an important enzyme for scavenging ROS. This enzyme dismutates two $O^{-}{}_{2}$radicals to O_2 and H_2O_2. Increase in SOD activity when exposed to Cr has been reported in *Oryza sativa*, *Solanum nigrum*, *Parthenium hysterophorus*, *Ocimum tenuiflorum*, *Zea mays*, *Amaranthus viridis*, *Gossypium hirsutum*, *Pisum sativum*, etc. CAT generally localized in the peroxisomes is an important antioxidant enzyme that scavenge H_2O_2 to O_2. In plants Cr can induce or suppress CAT activity. Induction of CAT by exposure of Cr has been observed in *Zea mays*, *Gossypium hirsutum*, *Corchorus olitorius* and *Pistia stratiotes* whereas suppressed in *Triticum aestivum* and *Matricaria chamomilla*. Glutathione occurs in various subcellular organelles, neutralize Cr-mediated enhanced generation of ROS through ascorbate-glutathione cycle and has been reported in *Oryza sativa*, *Salvinia natans*, *Pistia stratiotes*, *Brassica napus*, etc. (Shahid et al. 2017).

23.8 Health Risk by Cr Exposure

Chromium uptake by plants and accumulation in edible plant parts and their consumption by humans and animals can induce numerous health risks. Vegetables may accumulate heavy metals in their tissues more than maximum permissible limit. Vegetables and fruits are good source of essential micro- and macronutrients, and some can accumulate Cr in their tissues that may present a possible risk to the human health (Rani et al. 2016). Through the consumption of Cr-contaminated vegetables, respiratory, carcinogenic, renal, hepatic, gastrointestinal, cardiovascular, haematological, reproductive and developmental, genotoxic and mutagenic disorders can happen in human beings. Soil and plant enrichment factor (EF), translocation factor (TF), estimated daily intake (EDI), hazard quotient (HQ), maximum allowable level of plant consumed (MALPC), bioaccumulation potential (BAP), health risk index (HRI) and lifetime cancer risk (ILTCR) (Noli and Tsamos 2016; Rehman et al. 2016) are different parameters to estimate Cr health risk to humans as a result of consumption of Cr-contaminated food.

In contrast, it is believed that small quantity of Cr III is required for human metabolism and presents no human risk at low-level consumption of vegetables. Cr-induced health risk depends on soil contamination status and plant type. Chen et al. 2014 reported that bioaccumulation potential of Cr in human correlates with total soil Cr for *Allium cepa*, *Brassica rapa* and *Allium sativum*. But people eat mix vegetable and that may cause health risk. Therefore, choice of consumption of vegetables should be strategic and well managed under Cr-contaminated sites (Khalid et al. 2017).

23.9 Worldwide Case Studies

Wang et al. (2011) reported that the people living in Cr-exposed area of Hunan Province, China, are at risk of Cr-induced health hazards. Nabulo et al. (2012) deliberated health hazards of Cr for leafy vegetables grown at five different sites in peri-urban agriculture of Kampala City. Four out of five sites had HQ (10). Cherfi et al. (2015) calculated that the vegetables irrigated with heavy metal-contaminated water present potential health risk for consumers due to high estimated daily intake and targeted hazard quotient.

23.10 Conclusion

This chapter reviewed the biogeochemistry of Cr in soil-plant environment. In both environments (soil and plant), Cr exists in stable form as Cr III and Cr VI. With existence of chromium in soil, its phytoremediating mechanisms may vary significantly with its chemical speciation. Speciation and soil-plant transfer of Cr are governed by soil physico-chemical properties and microbial communities. Soil microbes help in reduction of Cr VI to Cr III, a less toxic form which refers as an efficient bioremediation technique. Cr can uptake through both active and passive way in plants. Translocation of Cr in plant is limited to specific part. In roots Cr retains in excess as compared to shoot due to restricted translocation. However, Cr hyperaccumulators can transfer high level of Cr to shoot tissues.

Cr toxicity alerts seed germination, plumule and radical growth, root elongation and plant development by interfering in physiochemical processes in plant. Cr-mediated hiked production of ROS causes lipid peroxidation, protein oxidation and genotoxicity. To combat the situation, plants have developed their own defence mechanism to detoxify effect of Cr in plants and make them more tolerant to Cr. Care should be taken while consuming Cr-contaminated food because it requires only in very specific amount by body otherwise can cause deleterious health risks.

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24 Biosorption: A Novel Biotechnological Application for Removal of Hazardous Pollutants

Anchal Singh and Pinki Saini

Abstract

Absorption is an emerging biotechnological innovation that involves removing/remediation of organic and inorganic substances like heavy metals, pigments, dyes, phenolics and pesticides from aqueous solution by economical alternate biological materials. A number of biomasses of different genera have been recognized to possess good biosorption capacity. It includes living or dead microorganisms like algae, bacteria, fungi and yeast and their components such as exopolysaccharides, seaweeds and plant materials and industrial and agricultural waste; natural residues are employed as sorbent in biosorption process. Biosorption process involves a solid phase and liquid phase (solvent) containing a dissolve species to be sorbed (sorbate/metal ions). It is a physicochemical, passive, rapid, reversible and metabolic-independent process. The binding characteristics of biological materials are attributed to the functional groups present on their surface. Mechanisms involved in the biosorption process include complexation, adsorption-complexation on surface and pores, ion exchange, membrane filtration, microprecipitation, heavy metal hydroxide condensation onto the biosurface and surface adsorption. Time, temperature, pH, concentration of organic pollutants and biomass in solution have a significant role in this process. It is an excellent way to treat industrial waste effluents, precious metal recovery, offering significant advantages like the low-cost, availability, profitability, ease of operation, efficiency and effectiveness and eco-friendly biological method. The aim of this article is to bring out the role of biosorption in the treatment and management of toxic waste.

Keywords

Biosorption · Biosorbent · Physiochemical · Chemosorption · Microprecipitation

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24.1 Introduction

Water is a natural resource for the growth and survival of all living organisms and development of culture and economics. Human activities such as increase urbanization, population growth, industrial production, climate change and other factors make the water quality degrades and pollute it beyond the level that it is challenging to removal of pollutants from the sources. There are two broad classes of contaminants/pollutants: organic and inorganic. The category of organic contaminants include industrial solvents, insecticides, pesticides and food processing wastes which can be degraded, whereas inorganic pollutants such as metals, fertilizers and industrial discharges, etc. are ineradicable and will be present indefinitely in the environment, which may result in probable accumulation in soil and water showing undesirable effect on biota and human health via the food chain (Sparks 2005). A number of industry such as printing, paper and pulp, tannery, textiles, petroleum, pesticides, paints and pharmaceuticals are the consequence of heavy metals pollution, one of the major environmental problems today that may pose serious health hazards (Glombitza and Reichel 2014). Heavy metals are naturally occurring elements and has high atomic weight between 63.5 and 200.6 (Aktan et al. 2013) and density at least five times greater than that of water, although it is present in the environment at low level, but in larger amount, it can be dangerous (Moraes et al. 2014). Mercury, lead, cadmium and chromium (VI) are regarded as toxic, but copper, nickel, cobalt and zinc show its toxicity on regular and long-term extensive use. Their toxicity depends upon the dose, route of exposure, chemical species as well as the age, gender, genetics and nutritional status of exposed individuals (Moraes et al. 2014). Food/water contaminated with heavy metal may have a determining effect on human and animal health even at low concentration, but gradual accumulation can lead to problem with the functioning of vital cellular components. Conventional processes consist of numerous limitations in the elimination of heavy metal from water as these are not cost-effective, not viable for treatment of low concentration, not renewable materials, not generation of toxic sludge and far away from their best possible performance (Popuri et al. 2007). These are precipitation, evaporation, electroplating, membrane processes, ion exchange, etc. Consequently it is needed to replace the method with biological, cheap and efficient method of treating metal-bearing effluents to provide a possible way out of metal removal from contaminated environment (Pandit et al. 2013). Hence the technology used to eliminate the heavy metal assist of biological material without generation of toxic sludge is known as biosorption.

24.2 Heavy Metals

Heavy metal contamination in water resources is one of the most serious problems in developing countries as well as a major environmental problem too; it's non-biodegradability, carcinogenicity and bioaccumulation property in soil, water and air accompanied by flora, fauna and human are disquiet. It contaminates terrestrial ecosystem in the course of soil while aquatic through sediments which affect the system via runoff, leaching and transport by mobile colloids (Adriano et al. 2004). The term heavy metals refers to metals and metalloids having an atomic density greater than 4 g/cm^3, atomic weight between 63.5 and 200.6, toxic and poisonous even at low concentration (Fu and Wang 2011). It is classified as toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (Au, Ag, PT, Pd, Ru, etc.), volatile metals (Hg, As, Sb, Se) and radionuclide (U, Ra, Th, Am, etc.) (Wang and Chen 2006, 2009). The common source of these pollutants includes natural, such as earth crust, rocks and metal restrains minerals, and anthropogenic sources which include mining, metallurgy, smelting, batteries, paint pigments, agriculture, pharmaceutical, domestic effluents, energy production units, microelectronics, sewage sludge and waste disposal (Ibrahim and Mutawie 2013). It's carcinogenicity increases oxidative stress in the body and leads to several health problems as liver and kidney damage, cardiac arrest, GIT and neurological disorders (Lung et al. 2015).

24.3 Biosorption: An Emerging Alternative Solution

Biosorption can be defined as the passive uptake of toxicants, exclusion or binding of metal or metalloid species, compound and particulates, organic and inorganic from aqueous solution through metabolically independent or physiochemical pathway by low cost dead/inactive biological materials or by materials derived from biological sources (Wang and Chen 2009; Gadd 2009). Biosorption process involves a solid phase (sorbent or biosorbent material) and liquid phase (solvent, normally water) containing a dissolve species to be sorbed (sorbate, metal ions). Biosorption is made up of two words bio and sorption; bio means the association of biological material, and sorption means both absorption and adsorption. Absorption involved both physical and chemical phenomenon in which molecules or ions enter some bulk phase (liquid and solid). On the other hand, adsorption is a physical process that involves bonding of molecules or ions onto the surface of another molecule. It is most economical, eco-friendly and highly efficient method, minimizes the chemical-biological sludge and regenerates biosorbent with possibility of metal recovery. It is analogous to conventional ion-exchange technology, where dead organisms along with waste bioproducts may be used. Live biomass utilized and intended for sorption process is known as bioaccumulation, i.e. metabolism-dependent active process. Algae (Salim et al. 2015), bacteria (Halttunen et al. 2007; Kinoshita et al. 2016), fungi (Amany et al. 2015) and cellulosic waste (Farooq et al. 2016; Abdolai et al. 2016) that include different wastes of food products have shown their capability to act as biosorbent of heavy metals (Kumar et al. 2014). Biosorption of heavy metal removal has also been reported involving the use of either laboratory-grown microorganism (biofilms of microorganisms or exopolysaccharide) or biomass generated by different food processing industries or agricultural wastewater treatment units (Shuhong et al. 2014). Studies reported that biosorption has the following advantages over other conventional methods: chelating metal at very low concentration, i.e. less than 100 mg/l, raw material is easily available and fiscal due to utilize action of waste products and dead biomass, no requirement of costly growth media and rapid process; metal loading is high due to ion-exchange process of nonliving materials; and process is reversible as sorption and desorption can take place, minimizing chemical and biological sludge. Drawback of biosorption including desorption of the metal from the biosorbent material has to be done for further use; the characteristic of biosorbents are difficult to be biologically controlled.

24.3.1 Biosorption Agents/Biosorbents

Biological material or biomasses having the capability to eradicate heavy metals are termed as biosorption agent or biosorbent. The potential sorbents like bacteria, yeast, fungi, algae, exopolysaccharides, sawdust, seed shells, sugar beet, pectin, gels, potato peels, straw and bran of wheat, etc. are used because of its economical and high efficiency (Abdelfattah et al. 2016). Biosorption are considered to be economical adsorbent, feasible and can be obtained from the various industries as a waste product. The adsorption activity towards the metal removal is due to the presence of functional groups like alcohol, aldehydes, ketones, carboxylic, ether and phenolic groups, etc. present on their surface. Factors affecting the potential of biomass in adsorption process are pH, temperature, adsorbent dosage, metal concentration and contact time, etc.

24.3.1.1 Bacteria as Biosorbent

Wastewater treatment using bacteria is a good biological approach. Bacteria have certain characteristics like smaller size, availability and flexibility. Numerous species of bacteria have potential to remove pollutants that are not biodegradable like metal ions and dyes. The cell structure, its biofilm capacity (bacterial biomass are usually used in the form of binding or supporting material to the adsorbent for the removal of heavy metals from the aqueous solution, e.g. biofilm of *Escherichia coli* and *Bacillus* sp. for removal of Cr (VI)) and also exopolysaccharide produced property which has great importance in heavy metal quenching (Khan and Joseph 2015; Toole et al. 2000). Cell wall shape and strength are primarily due to peptidoglycan, which is rigid, porous and amorphous material, and the core of which is very similar in all bacteria (Wang and Chen 2009). Peptidoglycan is a liner polymer of alternating units of two sugar derivatives, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). Peptidoglycan also contains several different amino acids, with D-glutamic acid, D-alanine and meso-diaminopimelic acid to be the major ones. It rapidly provides amino, carboxylic, phosphate and sulphate groups and has great importance in binding heavy metals due to their small size, ubiquity, capability to grow under controlled condition and resistance against varying environmental conditions. Bacterial species such as *Lactobacillus*, *Pseudomonas*, *Streptomyces*, *Escherichia*, *Micrococcus*, *Actinomycetes* (Ni^{2+}, Cr^{6+} and Zn^{2+}), etc. have been tested for metal quenching from aqueous solutions. Bacteria have developed a number of competent system for detoxifying metals ions and resistance mechanism for their survival (Mrvcic et al. 2012).

24.3.1.2 Algae as Biosorbents

The number of researches has been done on living and nonliving algal biomass for the elimination of heavy metals from wastewater. The adsorption capacity of living biomass is limited as compared to dead in the case of the heavy metal removal because the adsorption process is taking place in the growth phase and the heavy metal uptake also takes place in this phase only which is considered as an intracellular process and adsorption mechanism are more complicated in case of it, but in the case of nonliving algal biomass, the extracellular process is carried out because the metals get adsorbed on the surface of the cell wall. Algae are efficient and cheap biosorbent, required less nutrients being autotrophic, have high sorption capacity and are readily available in larger quantities in the oceans (Brinza et al. 2007), produce large biomass, do not produce toxic substance and unlike other biomass and microbes, such as bacteria and fungi. Algae are classified as green algae or freshwater algae which refer to micro-algal, brown algae or marine algae which refer to micro-algal and red algae. Brown algae have the higher metal-bearing capacity compared with red and green algae. The statistical analysis reported that biosorption potential of algae is about 15.3–84.6% which is higher as compared to other biosorbents. Using algae as biosorbent material has been less compared with the other kinds of biomass, especially fungi (15%) and bacteria (85%) (Kumar et al. 2014). Celluler component of algae includes different functional group, contained in cell proteins and sugars, which enhances the biosorption capacities, and they are carboxyl, amine, imidazole, phosphate, sulphate, sulfahydril and hydroxyl (Oyedepo 2011). It produces phytochelatin which synthesizes in response to toxic heavy metals, and stress binding of metal ions on algal surface depends on ion-exchange method where Na, Mg and Ca become displaced by heavy metal ions, complexion between metal ions and various functional groups such as carboxyl, amino, thiol, hydroxyl, phosphate and hydroxy-carboxyl, which can interact in coordinated way with heavy ions and algal species. Certain environmental factors like pH, temperature and contact time, etc. can influence the adsorption capacity of nonliving algae. Desorption of algal biomass can be done using HNO_3, HCl and EDTA 2Na which is the major advantage of the algal biosorption process for the recovery of biomass and makes the adsorbent as reusable. The usage of algae in environmental applications was found to have dual use in wastewater treatment and biofuel production.

24.3.1.3 Fungi as Biosorbent

Fungi are a large and diverse group of eukaryotic microorganisms, competent and economic for toxic metal removal from aqueous solution through biosorption. It can grow in natural environmental conditions. It is waste biomass belongings of antibiotic and food industries. Filamentous fungi and yeasts have been observed in many instances to bind metallic elements. Excess cell wall material and extracellular enzymes have the capacity to sorbed metals. The cell wall is made up of chitin, glucan, mannan, proteins and other polymers like carboxyl, phosphoryl, hydroxyl and imidazole functional groups which make the fungi more potential for adsorption process. Fungi can absorb the heavy metals through the mechanisms like intracellular precipitation, valence transformation, ion exchange and complexation. Some studies showed fungi and yeasts for the removal of toxic metals (such as lead and cadmium) from wastewater, Zn (II), CO (II) and Cd (II) from ternary mixture through dried *Aspergillus niger* biomass and recovery of precious metals (such as gold and silver) from process waters (Kapoor 1997). Some researches elaborate the use of fungal biomass composite with clay materials like smectites and kaolinites which have the physical and chemical stability towards the heavy metals. Fungal dead biomass composite with bentonites is found to remove Ni (II) and Zn (II) from aqueous media. Both living and dead fungi possess a significant ability to toxic and precious metals recovery. Metal uptake takes place through both intracellular and active binding and extracellular passive-binding mode. Fungi are relatively easy to grow in fermenters and so appropriate for large-scale production, and separation is also easy due to its filamentous structures along with less sensitive towards nutrition, aerobic and anaerobic condition, pH and temperatures (Leitao 2009). Fungi are utilized in two forms like mobilized condition and in immobilized cells. In the last decades, the use of immobilized systems of fungal cells for the metal uptake in the adsorption process took place because they are easy to use and handle. Adsorption process using immobilized cells *of Aspergillus niger* was evaluated for the removal of Cu^{2+}, Mn^{2+},Zn^{2+}, Ni^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}) from wastewater. To overcome the loss of biosorbent after regeneration process, they immobilized the fungal biomass with polymer matrixes (PVA and Ca alginate gels). The results showed that the removal efficiency of Ca alginate-immobilized biomass was found to be higher than the PVA-immobilized biomass in the remediation of the aforementioned heavy metals.

24.3.1.4 Biopolymer-Based Absorbent

Biopolymers are polymeric biomolecules produce by living organism. It contains monomeric units that are covalently bonded to form larger structures. There are three main classes of biopolymers: polynucleotide (long polymers composed of nucleotide monomers), polypeptides (short polymers of amino acids) and polysaccharides (linear-bonded-polymeric carbohydrate structures). The biopolymers are the main constituent of living cell structures and contain numbers of functional groups in their structure, play a vital role in biosorption process. These are natural biopolymers that participate in biosorption process, along with these there are some synthetic biopolymers also introduced like alginate which is environmental friendly and extensively used biopolymer in biosorption process. Calcium alginate pallets exhibit higher absorption capacity and can efficiently remove anionic contaminant from water solution. It is encapsulate with raw and untreated biomass or other materials to enhance the biosorption property such as magnetite which lead the formation of multifunctional sorbent, persist magnetic property and can remove both cationic heavy metals with the maximum adsorption capacities of above a few mmole per gram of sorbent and anionic contaminants like arsenic (Lim et al. 2008).

24.3.2 Low-Cost Adsorbents

Low cost adsorbents along with aforementioned adsorbents usage of low-cost adsorbents like agricultural wastes, industrial by-products and natural substances for the removal of heavy metals from wastewater. Low cost adsorbents include activated carbon, and in elastic double network polyvinyl alcohol/polyacrylic acid double network gel (PVA/PAA) adsorbent which was synthesized through a simple two-step method for the elimination of Cd(II) and Pb(II), peat was used by (Bartczak) for the removal of nickel (II) and lead (II) ions from aqueous solution and acrylonitrile grafted banana peels (GBPs) for the elimination of hexavalent chromium Cr(VI).

24.3.3 Agricultural and Plant Food Products Waste

The biosorption capacity of different agricultural waste and cellulosic content has high potential for biosorbed heavy metal from water. There are number of by-products such as sugarcane grass, soya bean hulls (Marshall et al. 2000), walnut hulls, cotton seed hulls and corn cobs (Reddad et al. 2002), straw, bran of wheat, banana, lemon and orange peel (Kelly et al. 2012), tea waste, maple leaves and mandarin peels (Abdolai et al. 2016), peanut husk (Abdelfattah et al. 2016), wheat and barley straw, rice husk hull and straw (Asadi et al. 2008), fruit rind-pulp and seeds, etc. which are used for biosorption process. Chemical composition of cell wall like cellulose, pectin, lignin, hemicelluloses, other polysaccharides, lipid, protein, sugar, water and many other compounds having various functional groups plays a vital role in metal sorption.

24.4 Bioabsorption: The Mechanism

The biosorptionprocess involves a solid phase (sorbent or absorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be saved (sorbet, metal ions) due to higher affinity of the sorbent for the sorbate species; the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. Biosorption is physiochemical process, which comprise of mechanisms such as absorption, adsorption, ion exchange, surface complexation and precipitation. The ability of microorganisms, for example, bacteria, fungi, algae and plant biomass to remove heavy metal ions or promote their transformation to less toxic forms, has been identified by various environmental scientists (Chaney et al. 2007; Singh et al. 2008; Yu et al. 2000). The complex structure of biomass implies that there are many ways by which these biosorbents remove various pollutants. Several factors are found to influence on the mechanism of metal biosorption such as the state of biomass (living or nonliving);

types of biomaterials; properties of metal solution chemistry; and environmental conditions such as pH, temperature, etc.

Living and nonliving biomass follow different mechanisms. Nonmetabolism dependent is followed by metabolism dependent, extracellular accumulation/precipitation, cell surface sorption/precipitation and intracellular accumulation (Neethu et al. 2015). During nonmetabolism dependent process can be rapid and reversible; there is physicochemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption (Ahalya et al. 2003). Cell wall of microbial biomass consisting polysaccharides, proteins and lipids has abundant metal-binding groups such as carboxyl, sulphate, phosphate and amino groups (Sardrood et al. 2013). Second the metabolism-dependent intracellular uptake, active biosorption or bioaccumulation and metal ions are transported across the cell membrane. A living cell requires both processes for metal transport and deposition, but nonliving cells in passive mode, i.e. metabolism independent, independent of energy, follow metal-binding mechanisms such as complexation, ion exchange, physical adsorption, etc.

24.4.1 Physical Adsorption

The process in which ions are transferred from a liquid phase to solid phase usually involves (i) boundary layer mass transfer across the liquid film surrounding the particles; (ii) internal diffusion/mass transport within the pores/solid diffusion; and (iii) adsorption within the particle and on the external surface. According to Allard et al. (1991) sorption may occur through two pathways: (i) physical adsorption, it is rapid and reversible and due to nonspecific attraction force (van der Waals force), and (ii) electrostatic adsorption due to coulombic attraction forces between charge solute species and adsorbent phase, which is usually rapid and largely reversible.

24.4.2 Complexation

The process involves metal removal through interaction between metal and the active group on the cell wall. It takes place by a complex formation on the cell surface. Complexation plays an important role in metal-ligand and sorbate-sorbent interactions. *Pseudomonas syringae* was found to be accumulating calcium, magnesium, cadmium, zinc, copper and mercury by complexation process.

24.4.3 Ion Exchange

Exchange of ions takes place between an electrolyte solution and a complex. In most cases the term is used to denote the processes of purification, separation and decontamination of aqueous solution via exchange of ions. The precise mechanism may range from physical binding followed by chemical binding. Biosorption of Pb

(II) and Cd (II) by *A. rubescens* and *L. scrobiculatus* biomasses took place due to chemical ion exchange.

24.4.4 Membrane Filtration

It is widely used in chemical and biotechnology processes; the process has valuable means of filtering and cleaning wastewater. There are a number of different methods of membrane technology. The most effective is pressure-driven membrane filtration. Other includes reverse osmosis, nanofiltration, ultrafiltration and microfiltration.

24.4.5 Microprecipitation

Refer to immobilization of metal species outside and inside the cells, for example, extracellular polymeric capsule or component.

24.5 Factors Affecting Biosorption

24.5.1 Effect of pH

It is possibly the most significant factor in biosorption process as it regulates the chemistry of sorbate, activity of functional group present on biosorbents and chemical properties of metallic ions (Gao and Wang 2007), microbial metabolism, growth and activity of cell functional groups. Mostly metal sorption is enhanced with pH in a particular pH range. With low-pH value, production of large number of H_3O^+ which occupies the binding sites enhance the electrostatic repulsion of functional groups in the cell surface and enhance sorption of anionic metals, while hydrogen ions inhibit the binding of metal cation through competing for binding sites due to strong repulsive force. As pH increase there is decrease in H_3O^+, and probability between metal cation and cell functional groups increases. It is found that removal of lead was high (55%) at pH 2, highest removal (95%) at pH 6, but as pH increases more than 6, precipitation of metals occurs, and reduction of absorption occurs (Halttunen et al. 2007)

24.5.2 Effect of Temperature

Usually the binding process is temperature dependent, as temperature increases it enhances the biosorptive removal of activity and kinetic energy of the adsorbate, but sometimes very high temperature may damage the physical structure of biosorbent (Park et al. 2010). The sorption capacity of biosorbent for metal ions depends on the interaction between biosorbent and metal ions which are exothermic or endothermic.

24.5.3 Influence of Contact Time

Biosorption which consists of two main processes, extracellur adsorption and intracellular uptake, is called bioaccumulation. Extracellur adsorption process is rapid and metabolism-independent process. It involves surface conjugation such as surface complexation and ion exchange, and metal transport occurs within few minutes as equilibrium is established. As chemical reaction starts there are abundant available empty binding sites on the biosorbent and high concentration of metal in solution, so the rate of metal uptake is very fast initially due to excited number of available sites present on biosorbent surface and decreases until equilibrium is reach (Abdolai et al. 2016). The latter stage is slow metabolism dependent and time-taking. It involves active transport and transmembrane transport, and metal uptake can be possible from surface into the cell through metabolism and vector. Rapid biosorption of metal ions was reported within 30 min, and maximum removal was observed in 2 h (Akar and Tunali 2006).

24.5.4 Biomass Concentration and Binding Sites

The dosage of biosorbent strongly influences the extent of biosorption. Metal removal increases with increasing the biomass concentration generally increased the surface area of the biosorbent, which in turn increases the number of binding sites and functional group on the surface of biosorbent conversely; the quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent dosage, which may be due to the complex interaction of several factors. An important factor at high biosorbent dosages, overlapping and coaggregation of biomass occurs due to electrostatic interaction, responsible to cut down the binding sites, that is why the available solute is insufficient to completely cover the available exchangeable sites on the biosorbent, usually resulting in low-solute uptake (Brenner et al. 2005). Many factors can affect biosorption like the type and nature of the biomass, freely suspended cells or immobilized preparations and living biofilms, etc.

24.5.5 Effect of Initial Pollutant Concentration/Metal-Ion Concentration

It is also an important factor for biosorption process. If the initial concentration of metal is limited and within the range of biosorbents or cell-resistant capacity, then sorption capacity increases, but if the concentration is too high in case of cell, it produces toxic effect like inhibiting the growth and metabolism leading to cell rupture. Actually initial metal-ion concentration provides a necessary driving force to overcome the resistance of metal ions. So if initial pollutant concentration is increasing, it increases the quantity of biosorbed pollutant per unit weight of biosorbent but decreases its removal efficiency (Park et al. 2010).

24.5.6 Effect of Pretreatment

In biosorption process the sorption capacity mainly depends on amount and accessibility and chemical state of the binding site and metals (Ahemad and Kibret 2013). Pretreatment and processing are an effective technique to enhance the binding sites enhancement, modification, polymerization and biosorption capacity. It is generally used to clean up the biomass to enhance the biosorption potential. There are number of methods such as heat treatment acid and alkali treatment, cross-linking of organic solvent, enzyme treatment and incapsulation which shows significant role in biosorption process. Study reported that the autoclaving of bacterial biomass enhanced the sorption capacity of metal ions; this is because of rupture of cell wall which exposes more number of potential binding sites (Paul et al., 2012). The aim of pretreatment is to increase the number of binding sites and enhance affinity and accessibility.

24.5.7 Particle Size

The smaller the particle size, the higher the biosorption effect because small particle can expose more contact surface. It is constructive for batch processes, not for column process due to its low-mechanical strength and clogging of the column (Park et al. 2010) (Tables 24.1, 24.2 and 24.3).

24.6 Desorption

Desorption of loaded biomass facilitates reuse, recovery and/or containment of sorbed materials of biomass, although it is desirable that desorbing agent does not significantly damage or disgrace the biomass (Gadd and White 1992). Desorption provides the possibility of regeneration of biomass, decrease overall process cost and continuous supply of biomass. A successful desorption depends on the mode of removal of metal ions and maintained mechanical stability of biomass. Generally most biosorbents exhibit an ion-exchange method for cationic ions, and mild acidic condition is required for desorption process. Desorption with acid is most useful because acidic solution is one of the common waste flush out through industries, and these waste have tendency to regenerate biosorbent. But high acidity could lead aggregation and integrity of biomass, lead decrease performance of biosorbents. $CaCl_2$ and EDTA are effective desorbents when complexation, chelation and microprecipitation mechanism are used; these require extensive screening for efficient desorption. In some cases desorption treatments may improve further sorption capacities, although in other hand it may be a loss of efficiency of the biomass. A

Table 24.1 Heavy metals, sources and associated health risks

Heavy metal	Sources	Health risks	Reference
Cadmium	Metal smelting and refining, phosphate fertilizers, welding, paint pigments, pesticides, plastics, polyvinyl and copper refineries, plastic	Renal dysfunction, proteinuria, obstructive lung disease, cadmium pneumonitis, bronchiolitis, chest pain, cough with foamy and bloody sputum and death of lung tissues. Bone defects as osteomalacia, osteoporosis and spontaneous fractures. Myocardial dysfunctions, nausea, vomiting, abdominal cramps, dyspnoea and muscular weakness	INECAR (2000), ECE (2002) and Young (2005)
Lead	Automobile batteries, petrol-based materials, pesticides, paints, pigments, electroplating, mining	Dysfunctions in the kidneys, joints and reproductive systems, cardiovascular system, gastrointestinal tract (GIT), acute and chronic damage to the CNS and PNS, severe and permanent brain damage, haematuria, anaemia, anorexia, malaise. In children poor development of the grey matter of the brain, resulting to poor IQ. Acute and chronic effects of lead result to psychosis	Lung et al. (2015), INECAR (2000) and Udedi (2003)
Mercury	Paper industry, emissions from industries producing caustic soda, thermometers, adhesives, paints, light bulb industry, wood preservatives, leather tanning, ointments-producing industry	Inorganic forms of mercury cause spontaneous abortion, congenital malformation and GI disorders (like corrosive esophagitis and hematochezia). Organic forms causes erethism, acrodynia/Pink disease, gingivitis, stomatitis, neurological disorders, total damage to the brain and CNS and congenital malformation	Lung et al. (2015)

(continued)

Table 24.1 (continued)

Heavy metal	Sources	Health risks	Reference
Arsenic	Volcanic, eruptions, soil erosion, semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, automobile exhaust, industrial dust and dyes	Arsenic inhibits the production of adenosine triphosphate; high-level exposure can cause death. Arsenic toxicity disorder, which confused with Guillain-Barre syndrome	INECAR (2000) and Granados-Correa et al. (2009)
Nickel	Metal refining, galvanization, paint formulation and powder, batteries processing units and superphosphate fertilizers, porcelain enamelling, stainless steel pipes	Oxidative stress, free radical formation in tissues, DNA mutation, neurotoxin, kidney, lung and liver damage along with reproductive damage, chronic bronchitis and lung cancer	Das et al. (2008), Akhtar et al. (2004) and Ozturk (2007)
Copper	Electroplating industry, metal refining, plastic industry and industrial emissions, copper polishing, paints	Nausea, vomiting, abdominal pain, kidney damage, anaemia, immunotoxicity and developmental toxicity, dizziness, diarrhoea	Merker et al. (2005) and Papandreou et al. (2007)
Zinc	Rubber industries, paints, dyes, wood preservatives and ointments, brass manufacturing, plumping	Zinc shows same signs of illness as does lead, mistakenly diagnosed as lead poisoning. Long term exposure causes impairment of growth and reproduction. The clinical signs of zinc toxicosis have been reported as vomiting, diarrhoea, haematuria, icterus (yellow mucus membrane), liver failure, kidney failure and anaemia	INECAR (2000) and Nolan (2003)
Chromium	Electroplating industry, leather, chrome plating, petroleum refining, tanning, textile manufacturing and pulp processing units. It exists in hexavalent and trivalent forms, textile, dyeing, steel fabrication	Carcinogenic, mutagenic, teratogenic, epigastric pain, nausea, vomiting, severe diarrhoea, lung tumours, etc.	Dupont and Guillon (2003), Olguin et al. (2013), Kobya (2004) and Shah et al. (2009)

Table 24.2 Recommended allowance of heavy metal according to FSSAI and WHO in drinking water

Heavy metal	Max. acceptable conc. (WHO 2004)	Max. acceptable conc. (FSSAI 2006)
Cadmium	0.003 mg/l	0.01 mg/l
Lead	0.01 mg/l	0.01 mg/l
Mercury	0.001 mg/l	0.001 mg/l
Arsenic	0.01 mg/l	0.05 mg/l
Nickel	0.01 mg/l	0.02 mg/l
Copper	2 mg/l	0.05 mg/l
Zinc	5 mg/l	5 mg/l
Chromium	0.05 mg/l	0.05 mg/l

number of substances have been used as metal desorbents including acids, alkalis and complex agents depending on the substance sorbed, process, requirements and economic considerations.

24.7 Conclusion

Biosorption is an alternative emerging method to conventional system for removal of toxic metals from industrial effluents. It offers several advantages, including cost-effectiveness, high efficiency, minimization of chemical/biological sludge and regeneration of biosorbents with possibility of metal recovery. Most significantly, the metal removal capacity of biological biomass is good or better than other conventional adsorbents. The new biological-based technologies need not necessarily replace conventional treatment approaches but may compliment them. Further investigation in structural studies of biosorbents, multi-metal studies, mechanistic modeling, recovery of metal ions, enhancement of biosorption capacity through modification of biosorbents and continuous flow studies is required for process scale up and enhanced industrial applications.

Table 24.3 Heavy metal biosorption by different microbial species and agriculture wastes

Methods of heavy metals removal	Metal ions	Biosorbents	Biosorption capacity (mg/g)	Reference
Biosorption of metals by bacterial species	Cadmium	*P. freudenreichii JS*	65.8	Ibrahim et al. (2006)
	Lead	*L. rhamnosus GG*	107	Halttunen et al. (2007)
	Mercury	*Enterobacter cloacae*	43	Rani et al. (2010)
	Arsenic	*L. casei*	0.312	Halttunen et al. (2007)
	Nickel	*Pseudo monas species*	556	Liu et al. (2004)
	Copper	*L. brevis*	26.52	Mrvcic et al. (2009)
	Zinc	*L. mesenteroides*	27.10	Mrvcic et al. (2009)
Biosorption of metals by algal species	Cadmium	*Ulva lactuca* sp.	43.02	Hajar (2009)
		Sargassum sp.	84.7	Lodeiro et al. (2004)
	Lead	*Spirogyra* sp.	140	Viraraghavan and Srinivasan (2011)
	Mercury	*Spirogyra hyalina*	39.2	Kumar and Oommen (2012)
	Arsenic	*Spirogyra hyalina*	8.7	Kumar and Oommen (2012)
	Nickel	*Ascophyllum nodosum*	50	Çelekli et al. (2010)
	Copper	*Spirulina platensis*	67.93	Brinza et al. (2007)
	Zinc	*Brown marine microalgae*	19–32	Freitas et al. (2008)
	Chromium	*Sargassum muticum*	34.10	Li et al. (2010)
		Sargassum greville	85.3	Kumar and Oommen (2012)
Biosorption of metals by fungal species	Cadmium	*Penicillium simplicissimum*	52.50	Martins et al. (2015)
	Lead	*Penicillium*	204	Tan et al. (2004)
	Mercury	*Saccharomyces cerevisiae*	64.2	Zeng et al. (2012)
	Nickel	*Penicillium chrysogenum*	260	Tan et al. (2004) and Hassan and Kassas (2012)
	Copper	*Aspergillus niger*	34.4	Zeng et al. (2012)
	Zinc	*Penicillium simplicissimum*	65.60	Martins et al. (2015)
	Chromium	*Aspergillus niger*	36.5	Wang et al. (2007)

(continued)

Table 24.3 (continued)

Methods of heavy metals removal	Metal ions	Biosorbents	Biosorption capacity (mg/g)	Reference
Biosorption of metals by cellulosic and agriculture wastes	Cadmium	Tea waste, maple leaves and mandarin peel	31.73	Abdolai et al. (2016)
		Rice straw	13.89	Li et al. (2015a, b)
	Lead	Tea waste, maple leaves and mandarin peel	76.25	Feng et al. (2011)
	Nickel	Orange peel	9.82	Torres-Ronda and del Alcázar (2014)
	Copper	Tea waste, maple leaves and mandarin peel	41.06	Abdolai et al. (2016)
	Zinc	Tea waste, maple leaves and mandarin peel	26.63	Abdolai et al. (2016)

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Part IV

Agriculture, Food, Nutrition and Health Security

25

Emerging Dichlorvos-Based Air Freshener Pertube Kidney Function in Male Albino Rats

E. I. O. Ajayi, J. B. Adetunji, M. A. Adeleke, A. O. Onilejin, M. O. Idrees, and T. E. Babalola

Abstract

Dichlorvos is an active ingredient, which is predominantly present in all synthetic and local organophosphorus pesticides used indiscriminately in Nigeria homes and farms. The deleterious effects of this toxicant have been severally reported. Recently, some dichlorvos-based air fresheners have been introduced into the market both for insect eradication and aromatic fragrance, by spraying as microdroplets in homes and offices without the need to vacate the space. The aim of this study, therefore, was to investigate the effects of two variants of dichlorvos-based aerosols, Sharp Action® (insecticide) and Choice Double Action® (air freshener) on kidney function indices of experimental rats compared to DD Force®. Thirty-six male albino rats weighing 180–200 g were randomly divided into six groups: Sharp Action® (SA_1,1:12; SA_2, 1:24), Choice Double Action® (CDA_1, 1:0.5; CDA_2, 1:1), DD Force® (DDF, 25 ng/kg) and Control, respectively. The three commercial samples were orally administered to rats for 21 days, and following overnight fasting, the rats were sacrificed. Blood was collected for biochemical analysis, while kidney was excised for organ-body weight assessment. The results obtained showed that the organ-body weight ratio significantly increased in all treated groups (4.4×10^{-3}, 4.1×10^{-3}, 5.2×10^{-3}, 4.9×10^{-3}, 4.8×10^{-3}, respectively) compared to the control (3.3×10^{-3}). Also, serum urea and creatinine significantly increased ($p < 0.05$) in CDA_1 and CDA_2 (8.39 ± 1.14 mg/dl and 9.55 ± 1.08 mg/dl; 7.38 ± 1.11 mg/dl and 8.07 ± 1.17 mg/dl, respectively) similar to DD Force®

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(6.40 ± 1.02 mg/dl and 7.90 ± 1.18 mg/dl). These were higher compared to SA_1 and SA_2 (5.98 ± 1.01 mg/dl and 6.78 ± 1.05 mg/dl; 5.40 ± 0.09 mg/dl and 6.40 ± 1.03 mg/dl) and control (4.89 ± 0.75 mg/dl and 5.83 ± 0.69 mg/dl). Serum albumin and total protein were significantly lowered by the toxicant (50.48 ± 1.73 g/l and 153.95 ± 8.09 g/l; 53.66 ± 1.33 g/l and 139.46 ± 9.59 g/l; 36.93 ± 2.41 g/l and 81.49 ± 4.38 g/l; 41.89 ± 2.79 g/l and 89.83 ± 3.72 g/l; 41.32 ± 1.10 g/l and 89.66 ± 1.38 g/l, respectively) when compared with the control (66.30 ± 3.63 g/l and 216.30 ± 6.87 g/l, $p < 0.05$). These results suggest that kidney function and protein synthesis were seriously impaired by all the dichlorvos samples, more importantly by Choice Double Action®. Embracing these dichlorvos-based air fresheners may increase the incidence of chronic kidney disease in the country arising from its nephrotoxicity and ability to damage the kidney.

Keywords

Dichlorvos · Pesticides · Inflammation · Microdroplets · Nephrotoxicity · Chronic kidney disease

25.1 Introduction

Dichlorvos is an active ingredient, which is predominantly present in all synthetic and local organophosphorus pesticides. These compounds have been extensively used for a few decades in agriculture for crop protection and pest control, thousands of these compounds were also screened and over one hundred of them are marketed for these purposes (Haddad and Winchester 1990; Mansour et al. 2009). A great proportion of the acute poisoning cases are caused by direct or indirect exposure to the active substance present in these organophosphate compounds (Namba et al. 1971). It operates by inhibiting the enzyme acetylcholinesterase, which allows acetylcholine to transfer nerve impulses indefinitely and causing an array of symptoms such as weakness or paralysis (Kamrin 1997; Yair et al. 2008). It was reported that close to one million of deaths and chronic diseases result from organophosphorus poisoning worldwide (Environews Forum 1999). The symptoms of exposure to these compounds are weakness, headache, blurred vision, salivation, sweating, nausea, vomiting, diarrhoea and abdominal cramps. Hence, there are some locally made insecticides, which are widely sold in the open markets alone or and sometimes mixed with air freshener, for use against the threat caused by insects in the environment.

In Nigeria, Sharp Action® and Choice Double Action® are trade names for locally made insecticides and air freshener used to totally eradicate insects and as an aromatic fragrance dispersed by spraying as microdroplets in homes and offices without the need to vacate the space. These preparations contain dichlorvos as an active ingredient, which has been reported toxic to humans (Peter and Cherian 2000), but their recognition, acceptance and wide spread proliferation in Nigeria have been due to cheap production cost, usefulness, convenience and affordability (Okeniyi and Lawal 2007). Microdroplets have been reported to linger in the air depending on the ventilation of the space where it is applied, and in this case, these products are advocated for use with no need for the user to neither vacate the space nor shut the windows in order not to compromise ventilation when they are applied, thus meeting the need to supply outdoor air and remove extra heat, humidity and contaminants from occupied spaces to meet health and comfort requirements (Qian and Zheng 2018). However, a problem arises when homes and offices are not properly ventilated or the space is overcrowded.

This study, therefore, was to investigate the effects of two variants of dichlorvos-based aerosols, Sharp Action® (insecticide) and Choice Double Action® (insecticide air freshener) on kidney function indices of experimental rats compared to DD Force® (Standard dichlorvos pesticide).

25.2 Materials and Methods

25.2.1 Chemicals and Reagents

DD force (2,2-dichlorovinyl dimethyl phosphate, 1000 g/LEC) was obtained from Jubal Agrotech Ltd., Nigeria. Sharp Action and Choice Double Action were bought from Olu-Ode market, a local market in Osogbo, Osun State. Alkaline phosphate, alanine and aspartate aminotransferase, bilirubin, urea, creatinine and albumin were all product of Randox Laboratories, UK. BSA was a product of Sigma-Aldrich, USA.

25.3 Laboratory Animals

Thirty-six healthy male rats weighing between 180 and 200 g were procured from the Animal House of the Department of Physiology, University of Ibadan, Nigeria. The animals were housed in metabolic cages with access to rat pellets and tap water in the Central animal house of the Department of Biochemistry, Osun State University, Osogbo, Nigeria. They were acclimatized for 2 weeks before the commencement of oral administration of toxicants.

25.4 Preparation of Dichlorvos-Based Aerosols

The dichlorvos-based aerosols were prepared in strict adherence to manufacturer instructions. One millilitre of DD Force® (DDF Standard) dissolved in 400 ml of distilled water. Sharp Action® was prepared to in two strengths (SA_1, 1 ml in 12 ml of distilled water and SA_2, 1 ml in 24 ml of distilled water), and Choice Double Action® was also prepared in two strengths (CDA_1, 1 ml to 1 ml of distilled water, and CDA_2, 1 ml in 0.5 ml of distilled water).

25.5 Animal Grouping and Toxicant Administration

The animals were grouped into six consisting of six rats each, viz. SA_1–1:12 group and SA_2–1:24 group, CDA_1 group, CDA_2 group, DDF – 25 ng/kg group and Control group, respectively. The animals were orally administered daily with 0.5 ml of the dichlorvos-based aerosol or distilled water for the various groups for a period of 21 days using oral cannula.

25.6 Preparation of Serum and Tissue Homogenates

The blood was collected from the animals prior to sacrifice through ocular puncture into plain bottles and kept on the bench to coagulate for 10 min and was spun at 3000 rpm for 10 min using a table centrifuge. The serum was collected with a Pasteur pipette into a clean dry sample bottles and stored at 4 °C for biochemical assays. The liver and the kidney were subsequently excised and weighed for the determination of the organ-body weight ratio.

25.7 Determination of Biochemical Parameters

The serum albumin was determined by the method described by Doumas et al. (1971), the method of Tietz et al. (1994) was adopted for total protein determination, and the methods described by Butler (1975) and Veniamin and Vakirtzi-Lemonias (1970) were adopted for urea and creatinine determination, respectively. The aminotransferases and alkaline phosphatase activity was determined adopting the method of Reitman and Frankel (1957).

25.8 Statistical Analysis

Data were expressed as means of six replicates, taking into account their standard deviations. They were subjected to one-way ANOVA using Duncan Multiple Range test analysed by SPSS software (version 20.0).

25.9 Results

The dichlorvos-based aerosol from Sharp Action® (SA_1 and SA_2), Choice Double Action® (CDA_1 and CDA_2) as well as DD Force® (DDF) administered to the rats for 21 days, caused significant ($p < 0.05$) increases in the weight of the kidney in all the groups when compared with the control (Fig. 25.1). On the other hand, significant decreases were observed in the liver-body weight ratios for all group administered dichlorvos-based aerosols when compared with the control (Fig. 25.1).

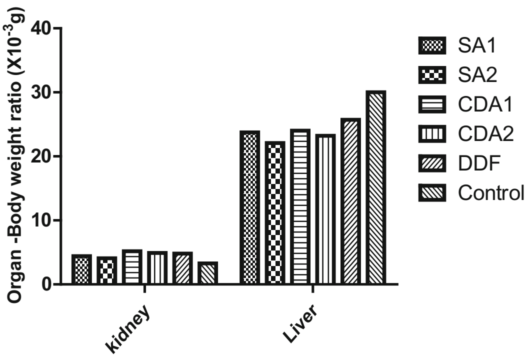

Fig. 25.1 Effect of dichlorvos-based aerosol on organ-body weight ratio

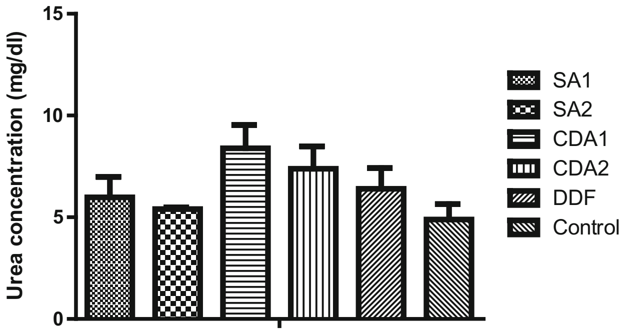

Fig. 25.2 Effect of dichlorvos-based aerosol on serum urea in male Wistar rat

The results obtained also revealed that the administration of the dichlorvos-based aerosol SA_1, SA_2 and CDA_1 significantly ($p < 0.05$) decreased the concentration of urea in the serum, whereas the reverse was the case with CDA_2 and DDF, compared with the control (Fig. 25.2). It was observed that the administration of the dichlorvos-based aerosol caused significant elevation in the concentration of creatinine in all the groups, compared with the control, with the highest recorded for CDA_2 (Fig. 25.3).

A significant ($p < 0.05$) reduction was noticed in serum albumin, bilirubin and total protein concentration for all groups administered dichlorvos-based aerosols, compared with the control (Figs. 25.4, 25.5 and 25.9).

There were decreases in the activities of alanine aminotransferase in the serum of the rats administered SA_1, SA_2, CDA_2, and DDF, while an increase was observed for CDA_1 group, compared with the control (Fig. 25.6).

Figure 25.7 reveals that the dichlorvos-based aerosol formulations SA_2, CDA_1, and CDA_2 caused significant reductions in the activities of aspartate aminotransferase in the serum of the rats, compared with the control. However, SA_1 and DDF caused increases in the activity of the same enzyme in the serum. An increase was

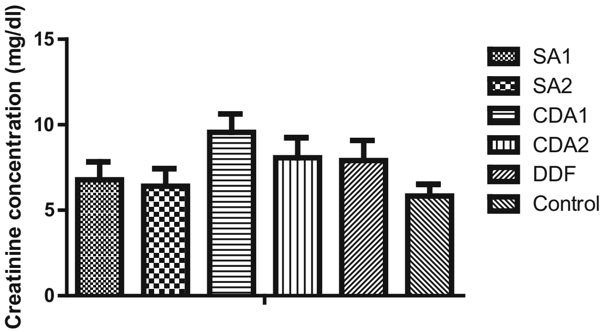

Fig. 25.3 Effect of dichlorvos-based aerosol on creatinine in male Wistar rat

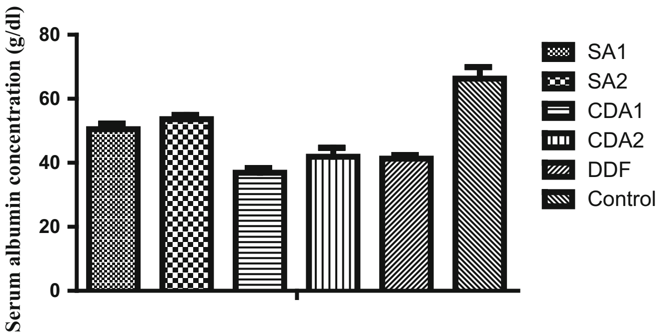

Fig. 25.4 Effect of dichlorvos-based aerosol on serum albumin level in male Wistar rat

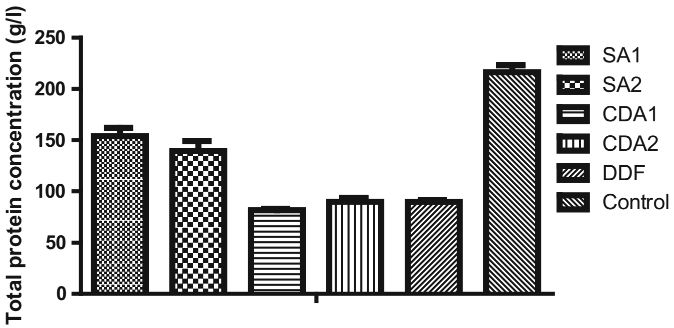

Fig. 25.5 Effect of dichlorvos-based aerosol on serum total protein in male Wistar rat

observed in serum alkaline phosphatase activity in rats administered SA_1, CDA_1, and CDA_2 compared with the control. But there was a decrease its activity in the SA_2 group, whereas DDF group showed no significant difference compared to the control group (Figs. 25.8 and 25.9).

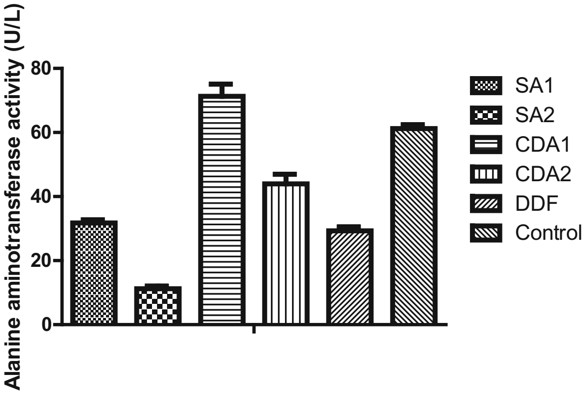

Fig. 25.6 Effect of dichlorvos-based aerosol on the activity of alanine aminotransferase of male Wistar rats

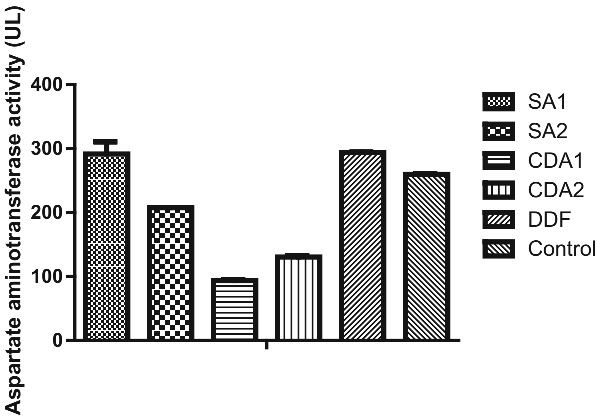

Fig. 25.7 Effect of dichlorvos-based aerosol on the activity of aspartate aminotransferase

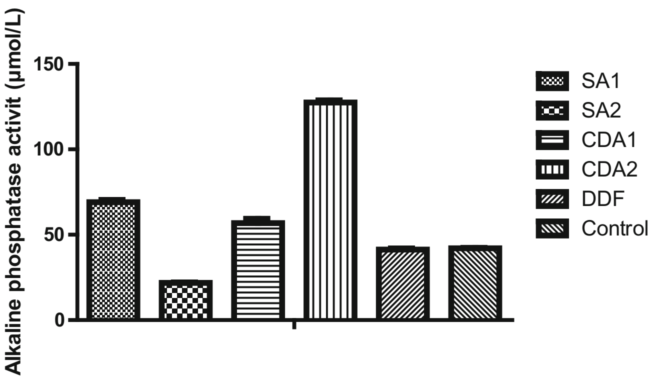

Fig. 25.8 Effect of dichlorvos-based aerosol on the activity of alkaline phosphatase

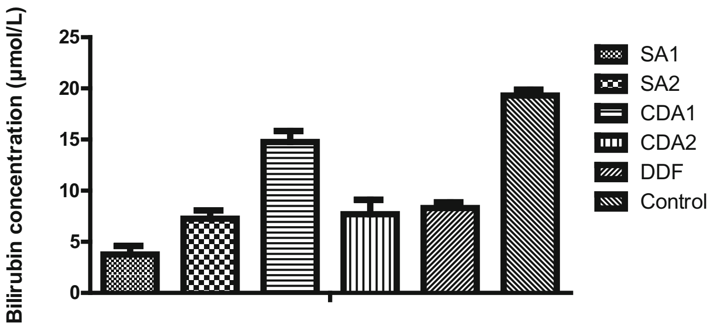

Fig. 25.9 Effect of dichlorvos-based aerosol on bilirubin concentration of male Wistar rats

25.10 Discussion

The use of insecticides and pesticides whose active ingredient is dichlorvos has been reported to interfere with a number of biochemical processes. The inhalation of dichlorvos due to indiscriminate exposure has resulted in the appearance of some toxic symptoms ranging from dizziness, weakness, salivation, nausea, headache and muscle weakness following exposures via gastrointestinal and dermal routes. The mechanism of action of dichlorvos involves its binding to the active site of acetylcholinesterase, leading to accumulation of acetylcholine and later over-activation of cholinergic receptors at the neuromuscular junctions, the autonomic and the central nervous systems (Paudyal 2008). It was also reported that the mechanism for toxicity of pesticides generally is through the induction of oxidative stress, thereby leading to necrosis and apoptosis as well as changes in metabolic and vital functions of the cells (Abdollahi et al. 2004).

The administration of the dichlorvos-based aerosols at all the concentrations tested caused increases in the kidney-body weight ratios. This suggests that exposure to this formulations through domestic use is capable of inducing kidney damage. On the other hand, there was a reduction in the liver-body weight ratios following oral administration of the dichlorvos-based aerosols. This might be due the selective toxicity of the toxicant on tissues. This finding agrees with the reports of Barma et al. (1990) and Ambali (2009) that a drastic loss in weight of rats exposed to chlorpyrifos is a clear indication of its toxicity.

Enzymes are widely distributed and more concentrated in the organs, where they are important for many metabolic processes in living cells, but they may leak out into the blood, in cases of tissue impairment or damage. The alanine and aspartate aminotransferases are cytosolic enzymes which are more concentrated in the liver. Therefore, reductions observed in the concentrations of these enzymes after the administration of the dichlorvos-based aerosols show that there is a reduced rate of synthesis of the enzymes. This may invariably suggest that the level of dichlorvos in the formulated aerosols might not pose any threat to the liver at the concentrations tested and over the number of days of exposure.

There was an elevation in the activities of alkaline phosphatase and aspartate aminotransferase of SA_1, DDF as well as in the alanine aminotransferase of CDA_1 following administration of the dichlorvos-based aerosols. It may therefore follow that these two aerosol formulations can cause damage to the tissues resulting in the leakage of the enzymes into the extracellular fluids (Akanji et al. 1993; Jimoh and Odutuga 2001).

Albumin is a major protein synthesized by the liver, and it is responsible for the transport of all other proteins in the blood. In the case of hepatocellular dysfunction, the liver is incapable to manufacture albumin at the usual rate even, while degradation continues as usual, leading to reduction in albumin concentration. The decrease in albumin caused by the administration of the dichlorvos-based aerosols might be attributed to liver dysfunction resulting from damage, diminishing or loss of albumin continuously from the body.

The kidney plays a crucial role in regulation of intracellular fluids, electrolytes and excretion of metabolic waste products. Kamal (2010) reported that overall body homeostasis is greatly dependent on the functional integrity of the kidney. Therefore, assessment of kidney biomarkers is a pointer to its current condition. As such, the increases observed for creatinine and urea in this study is an indication that the dichlorvos-based aerosols administered might cause a reduction in the performance of the nephrons and glomerular dysfunction. It also suggests reduced excretion and consequently may imply impairment in the normal functioning of the kidney. Chawla (1999) reported that renal dysfunction decreases tubular mass and could critically affect the performance and regulatory roles of the organ.

25.11 Conclusion

Embracing these dichlorvos-based air fresheners may increase the incidence of chronic kidney disease arising from its nephrotoxicity and ability to damage the kidney.

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Consumption of Green Chilli and Its Nutritious Effect on Human Health

26

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Abstract

Chilli (*Capsicum annuum L.*) is the most widely consumed spice and used in the cuisine of all countries. Green chilli has widespread acceptance around the world as a food and source of spices. They provide essential antioxidant and vitamins A, C and E for most of the world population. They are considered to be a good source of various nutritional compounds, such as flavonoids and mineral elements. Green chilli also produces high amounts of vitamins B1 (thiamine), B2 (riboflavin) and B3 (niacin). Green chilli showed antioxidant and antimicrobial properties. The group of pungent components is called capsaicinoids; the most commonly found in food are capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin. These compounds have strong physiological and pharmacological properties. Chillies are widespread as a neuropharmacological component in medical products due to its high content of capsaicin, protein, fixed oil, thiamine and ascorbic acid. Chilli, generally used as fresh and also in dried or powders form either whole or crushed or ground. The fresh fruit is also used in salad, pickle and canned product. It is an important ingredient for flavouring meat and meat products and vegetable soups. Chilli oleoresins and oils are also used for preparation of snack foods.

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Keywords

Chilli · Capsicum · Green · Capsaicin · Health

26.1 Introduction

Chilli (*Capsicum* spp.) is most the popular spice as well as vegetable crop for small and medium farmers in America, Africa and Asia. Green chilli has widespread acceptance around the world as a food and as a source of spices. They provide essential antioxidant vitamins, including the A, C and E for most of the world's population. It is a unique spice cum vegetable with a commercial value. It is extensively consumed throughout the world because of their colour, flavour and pungency. So that it is used for culinary, pharmaceutical and other purposes across the globe. With drastic changing scenario of domestic spice market, a wide variety of chilli and it is value added to the food product have gained wide popularity. Chilli available in India market can broadly be classified into (1) (red, green and multi-colour), (2) fresh processing (paste, sauce, canning, pickling), (3) dried spice (whole fruits and powder), and (4) industrial extracts (paprika oleoresin, capsaicinoids and carotenoids). Besides traditional nutritional food uses, there are many uses of chilli for different purpose like non-food (defence, spiritual, ethnobotanical) (Kumar et al. 2012; Meghvansi et al. 2010).

The extremely hot or burning sensation of green chilli is due to the chemical compound like capsaicinoids. It is only found in capsicum (Hoffman et al. 1983). The capsaicinoids present in the green chilli are predominantly capsaicin and dihydrocapsaicin making up to 80–90%. The ratio of capsaicin to dihydrocapsaicin ranges between 1:1 and 2:1 (Govindarajan and Sathyanarayana 1991). The capsaicin, in fruit of *capsicum chinense* found a very high in comparison with the other varieties of chilli (Sanatombi and Sharma 2008). Capsaicin showed pharmacological properties, exploiting the therapeutic potential (Prasad et al. 2005). Capsaicin has become a promising molecule for the development of a new generation of analgesic anti-inflammatory agents targeting the nociceptive primary afferent neurons (Szolcsanyi 2003). It has also been reported to inhibit the growth of prostate cancer cells (Mori et al. 2006). The anti-oxidative capacity of chilli is higher than ginger, garlic, mint and onion (Shobana and Naidu 2000), which may play an important role in the process of chemoprevention (Yu and Hall 1994).

26.2 History

There is historical evidence at sites located in southwestern Ecuador that chilli peppers were domesticated more than 6000 years ago (Perry 2007; BBC News Online 2007) and also it is one of the first cultivated crops in the Central and South Americas (Bosland 1996) that is self-pollinating. Christopher Columbus

(Italian explorer) was one of the first Europeans to encounter them (in the Caribbean Islands) and called them "peppers" because they look like white and black pepper of the *Piper* genus known in Europe countries, because of hot spicy taste. In the European countries, chilli is grown as botanical curiosities in the gardens. The monks experimented on the chilli for their culinary potential and also discovered the pungency, which is offered as a substitute for black peppercorns. Collingham (2006) reported Columbus brought the first chilli to Spain and write their medicinal effects in 1494.

26.3 Taxonomy of Chilli

The chilli is also called by synonyms as per area, region, countries and languages. According to species of chilli, it can be classified into three groups, viz. (1) pubescens complex, *C. pubescens*, *C. eximium* and *C. cardenasii*; (2) baccatum complex, *C. baccatum*, *C. praetermissum* and *C. tovarii*; and (3) annuum complex, *C. annuum*, *C. frutescens*, *C. chinense*, *C. chacoense* and *C. galapagoense*. A part from these groups of chillies, the following species of chillies is also considered as *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* (IBPGR 1983) (Table 26.1).

26.4 Physical Characteristics of Chilli

Chilli is found in different size, shape and colour (Teotia and Raina 1986). It has two basic important qualities, i.e. pungency that attributed to an alkaloid capsaicin and colour due to a pigment (Narayanan et al. 1980). The bulk of pungency and colour is concentrated in the pericarp. Chilli seed had high pungency and low colour fraction which are required by pharmaceutical industry. The morphology of chilli is reported by Raina and Usha (1996) in Plate 26.1.

Table 26.1 Taxonomy of chilli

Kingdom	Plant
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Capsicum
Species	*Annuum*

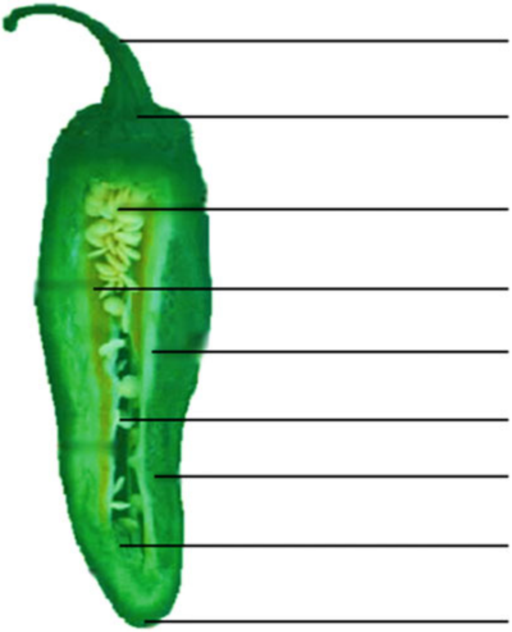

Plate 26.1 Cross-sectional view of green chilli

26.5 Functional Properties of Chilli

The quality of green chilli can be determine by a number of different parameters such as colour, hotness, ascorbic acid content and volatile flavour compounds (Henderson 1992).

26.5.1 Chemical Composition

The chemical composition of the fresh green chilli is presented in Table 26.2 according to Alsebaeai et al. (2017a, b), and also the chemical co-composition of green chilli powders is presented in Table 26.3.

Isabelle et al. (2010) reported that chilli contains nutrients like moisture 90.21 (%), H-ORAC 18.39 (l mol TE/g FW), TPC1.07 (mg GAE/g FW), ascorbic acid 650.0 (l g AA/g FW), capsanthin 0.23 (l g/g FW), capsorubin 0.42 (g/g FW), capsaicin 18.98 (g/g FW) and dihydrocapsaicin 12.71 (g/g FW) and also analysed that the red chilli contains moisture 85.78 (%), H-ORAC 27.75 (mol TE/g FW), TPC 2.77 (mg GAE/g FW), ascorbic acid 2177.3 (g AA/g FW), capsorubin 0.52 (g/g FW), capsanthin 19.46 (g/g FW), dihydrocapsaicin 19.82 (g/g FW) and capsaicin 26.79 (g/g FW); vitamin and mineral content in capsicum (per 100 gm) is phosphorus (78 mg), calcium (29 mg), potassium (374 mg), iron (1.2 mg), thiamine

Table 26.2 Chemical composition of fresh green chilli

Compounds	Raw green chilli
Moisture (%)	85.69 ± 0.74
Ash (%)	0.824 ± 0.12
Reducing sugar (mg)/g	1.77 ± 0.02
Protein (%)	5.83 ± 0.21
Fibre (%)	3.27 ± 0.12
Fat (%)	6.15 ± 0.33
Ascorbic acid mg/100 g	112.27 ± 0.35
Total phenol content (mg/100 gm)	13.25 ± 0.50

Mean ± standard deviations (n = 3)

Table 26.3 Chemical composition of green chilli powders (Alsebaeai et al. 2017a, b)

	Samples of green chilli powders		
Compounds	Lyophilize green	Tray green	Sun green
Moisture content (%)	8.99 ± 0.08	7.34 ± 0.11	8.23 ± 0.20
Ash (%)	6.80 ± 0.156	7.37 ± 0.718	9.01 ± 0.252
Fat (%)	5.23 ± 0.605	b5.00 ± 0.626	4.83 ± 0.300
Protein (%)	16.87 ± 0.208	15.17 ± 0.057	15.10 ± 0.265
Ascorbic acid mg/100 g	165.20 ± 0.200	130.50 ± 0.100	90.70 ± 0.300
Fibre (%)	25.67 ± 0.115	25.63 ± 0.116b	25.47 ± 0.231
Reducing sugar (mg)/g	80.62 ± 0.327	81.19 ± 0.327	96.80 ± 0.327

Mean ± standard deviations (n = 3)

(0.22 mg), niacin (4.4 mg) and riboflavin (0.36 mg). Chillies are also rich in B complex group of vitamins such as riboflavin, niacin, pyridoxine (vitamin B6) and thiamin (vitamin B1). Alsebaeai et al. (2017a, b) studied the effects of drying methods on the quality and characterization of green chilli powder, and they found that lyophilize drying method is the best in comparison with other drying method used in his study; also they found capsaicin contents were found in all dried green chilli trials varied from 0.069 to 0.135 mg/100 g. Alsebaeai et al. (2016) found that lyophilize green chilli powders are rich in bioactive compound in comparison with other sample produced by different drying methods used in his study.

26.5.2 Capsaicinoids

Diaz et al. (2004) reported that capsaicinoid is also called non-volatile alkaloids; responsible for the pungency of the *Capsicum*, it contains amides of branched chain fatty acids (C9–C11) and vanillylamine. Every capsaicinoids are present in their own group like length of the aliphatic side chain, presence or absence of a double bond, branching point and their relative pungency. Approximately 90% of the chilli fruits pungency is due to capsaicin and dihydrocapsaicin. Bennett and Kirby (1968) are also suggested the types, amount and their chemical structure in the given table.

26.5.3 Properties of Capsaicin

Yao (1992) achieved that capsaicin ($C_{18}H_{27}NO_3$) has many characteristics like odourless and white crystal (rectangular plates, scales in petroleum ether) with sharp burning pungency. It is also slightly soluble in hot water and carbon disulphide solution but extremely soluble in organic substance like ether, alcohol, benzene and chloroform and insoluble in water. It is fairly resistant to acids and alkali solutions at room temperatures (Table 26.4).

26.6 Chilli Products

There are different chilli preparation viz. Green chilli oil macerate, Green chilli essential Oil, Green chilli powder, Green chilli extract shown in fallow.

(a) Fresh green chilli: it can be used in different purpose.
(b) Green chilli essential oil: oil obtained by steam distillation.
(c) Green chilli powder: obtained by crushing cloves.
(d) Green chilli extract: obtained by soaking the chilli in alcohol.

26.7 Usage of Green Chilli

26.7.1 Nutrition Use

26.7.1.1 Beverages

Beverage and juice products are fortified with capsaicin as bioactive compounds. There are two products in the market, viz. Sweet launched in 2011 and Prometheus Springs Elixirs launched in 2007.

26.7.1.2 Food Flavourant

Dried chillies are extensively used as a spice in cuisine as curried species in India and abroad. Grinding roasted dry chilli with other condiments such as cumin, coriander, farinaceous and turmeric matter makes curry powder. It is also used as ingredients for seasoning of fish egg, meat products, pickles, sauces, chutneys, frankfurters, sausages, etc.

Table 26.4 Properties of capsaicin

Properties	Quantity/temperature
Molecular weight	305.4118 g/mol
Boiling point	210–220 °C
Melting point	65 °C
Sublimate	115 °C

26.7.2 Medicinal Use

26.7.2.1 It Can Be Used as Source of Antioxidant

Rice-Evans et al. (1995) reported that the composition of chilli mainly depends on the stage of maturity and variety of chilli, Chilli contains phytochemicals, bioactive compound, pharmacological characterizations such as antioxidant, anti-allergic, anticarcinogenic and anti-inflammatory activities. Many researchers have also present their result as ripe red chillies are naturally rich in ascorbic acid (vitamin C) and provitamin A (Kidmose et al. 2001), and it neutralizes free radicals in the human body that will reduce the risk of diseases and cardiovascular and arthritis disease; similarly it acts as anti-cancer (Nishino et al. 2009). Rao and Rao (2007) found that carotenoids are fat-soluble antioxidants found in chilli. In addition to these results, several researches have demonstrated the antimicrobial activity of chilli. Ademoyegun et al. (2011) showed in their result that carotenoids are also responsible for orange and red colours in vegetables. The carotenoids in chillies include pigment like capsanthin and carotene. Ademoyegun et al. (2011) demonstrated that the yellow-orange colour of chillies is because of α- and β-carotene, β-cryptoxanthin, lutein and zeaxanthin. Perera and Yen (2007) reported that consumption of carotenoid-rich foods reduces the incidence of several disorders such as cardiovascular diseases, cancers, cataracts, age-related macular degeneration and diseases related to compromised immune function and other degenerative diseases. Surh and Seoul (2002) noted that plant phenols include simple phenols, anthocyanins, flavonoids, lignins, lignans, tannins and stilbenes. Phenolic compounds function as antioxidants with activities similar to vitamins E and C and β-carotene. A wide variety of spice-derived phenolic compounds, such as capsaicin, possess potent anticarcinogenic and anti-mutagenic properties.

26.7.2.2 It Can Be Used as Source of Antimicrobial

Chilli fruits are added at a small quantity to produce a characteristic taste of cuisine of Indonesia (West Sumatran). It is reported that cuisine using a large amount of chilli could help in preservation of food for long period without spoiling. Only a special variety of chilli was used, i.e. *Capsicum annuum* L.; there are different varieties like (var. *C. longum* which has a curly shape) showed antimicrobial action. Chilli (*C tincture*) was previously reported by Siehta et al. (1984) that is able to inhibit the growth of species *Bacillus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus.*

According to Molina-Torres et al. (1999), less quantities of capsaicin (pure, from Sigma Aldrich) 25 μg/ml had a high inhibitory effect towards *Bacillus subtilis*; also they found that capsaicin at concentrations more than 200 or 300 μg/ml only inhibit the growth of *E. coli*. Also they studied the effect of *Capsicum* extract on *S. typhimurium* inoculated in minced beef. The minimum level concentration of the chilli extract was 1.5 ml/100 g of meat. The combination of chilli extract and sodium chloride tested was not successful to eliminate *Salmonella*. This could be justified by the fact that *Salmonella* is tolerant to salt. Careaga et al. (2003) estimated the antimicrobial effect of *Capsicum* extract on *P. aeruginosa* inoculated in minced beef. Inhibitor of *P. aeruginosa* growth was found between 0.06 and 0.1 ml/100 g meat, also they observed that the bacteriostatic effect between 0.5 and 1.5 ml/100 g meat. The combination of sodium chloride and chilli (*C. annum*) extract tested eliminated *P. aeruginosa* after 3 days of storage.

Soetarno et al. (1997) found that the ethanol extracts of the fruits of three kinds of chillies showed similar effect in their antimicrobial activities against and Gram (−) and Gram (+) bacteria and fungi, although they contained different level of bioactive compounds. Bioautographic tests indicated that capsaicin was the main antimicrobial component. There are also two non-polar components of ethanol extract also responsible for the antimicrobial activity, and they assume that compounds responsible on the inhibitor of *Pseudomonas aeruginosa*.

26.7.2.3 It Can Be Used as Source of Antidiuretic

Okumura et al. (2012) investigated the effect of caffeine and capsaicin on the blood glucose level of obese/diabetic model mice. They found that the blood glucose level of KK-A(y) obese/diabetic mice decreased significantly after feeding with less than 0.0042% capsaicin. The glucose level on the blood of control mice was 234.4 mg/dL at 10 days after feeding, and it increased to 500.6 mg/dL after 28 days, whereas levels after intake of capsaicin increased from 131.6 mg/dL to 255.1 mg/dL. The antidiabetic activity of capsaicin was observed by a decrease in total drinking water and also increases of the blood insulin level.

26.7.2.4 It Can Be Used as Pain Relief

Chilli compound (capsaicin) helps in reducing pain from rheumatoid arthritis or fibromyalgia and inflammatory heat and noxious chemical hyperalgesia (Fraenkel et al. 2004). Capsaicin is also the key ingredient in Adlea (Adlea is product candidate for the treatment of site-specific moderate to severe pain), a drug which is in Phase 2 trial as a long-acting analgesic to treat osteoarthritis pain and post-surgical (Remadevi and Szallisi 2008). Capsaicin is also an ingredient of some over-the-counter pain reliever creams at a concentration of 0.075% or lower.

26.7.2.5 It Can Be Used as Anticarcinogenic

The World Cancer Report (WCR) reported that cancer rates would increase at an alarming rate in the global scope. In this report, cancer rates could further increase by 50–15% million new cases in the year 2020. Thus, it is increasingly important to find drugs to inhibit the growth of cancer. Recently, Park and Kim (2007) said that capsaicin is proved to have great activity for inhibiting various tumour cells. Besides, capsaicin from chilli also decreased the growth of human hepatic carcinoma cells (Huang et al. 2009), leukemic cells, prostate and nasopharyngeal in vitro because of its ability to mediate cell cycle arrest and induce cell apoptosis. Capsaicinoid compounds are a group of chemical compounds in chilli peppers and are responsible for stimulated tartly flavour. Also capsaicin is the main component of capsaicinoids in chilli peppers (Govindarajan and Sathyanarayana 1991).

Capsaicinoid compounds have various anti-cancer effects, which have been put forward for a period of time. But currently it still stays in the stage of lab work (animals experiment and in vitro experiment). It will take a long way to go before entering the clinical stage. Besides, many researchers reported that capsaicin has carcinogenic effects, such as enhancing tumour cell migration and proliferation by the treatment with low concentration of capsaicin (Yang et al. 2013). Mennen et al. (2005) investigated that the anticarcinogenic activity of phenolic compounds is because to the inhibition of *N*-nitroso compound formation in vitro.

26.8 Conclusion

It can be concluded that the green chilli has many properties that can improve the human health like anti-cancer treatment or pain relief. It can be used as fresh, dried or powders. The fresh fruit is also used in salad, pickle and canned product. It is an important ingredient for flavouring meat and meat products and vegetable soups.

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Synthesis, Characterization, and Evaluation of Toxicity of Melatonin-Loaded Poly (D,L-Lactic Acid) Nanoparticles (Mel-PLA-Nanoparticles) and Its Putative Use in Osteoporosis

27

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Abstract

Melatonin-loaded PLA nanoparticles are nowadays important in biological system for its biodegradable nature for targeted drug delivery. Hence, aim of the study is to note applicability and toxicity of Mel-PLA nanoparticles in osteoporosis. Different concentrations of melatonin and PLA were prepared by dissolving in dichloromethane (DCM). The final dried nanoparticles were used for structural analysis by SEM, TEM, and FTIR. Toxicity and immunological impact of nanoparticles were evaluated on rats: control and nanoparticle treated ($n = 5$/group) for 7 days. Afterward animals were sacrificed and blood, liver, and kidney were collected. A fraction of blood was processed for TLC, DLC, and %LC, and the remaining was centrifuged at 3000x g at 4 °C for 30 min. Separated plasma was used for measurements of IL-2, IL-6, TNF-α, IFN-γ, IL-1β, urea, creatinine, and BUN. Both plasma and tissue homogenates were used for AST, ALT, ACP, and ALP estimations. We noted significantly high ($p < 0.05$) levels of TLC, DLC, %LC and IL-2, and TNF-α upon treatment. The rest of the parameters were found to be significantly low (IL-6, IL-1β, AST, ALT; $p > 0.05$) or unaffected (IFN-γ, ACP, and ALP). From our preliminary study,

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we may conclude that we have synthesized Mel-PLA nanoparticles and their effects were nontoxic to animals.

Keywords

Mel-PLA nanoparticles · Characterization · Immune effect · Synthesis · Toxicity

Abbreviations

ACP	Acid phosphatase
AFM	Atomic force microscopy
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
DCM	Dichloromethane
DSC	Differential scanning calorimetry
FTIR	Fourier-transform infrared spectroscopy
IFN-γ	Interferon gamma
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-6	Interleukin-6
LFT	Liver function test
Mel	Melatonin
PLA	Poly lactic acid
RFT	Renal function test
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TNF-α	Tumor necrosis factor-alpha

27.1 Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced and released by the pineal gland, which has potential to regulate neuro-immune modulation, anti-cancerous, apoptosis, and cell division by scavenging free radicals (Tan et al. 2007; Hardeland et al. 1993). The immune system being an open and multidirectional system has greatly evolved to interact with the endocrine system to combat with the environmental threats. Recently the important role of melatonin has been carried out into the area of neuroendocrine-immunology in reference to bone deformities (Maestroni et al. 2002). Melatonin receptors are present on the lymphocytes (Drazen and Nelson 2001). This hormone is well established in the regulation of apoptosis and cell division by enhancing the secretion of mitogens (Xu et al. 2007). The effect of melatonin as an antioxidant and antiapoptotic had been proved in a seasonal breeder *Funambulus pennanti* (Ahmad and Haldar 2010). The potential clinical benefit of the use of melatonin as an antioxidant and antiapoptotic will be helpful for treatment of many degenerative diseases, such as osteoporosis (a bone degenerative disease) and Alzheimer (a neurodegenerative disease). Considering the important role of melatonin, a proposal has planned to encapsulate the melatonin with biodegradable polymeric nanoparticles and its controlled delivery with desired release kinetics. Biodegradable polymeric nanoparticles have been extensively used with great interest in the area of nano-biotechnology as delivery systems for active molecules and drugs for therapeutic use (Pandey et al. 2013), due to their controlled and sustained-release properties and biocompatibility with tissue and cells (Cheng et al. 2007; Kim and Kang 2008). The current proposal is based on the controlled release of melatonin by encapsulating with biodegradable polymeric nanoparticles. The polymeric nanoparticles have the potential to act as a carrier of active molecules and drugs at target sites, protecting them from physiological environment and increase their biological activity. So we have planned a melatonin and melatonin loaded polymeric biodegradable nanoparticles in regulation of osteoporosis and bone deformities in rodents.

27.1.1 Why Polymeric Nanoparticles?

The polymeric composition (hydrophobicity, surface charge, and biodegradation profile) of the nanoparticles, any adjuvant substances, and the associated active molecules and drugs (molecular weight, charge, localization in the nanospheres by adsorption or incorporation) have a great influence on the active molecules and drugs absorption, bio-distribution pattern, and elimination. The polymeric nanoparticles technology used in the recent years has great significance in improving the efficacy of the active molecules and drugs. Nanoparticles can be prepared from a variety of biodegradable polymers such as synthetic as well as natural polymers. The choice of materials depends on several factors including (i) size and morphology of the nanoparticles; (ii) surface charge and permeability of the nanoparticle; (iii) degree of biodegradability, biocompatibility, and cytotoxicity; and (iv) drug loading and desired release profile.

27.1.2 Rationale of the Study

The main aim of this proposal is to improve the osteoporosis conditions (either post-estrus or induced) in rodents by developing melatonin-loaded polymeric nanoparticles and their controlled delivery in rodents as requirement. (i) Osteoporosis is not only an indigenous problem, but also it is well prevalent throughout the world. Except for Ca^{+2} supplementation, there is no cure suggested for the treatment of this clinical condition. (ii) Till date, age factor and menopause are regarded as the main cause of osteoporosis particularly in humans. But, in this regard, further literature and clinical investigations are completely lacking. (iii) The improvement of osteoporosis by controlled delivery of melatonin-loaded nanoparticles is the most untouched area of research, and during the literature survey we have found very few literatures.

27.2 Current Status of Research

27.2.1 National Status

In recent years, rapid scientific and technological advancements have been made in controlled release of active molecules and drugs using biodegradable polymer-based nanoparticles. To overcome the undesirable side effects, and to protect the efficacy of active molecules and drugs from physiological environment, controlled delivery approach is necessary in order to get optimum therapeutic results. At present, scientists are involved to develop biodegradable polymeric nanoparticles by using various biodegradable polymers such as poly- (lactic-co-glycolic acid; PLGA), poly (D,L-lactic acid; PLA), poly (ε-caprolactone; PCL), chitosan gelatin, and poly-alkyl-cyanoacrylates (Gou et al. 2009; Zheng et al. 2010; Jain et al. 2006; Kumari et al. 2011; Pandey et al. 2014) for target delivery of active molecules and drugs related to cancer, diabetes, malaria, and other harmful diseases. Our lab is engaged in exploring immunomodulatory role of melatonin considering the bone marrow functioning in rodents and birds (Vishwas et al. 2013, 2014). Recently we have published our findings in direction of melatonin's immune protection because of its anti-oxidative and antiapoptotic action via inhibiting caspase-3 activity in spleen lymphocytes of seasonal breeders under radiation-induced stress (Sharma et al. 2008) and also by bone marrow functioning. In India very few groups are involved in bone deformity study (Compston 2002). The reasons behind the mechanism of melatonin action in bone anomalies (as suggested by Jäger and Kuchroo 2010) have never been explored at national/international level till date. To the best of our knowledge, there is no any information available in India concerning synthesis of melatonin-loaded polymeric nanoparticle and its delivery approach in comparison to melatonin except for the report of Pandey et al. (2014).

27.2.2 International Status

The role of melatonin in execution of different physiological functions like modulation of reproduction (Haldar et al. 1992) and immunity (Reiter et al. 2000) is very much well evident. Reports are available on melatonin functioning in bone marrow functioning in modulation of immunity (Vishwas et al. 2013). But, unfortunately, till date a single literature is not available even at international level, regarding the therapeutic use of melatonin or melatonin-rich compound in therapeutic use of melatonin in bone deformities study. Further, internationally, very few groups are working in the area of melatonin-loaded polymeric nanoparticles. Melatonin-loaded polysorbate 80-coated Eudragit S100 nanopanicles provided an increase in the in vitro effect of melatonin against lipid peroxidation in comparison to the melatonin in aqueous solution (Schaffazick et al. 2005). Hafner et al. (2009) developed melatonin-loaded lecithin/chitosan nanoparticles for the transmucosal delivery of melatonin and observed that nanoparticles enhance melatonin transport across the epithelial barrier.

27.3 Hypothesis

It is evident from previous literature (Liu et al. 2014; Tamimi et al. 2012) that melatonin may impair bone softening by activating Bone Morphogenetic Protein 15 (BMP-15). In India (about 50%), almost 45% population of post-menopausal women of the world are suffering from impaired bone functioning and fragile bone disease (FBD; WHO 2009). This is due to low level of estrogen in circulation which in turn affects the Ca^{+2} absorption from blood. Further, the cesarean deliveries of mothers also lead to impaired bone functions just after menopause. Till date, there is no literature available regarding the proper management of bone fragility in females particularly after menopause except for some commercially available Ca^{+2} supplementation. Taking this background the present dissertation is divided into different parts which are in progress. Since the studies are underway (unpublished data), we will be providing the glimpses of results in terms of levels of significance wherever necessary.

27.4 Experimental Approaches

27.4.1 To Investigate the Possibility of Developing and Optimization of All Conditions for Preparations of Melatonin-Loaded Polymeric Nanoparticles

Depending on the physicochemical characteristics of melatonin, it is essential to choose the polymer and the method of preparation to achieve an efficient entrapment and controlled delivery of the melatonin. Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable. The method is based on the use of an organic matter on continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse micro-emulsion. During preparation of nanopanicles, surfactants or protective soluble polymers may be used to prevent aggregation in the early stages of polymerization. To avoid toxicity, it is necessary to eliminate the organic solvents and surfactants from the prepared nanopanicles. Emulsification-solvent evaporation method may be used for emulsification or the polymer solution into an aqueous phase and evaporation of the organic solvent by inducing polymer precipitation as nanospheres. The solvent is subsequently evaporated by increasing the temperature under pressure with continuous stirring. This can be controlled by adjusting the stir rate, type, and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature.

27.4.2 Nanoparticles Preparation

The most suitable biodegradable polymer-based nanoparticles containing melatonin were prepared by emulsification and solvent evaporation/nano-precipitation method (Bilati et al. 2005). Briefly, known amounts of polymer and melatonin was added into the organic solvent (based on the nature of dispersed phase) and co-dissolved by stirring. The resulting, organic solution was added dropwise into the aqueous phase containing emulsifier under high speed stirring. Nanoparticle suspension was collected using an ultra-centrifuge (1, 50, 000 g, 30 min, 4 °C) and washed with distilled water at least three times. Organic solvent was removed from nanoparticles suspension by the rotor evaporation technique under reduced pressure at 40 °C and finally dried powder of nanoparticles obtained. The final dried nanoparticles were stored at 4 °C until further use.

27.4.3 Nanoparticle Characterizations

Characterization and evaluation of particle size, morphology, structure, and stability of melatonin-loaded nanoparticles was carried out by using SEM, AFM, FTIR, and particle size analyzer. Thermal characterization of pure polymeric nanoparticles, melatonin, and melatonin-loaded nanoparticles was carried out using differential scanning calorimetry (DSC).

27.5 Evaluation of Toxicity of Nanoparticles in Animal Models

27.5.1 Administration of Melatonin-Loaded PLA Nanoparticles in Animals

After synthesis of melatonin-loaded PLA nanoparticles, the therapeutic efficacy toxicity (if any) of nanoparticles were evaluated in rats. Animals were divided into two groups (control and nanoparticle treated; n = 5/group) with ad libitum access to commercially available animal palette and water. In the control group, animals were administrated with normal saline where the treated animals received melatonin-loaded PLA nanoparticles. After 7 days, animals were sacrificed, and blood and desired tissues (liver and kidney) were collected and were homogenized in respective buffers and were processed for evaluation of different immunological parameters and evaluation of toxicity in terms of liver function test (LFT) and renal function test (RFT).

27.5.2 Evaluation of Toxic Impacts of Melatonin-Loaded PLA Nanoparticle

Any kind of foreign chemical introduced in body can evoke different physiological side effects which are mainly of two types: (1) toxic effects and (2) immunological side effects. Thus, to evaluate the same, we assessed the LFT and RFT in both control and nanoparticle-treated groups.

27.5.2.1 Effect on Liver Function Test (LFT)

Serum glutamic oxaloacetic transaminase (SGOT or aspartate aminotransferase, AST) and serum glutamic pyruvic transaminase (SGPT or alanine aminotransferase) are stored in the liver under normal conditions. But, upon clinical conditions where the liver is injured (which may be induced due to any kind of drug treatment or due to alcoholic shock), the level of both SGOT and SGPT may be elevated. Thus, assessment of SGOT and SGPT are regarded as the universal markers of liver function. Upon nanoparticle treatment, we noted significantly low levels ($p > 0.05$) of SGOT and SGPT as compared to control in plasma and liver. This provided us the first clue that the nanoparticle treatment is not going to affect the most biochemically efficient organ of the body, i.e., the liver. Further, we were interested in evaluating the functioning of another physiologically important organ, i.e., the kidney, which was assessed by renal function test (RFT).

27.5.2.2 Effect on Renal Function Test (RFT)

The kidney is one of the most important organs of body actively and effectively participating in detoxification. Any kind of drug administration may hamper renal function as well as glomerular filtration rate (GFR); hence our next aim was to investigate the renal function test (RFT) by assessing plasma urea, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), and acid phosphatase (ACP). We noted significantly low levels ($p > 0.05$) of plasma urea, BUN, and creatinine levels in nanoparticle treatment groups; however the ACP and ALP levels were found to be unaffected as compared to control. Thus, our results have suggested that the nanoparticle treatment neither had negative impact on liver function nor had negative effect on kidney function. But, any kind of drug treatment (including the nanoparticles) can evoke immunological anomaly in the body. Hence, our next aim was to investigate the status of cell-mediated and humoral immune parameters upon nanoparticle treatment.

27.5.3 Evaluation of Immunological Effects of Melatonin-Loaded PLA Nanoparticles

27.5.3.1 Effect on Cell-Mediated Immune Parameters

The cell-mediated immune parameters are the first marker for any kind of immunogenic or hypersensitivity reaction. To assess the same, we noted significantly high ($p < 0.05$) levels of total leukocyte count (TLC), differential leukocyte count (DLC), and % lymphocyte count (%LC) upon nanoparticle treatment as compared to control. These results suggested that the elevated levels of peripheral cellular components of immunity may be due to some kind of hypersensitivity reaction because of nanoparticle treatment. To investigate the same, we noted humoral immune parameters, i.e., cytokines.

27.5.3.2 Effect on Humoral Immune Parameters

The open circuit of blood immune parameters is chemically coordinated by lymphokines, chemokines, and cytokines. Among them, the most effective are the cytokines which are producing the broadest spectrum coordinating a number of immunologically active cells. The cytokines are mainly of three types of pro-inflammatory (e.g., IL-2), anti-inflammatory (e.g., IL-6), and switch between pro- and anti-inflammation (e.g., TNF-α). We noted significantly high ($p < 0.05$) levels of IL-2 and TNF-α and significantly low ($p > 0.05$) levels of IL-6 and IL-1β (a cytokine directly associated with plasma cortisol) in nanoparticle treatment group in comparison to control. Further, IFN-γ (a particular cytokine marker for viral infection) was found unaffected both in control and nanoparticle treatment group.

27.6 Conclusion

From our preliminary study, we may conclude that we have successfully synthesized melatonin-loaded PLA nanoparticles which are neither having toxic (in terms of LFT and RFT) nor having immunological (in terms of cell mediated and humoral) side effects. Thus, our preliminary work is based on the synthesis and optimization of melatonin-loaded polymeric nanoparticles with desired panicle size, increased entrapment efficiency, and controlled release kinetics. The optimized formulation will be applied for treatment of constitutive and induced bone anomalies as well as a special focus on osteoporosis in rodents with relevance to post-menopausal mothers as they are the backbone of our family and society.

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Nutritional Physiology

28

O. T. Olaniyan and G. E. Okotie

Abstract

It is estimated that the world's population will increase tremendously by the year 2025 impacting great demand on food. New innovations in food science and technology are needed to meet and alleviate the problems of food insecurity and malnutrition of the ever-growing population. Many great approaches have been made through food fortification and functional food products to improve health and wellness through food science and technology. In 2011, the World Health Organization (WHO) estimated that globally 115 million (18%) children less than 5 years of age were underweight and 178 million children (28%) were stunted. A quarter of all children in developing countries suffer from malnutrition with the majority of them residing in Africa.

Significant deficiencies in the level of micronutrients, vitamins, minerals, protein, energy, iron, and iodine with ever-increasing population have been the major factors contributing negatively to the relative nutritional contents in individuals. To address these deficiencies, food fortification, supplementation, and dietary diversification must be deployed to mitigate risks associated with such important nutrients. Food fortification could be considered as a public health strategy to enhance nutrient intakes of a population. Over the years, fortification has been effective at reducing the risk of nutrient deficiency diseases such as beriberi, rickets, goiter, and pellagra. However, the world today is very different from when fortification emerged in the 1920s.

Over the years, infant formula and food fortification with high levels of minerals and vitamins have led to a rapid increase in vitamin and mineral intake

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among infants, children, and adults. This has led to a sharp increase in the prevalence of metabolic diseases such as obesity, diabetes, cardiometabolic disorders, endocrine and reproductive dysfunctions, and other related diseases associated with overnutrition especially in developed countries. Micronutrient fortification is suggested in any region where mild to moderate deficiency is prevalent, though, there is uncertainty about both the long-term benefit and the safety of micronutrient fortification in such regions, especially where the deficiency is mild. This reveals that consumption of a diet that is fortified with micronutrients for a long time can lead to adverse health effects. Studies have shown that nutrition plays a critical role in the prevention of chronic diseases, as most of them can be related to diet. Functional food enters the concept of considering food not only necessary for living but also as a source of mental and physical well-being, contributing to the prevention and reducing of risk factors for several diseases or enhancing certain physiological functions in human system.

Currently dairy products are excellent media to generate an array of products that fit to current consumer demand for functional food. Fermented dairy products enriched with probiotic bacteria have developed into one of the most successful parts of functional foods. A growing attention in basic research on the effect of probiotics in human health and the commercial advantages of the probiotic food biotechnology has been observed in the last 30 years. This increased research has resulted in significant advances in the understanding of the fundamental mechanisms by which probiotics may confer beneficial effects to the host. The food industry is especially active in studying probiotics because the gastrointestinal tract is one of the richest zones of biodiversity within the body with at least 450 known species of microorganisms commonly found there.

This book chapter points to a future in which specific and targeted probiotics help protect against diseases to which we are innately predisposed. There is no doubt that important application of genetic manipulation offers opportunity to enhance the current probiotic properties of an organism. However, only a careful scientific approach will ensure the success and acceptability of these genetically modified organisms (GMO) as probiotic foods through nutrigenomic science targeted to individuals, and as new evidence on diet–gene modulations becomes accessible and genotypic analyses are used to enhance the quality of medical care, there are enormous legal, ethical, safety, and psychosocial concerns that will need to be addressed. Only then are these food innovations likely to be an essential part of the universal agenda for contending malnutrition and other nutritional problems.

Keywords

Food fortification · Functional foods · Nutrition · Physiology · Probiotics

28.1 Introduction

Nutrition plays an important role in the prevention of disease and is vital to the restoration of health and quality of life. The phase of disease prevention is tailored toward dietary recommendation to the individual. In the last decades, major revolution in food science and technology was noticed due to the green revolution. In 2011, the World Health Organization (WHO) estimated that globally 115 million (18%) children less than 5 years of age were underweight and 178 million children (28%) were stunted. A quarter of all children in developing countries suffer from malnutrition with the majority of them residing in Africa.

Undernutrition in children less than 5 years of age increases the risk of mortality and morbidity due to diarrhea and increased risk of infections by an estimated 35% and 11%, respectively. This can lead to long-term consequences such as delay in educational, social, and economic growth and development. There has been some improvement in the reduction in the proportion of underweight children less than 5 years of age in developing countries from 30% to about 23% between 1990 and 2009; however, this is not sufficient to meet the Sustainable Development Goal to reduce the under-5 mortality rate by two thirds by the year 2030.

Despite the drop in the level of malnourished population in the developing countries, hunger and malnutrition are still the major challenges of these countries, thereby compromising the health status of the populace. Significant deficiencies in the level of micronutrients, vitamins, minerals, protein, energy, iron, and iodine with ever-increasing population have been the major factors contributing negatively to the relative nutritional contents in individuals. To address these deficiencies, food fortification, supplementation, and dietary diversification must be deployed to mitigate such risks associated with such important nutrients.

Food fortification in public health is an effective method of prevention and treatment of nutritional deficiencies. Fortification of food with essential nutrients has contributed positively and significantly in developed countries to virtually eradicating some disease conditions like rickets, pellagra, goiter, and beriberi (Olin 1924). A number of factors affect fortification of food like technological properties (O_2 permeability, pH, and moisture) leading to unacceptable flavor and taste. Dietary supplementation has the advantage of targeting risk groups with rapid results. This involves identification of potential risk groups and compelling them to adhere strictly to treatment regime. There are serious health concerns about the micronutrient of some supplements due to overconsumption. Dietary diversification involves consumer advocacy programs to encourage changes in dietary patterns. However, factors affecting food choice are complex, and therefore programs to affect changes in dietary patterns may be for long term.

28.2 Foods Fortified with Vitamins and Minerals for Health Maintenance

Fortification can be defined as a process of adding nutrients or non-nutrient bioactive molecules to edible products (food constituents, food, or supplements). Fortification is used in correcting or preventing widespread nutrient intake shortfalls and related deficiencies, to balance the total nutrient profile of a diet, to restore nutrients lost in processing, or to appeal to consumers looking to supplement their diet. Food fortification could be considered as a public health strategy to enhance nutrient intakes of a population. Over the years, fortification has been effective at reducing the risk of nutrient deficiency diseases such as beriberi, rickets, goiter, and pellagra. However, the world today is very different from when fortification emerged in the 1920s.

28.3 The Historical Perspectives

It all started in 400 BC by the Persian physician Melampus who proposed that iron (Fe) filings added to wine could increase the potency of soldiers (Mejia 1994). In the year 1831, the French physician Boussingault suggested that the addition of iodine to salt could prevent goiter. Fortification of salt with iodine was then introduced in Switzerland in the year 1923 to prevent goiter and cretinism among the populace (Blum 1997), and also shortly it was introduced in Michigan, USA, in 1924 (Sebrell 1974). The vivid fall in the occurrence of goiter that followed led to voluntary iodization of salt throughout the USA (Lee et al. 1999).

In the UK vitamin A was first supplemented with margarine voluntarily in 1927. This practice became mandatory, together with vitamin D, during the Second World War in order to achieve nutritional equivalence to butter. Currently in the UK, all fat spreads have added vitamins A and D, and many also include vitamin E. Between 1907 and the 1940s, it was estimated that 3 million cases and 100,000 deaths resulted from an epidemic of pellagra in the USA, initially thought to be caused by infection. The Nobel Prize for Physiology was awarded in 1929 for work in chickens demonstrating a nutritional etiology. Voluntary enrichment of bread and several grain products with niacin were executed in 1938. Mandatory fortification with thiamin, niacin, iron, and riboflavin followed in 1940. Consequently, pellagra had become almost non-existent by 1950 (Lanska 1996). In the year 1998, the US Food and Drug Administration introduced mandatory addition of folic acid to enriched grain products (like rolls, bread, tortillas, crackers, rice, and pasta) which will reduce the occurrence of neural tube defects in the newborn, cardiovascular disease, and cancers (Choi and Mason 2002).

1. *Water Fortified with Minerals (Ca++, Mg+, Fe+, and Zn)*

Fortification is the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting verified deficiency of one or more nutrients. Mineral-fortified water can contribute to mineral balance and overall nutrition (Cock and Bechert 2002). There are several factors responsible for the relative nutritional contents in individuals and populations; they include the amount of minerals per volume, as well as the volume, frequency, and regularity of consumption. Due to the increased demand on products that will improve wellness and health, individuals have placed high demand on natural products also known as functional foods (Fuhrman 2007; Schweizer 2010). They are called functional foods because of their ability to improve health and reduce disease conditions in the human body. Functional food helps the body in weight and cholesterol reduction, tension reduction, metabolism improvement, mental fitness and concentration, and maintenance of pregnant or postmenopausal balance, reduces liver damage, and lowers the risk of osteoporosis and anemia, severe nutrient deficiencies, and the death toll of cardiovascular diseases (Beristain et al. 2006; Roskell 2007).

It is well known that diets alone cannot meet the daily dietary requirements, hence the need for fortified food. Fortified foods with minerals, both (organic and inorganic) help the body in the maintenance of calcium homeostasis, hormonal regulation, enzymatic reactions, muscle contraction, nerve impulse transmission, synthesizing vitamins, and bone structure and improve digestion and absorption of nutrients, blood clotting, cell signaling processes, and other biological processes. Mineral-fortified water is recommended because of its bioavailability which is similar to that of dairy products and pharmaceutical supplements (Haynes 2008). It is also known that mineral-fortified water helps in the maintenance of bone mineralization, prevention of osteoporosis, immune responses, and cardiovascular function with no side effects. Fortified water is a functional beverage and can be classified into four groups, namely:

1. Enriched beverages
2. Sport and energy drinks
3. Nutraceuticals for specific medical or health benefits
4. Probiotics, found in dairy drinks

Dietary components consist mainly of oligosaccharides, polysaccharides, proteins, peptides, and glycoproteins that are fermented by the gut bacteria and subsequently produce short-chain fatty acids (SCFA) as their major end products; the majority of these SCFA are acetate, propionate, and butyrate. Water as a vehicle can deliver nutraceuticals and other supplements essential to produce a beverage. It is the major component of the body in extracellular and intracellular compartments. Alteration in water and electrolytes balance in the body is known to be responsible for pathophysiological conditions like fever, vomiting, or diarrhea. Report has shown that water is considered to be the suitable vehicle for fortification with minerals and vitamins. Water and electrolytes are constantly been lost through urine, perspiration, feces, and sweat. Also, vitamins and mineral bioavailability increases with their solubility in water (Goldberg 1994; Menard 2003).

2. *Salt Fortified with iodine*

Iodine is known to prevent endemic goiter. Harry Sloan from Cleveland was the first to propose the iodination of salt for human beings. Later Michigan State Medical Society in 1922 launched one of the world's first food fortification campaigns program in applied public health (Alfred 1932). Many years later salt fortification with iodine and vitamin A became commercially available for the prevention of goiter and night blindness.

3. *Milk Fortified with Vitamin D*

In 1919, Edward Mellanby discovered the efficacy of cod liver oil in the prevention of rickets in puppies. S. J. Cowell found that irradiated milk was much more effective than untreated milk in stimulating bone calcification in children. (Irradiation of milk converts inactive ergosterol into physiologically active vitamin D_2.) Cowell's discovery prompted Harry Steenbock's in 1929 invention of a milk irradiator for industrial use. Many years later different milk products with cod liver and other preparations were made commercially available for the prevention of rickets. The introduction of vitamin D-fortified milk into the marketplace appears to have taken a course similar to that of most new pharmaceuticals and other innovations (Dary 1998).

4. *Flour and Bread Fortified with Vitamin B and Iron*

Beriberi did not impact so much on the general public as an obvious and common health problem the way goiter and rickets had. Therefore the fortification program was thus lessened for the beriberi prevention. The deficiencies of vitamin B were highly prevalent in the developed countries in early 1900, thus providing incentive for fortification of cereal. Active efforts toward enriching cereal flours and products with iron and B vitamins began in the 1930s (Dary 1998). This became the common practice for the commercially available pharmaceuticals and cereal products many years later.

28.4 Bioavailability of the Particular Fortificant

The bioavailability of nutrients/fortificants refers to their absorption in the gastrointestinal tracts and physiological role in the body system. These issues of bioavailability are similar for obligatory and intended fortification processes. Nutritional physiology has identified several factors related to the issues of bioavailability:

- Vitamins and minerals are more easily absorbed in the gut into the body than other nutrients.
- Complex interaction between nutrients has influence on their individual level of absorption in the body.

- Food components have been found to enhance or inhibit the absorption of some nutrients in the body.
- Level of absorption in the body is dependent on the current nutritional status and health of the individual.

The bioavailability of the vitamin or mineral needs to be effective both with respect to its form and in the food that carries the vitamin or mineral as far as this is possible given the health status of the individual and their dietary intakes. The conditions are possibly more important for intended fortification where there is the potential for fortification with a range of vitamins and minerals that might interact or that may be added at cumulative levels greater than the recommended for daily intake.

28.5 Health Risks from Consumption of Micronutrient-Fortified Foods

Over the years, infant formula and food fortification with high levels of minerals and vitamins have led to a rapid increase in vitamin and mineral intake among infants, children, and adults. This has led to a sharp increase in the prevalence of metabolic diseases such as obesity, diabetes, cardiometabolic disorders, and other related diseases (Black et al. 2008). Micronutrient fortification is suggested in any regions where mild to moderate deficiency is prevalent, though, there is uncertainty about both the long-term benefit and the safety of micronutrient fortification in such regions, especially where the deficiency is mild (Carle et al. 2014). This reveals that consumption of a diet that is fortified with micronutrients for a long time can lead to adverse health effects. An adverse health effect has been defined as any impairment of a physiologically important function resulting in change in growth, morphology, physiology, development, or life span of an organism (Black 2003).

28.6 Functional Foods for Addressing Specific Health Need

All foods are functional to some extent because all foods provide aroma, taste, and nutritive value. However, foods are now being examined extensively for additional physiologic benefits, which may reduce chronic disease risk or otherwise optimize health. The positive effects of a functional food are to either maintain a state of well-being and health or reduce the risk of developing pathologic conditions. The initial step in the development of a functional food is the identification of a specific interaction between one and a few components of the food and a function (cellular, genomic, physiologic, and biochemical) in the organism that is potentially beneficial to the health.

A functional food component can be a macronutrient if it has specific physiologic roles (resistant starch or n-3 fatty acids) or an essential micronutrient if its intake is more than the daily recommendation. It can also be a food component that is of nutritive value, though it is not essential (some oligosaccharides) or is even of no nutritive value (live microorganisms or plant phytochemicals). Indeed, beyond its nutritional (metabolic requirements) value and function of providing pleasure, a diet provides consumers with components able to both modulate body functions and reduce the risk of some disease conditions.

1. *Gastrointestinal functions*: These functions include those that are associated with a balanced colonic microflora mediated by the endocrine activity of the gastrointestinal tract and dependent on the gastrointestinal tract's immune activity in control of nutrient bioavailability, transit time, mucosal motility, and modulators of epithelial cell proliferation.
2. *Antioxidant and redox systems*: These systems require a balanced and satisfactory intake of antioxidant (pro-) vitamins as well as non-vitamin food components such as polyphenols, phytosterol, carotene, and other natural antioxidant-based plant origins like proanthocyanidins, anthocyanins, flavonols, lycopenes, and isoflavones. Antioxidant and redox activities are important for cell and tissue function; therefore their imbalance is linked with several pathophysiological conditions like cancer, diabetes, obesity, and cardiovascular complications.
3. *Metabolism of macronutrients*: This concerns the metabolism of carbohydrates, amino acids, fatty acids, and the particular hormones modulating their metabolic pathways via insulin and glucagon balance or the synthesis of gastrointestinal peptides. This process is to reduce the risk of pathophysiologic effects linked with insulin resistance and cardiometabolic diseases which will involve interactions between nutrient intake and regulation of gene expression or an indirect interaction such as the reduction of hepatic lipogenesis by fructans.
4. *Neonatal development and early life*: Both the maternal and infant's diet can influence the development and growth of body structure and functions; examples are the importance of folic acid in the diet of pregnant women and the role of long-chain polyunsaturated fatty acids in the early stage of brain growth and development.
5. *Xenobiotic metabolism*: Modulated by a nonnutritive dietary component, such as phytochemicals. Such modulations may have important implications in the control of genotoxicity or carcinogenicity caused by chemical contaminants present in food and the environment.
6. *Mood and behavior/cognition and physical performance*: Questions have been raised about the role of food components on brain functions, but the boundary between nutritional and physiologic effects is not always easy to conclude. Furthermore, approaches for studying such effects are usually perceived as inadequate to generate data required for a reliable statistical analysis. The hunger and satiety center in the hypothalamus regulates feeding. Stimulation of hunger or feeding center causes uncontrollable hunger resulting into excessive feeding (hyperphagia) and obesity. The satiety center causes total loss of appetite. The feeding center is always active, but the satiety center constantly regulates the feeding center by inhibiting it.

These centers are regulated by the following mechanisms:

Glucostatic mechanism: The cells of satiety center function as glucostats or glucose receptors, which are stimulated by increased blood glucose level.

Lipostatic mechanism: When the size of adipose tissues increases, adipocytes produce and release an enormous quantity of leptin into the blood. While circulating through the brain, leptin crosses the blood-brain barrier and enters the hypothalamus to inhibit the feeding center.

Peptide mechanism: Many peptides are secreted by the body which can stimulate or inhibit feeding. One of such is ghrelin which is produced by the stomach during fasting and capable of increasing appetite by stimulating feeding.

Hormonal mechanism: So many hormones are capable of inhibiting feeding center by acting on the hypothalamus. Examples are oxytocin, glucagon, and pancreatic polypeptides.

Thermostatic mechanism: Temperature is known to inhibit feeding. During pathogenic infestation resulting in fever, food intake will decrease. Cytokines and thermoreceptors are known to play an active role in the inhibitory process.

28.7 Development of Diets That Matches Human Nutrient Requirements

The primary role of diet is to provide enough nutrients to meet metabolic requirements while giving the individual a feeling of satisfaction and well-being. However, beyond meeting nutrition needs, diet may modulate various functions in the body and may play detrimental or beneficial roles in some diseases.

Growth and Development Nutrition or diet has noticeable influences on gene expression, and the understanding of this interaction is an important concept to provide a basis for determining nutritional requirements on an individual basis. The effects of nutrition can be exerted at many stages of development between transcription of the genetic sequence and production of a functional protein. Genes are regulated by complex arrays of reaction elements that influence the rate of transcription. Nutrients and hormones either act directly to influence these rates or act directly through particular signaling transduction pathways. Metabolites of vitamins A and D, fatty acids, some sterols, and zinc are among the nutrients that influence transcription directly. Components of dietary fiber may influence gene expression indirectly through changes in hormonal signaling, mechanical stimuli, and metabolites produced by the intestinal microflora.

Metabolic Demand Metabolic demands during pregnancy are complicated by nutritional levels in tissues and body fluid compartments which normally alter due to hormone-induced metabolic changes in body functions. The nutritional intake in pregnant women normally increases to support the developing fetus for growth and development along with the associated changes in maternal structure and metabolism. During the period of fetal growth, fetal tissues need considerable amount of nutrients to provide energy and for immediate postnatal life which are obtained from the mother's diet. Maternal metabolism is readjusted through hormones, redirecting the nutrient to highly specialized maternal tissues specific for reproduction like mammary gland and placenta. Therefore fetal growth and development is largely dependent on the utilization of maternal nutrient, gene expression of factors promoting tissue growth and development, and hormonal network. The failure of materno-placental nutrient supply to meet the fetal nutritional demand can cause restriction in fetal growth and development. It has been shown that babies who are nutrition-deprived in utero are prone to developing hypertension, coronary heart disease, diabetes, cancer, obesity, and some other metabolic disorders later in life.

Micronutrients Micronutrient deficiencies are global challenges especially in the developing countries posing serious health and economic problem. Approximately more than two billion of the world's population suffers various deficiencies with a large number of diverse functional consequences throughout the lifetime. The most common micronutrient deficiencies are iron, iodine, and vitamins. These are caused by poor absorption and utilization or poor intake of micronutrient containing food.

Iron Iron deficiency and folic acid cause anemia which has been shown to increase maternal mortality and adverse pregnancy outcomes. Anemia is linked with high incidence of low birth weight in infants, premature delivery. One of the major causes of anemia in pregnancy is nutritional factor. Poor intake of animal and plant dietary iron causes poor bioavailability of non-heme and heme iron. The high physiological iron requirement during pregnancy is difficult to meet by diet alone. Therefore food supplements fortified with iron should be given to pregnant women as soon as pregnancy is confirmed.

Antioxidants Vitamins C and E are very potent antioxidants, and both may play a vital role in the protection of male germ cells against oxidative stress, a potential for a genetic mutation which in turn may lead to birth defects, and other diseases in an individual. A diet complete in antioxidant-containing fruits and vegetables may reduce the risk of disease. There are numbers of natural or plant-base antioxidant such as anthocyanidin, alpha-tocopherol, and beta-carotene.

Vitamin A and C Vitamin A deficiency can lead to visual defects. Prevention of vitamin A and C deficiencies can be ensured by regular intake of fruits and vegetables. Fortified food with vitamin A or C will also help in the prevention of eye damage.

Folate Folic acid plays an important role in the growth and development of the central nervous system during the early weeks of gestation, which is generally before the pregnancy is even confirmed. In a significant number of embryos, an inadequate supply of folate at this time leads to a failure of the primitive neural tube to close and to differentiate embryologically which could results in neural tube birth defects (NTD) such as anencephaly and spina bifida. Folic acid supplementation or consumption of food fortified with folic acid during the periconceptional period can markedly reduce the occurrence of severe embryonic, growth and developmental malformations.

Iodine Iodine deficiency during pregnancy can cause brain damage to the fetus leading to serious intellectual retardation, growth retardation, and brain disorders which will become apparent in childhood. This can be prevented or corrected in early stages of pregnancy. Salt iodination has been shown to be a low cost and effective way of preventing these deficiencies.

28.8 Improved Health and Wellness Through Food Science and Technology

New developments in food science and technology provide personalized tools to provide support to individuals in their quest for improved health. Innovations in food science and technology can influence individuals' food choices and improve nutrient intake. Food factors such as flavorings, processing, packaging, and genetic engineering may alter nutrient content. Adding flavors increases children's milk consumption and nutrient intake but does not adversely affect their weight. Herbs and herb blends add flavor to food and may increase the consumption of reduced sodium foods containing these ingredients. The nutrient content of many crops can be increased through traditional breeding practices (Ozzard et al. 2008).

In using food factors to drive food choice for health, one should also consider the role of food and meal satisfaction in eating behavior. In this perspective, satiety and the connection between the diet, gut, and brain are vital. Hunger and lack of satisfaction with food are among the top-ranked causes people do not adhere to dietary recommendations. Taste is a motivator of food intake; however, taste alone will not satisfy hunger and appetite. Likewise, a one-size-fits-all approach will not work (Mithen et al. 2003). Understanding the physiological regulatory pathways of satiety and food intake control and more so how these differ based on individual characteristics will help in getting closer to delivering satisfying foods and diets to which an individual can adhere. Satiety is defined as the satisfaction of hunger and appetite. Satiety is influenced by dietary factors such as energy (kJ); food form (liquid, solid); energy density (the energy content for the weight of food); macronutrient composition, weight, and volume of food; taste; and nutrient availability in a food system and associated nutrients, like fiber.

A single, optimal dietary composition for managing appetite, satiety, and body weight is unlikely to be developed (Burton-Freeman et al. 2002). For example, sex-specific differences should be considered in devising strategies for optimal food intake and body weight control. Studies in premenopausal, healthy weight women and men suggest that women respond to intestinal-phase cues for satiety, whereas men appear to respond to gastric-phase cues. Cognitive dietary restraint and obesity have also been reported to affect physiological satiety signaling, and, hence, dietary strategies to manage appetite and promote satiety and body weight control will require some level of accounting for individual characteristics, such as sex, age, body weight status, lifecycle stage (pre- or postmenopausal), and others (Burton-Freeman et al. 2004).

28.9 Sensory Physiology: Understanding of Chemosenses (Taste and Smell) to Meet Different Flavor Needs and Preferences

A sense is a faculty by which outside stimuli are perceived. Survival of organisms between bacteria and mammals depends largely on its ability to check its environment by chemoreceptive scrutiny mostly aimed at identifying nutrient sources and be alert of potential life threats or facilitating reproduction. The ability to perceive flavors starts in utero with the development and active functioning of the gustatory and olfactory systems in the first trimester. Fungiform, circumvallate papillae, and foliate appear by the 10th week of gestation, and taste cell synaptogenesis is increasingly obvious during weeks 8–13. Taste papillae are functionally mature by the beginning of the second trimester, and the distribution and numbers of papillae that are present during late gestation are strikingly similar to those seen in childhood and adulthood (Feeney et al. 2011). This serves as foundation for continuing development of food preference across the lifespan (Kim et al. 2005). Although much of food-preference development occurs during early childhood, food preferences continue to change during adolescence and adulthood (Lucchina et al. 1998). The factors that influence this change become more complex as the individual matures. Adult food preferences are associated with age, sex, health status, education and income, and the healthfulness of food preferences increases with increasing age (Pronin et al. 2007).

Also the development of the olfactory system begins during the first trimester. By the 8th week of gestation, the olfactory bulb has differentiated from the forebrain, and primary olfactory receptors have appeared (Sandell and Breslin 2006). Olfactory marker proteins, an indication of olfactory receptor maturity, are also present by the 28th–29th week of gestation. The nasal plugs blocking the nasal passages dissolve between the 16th and 36th week of gestation, allowing the nasal passages to be bathed in amniotic fluid in utero (Dinehart et al. 2006). It has been estimated that the human fetus swallows up to 500–1000 mls of amniotic fluid per day and actively inhales twice the volume near term. Olfaction ascends from receptors located on the epithelium of the nasal cavity, which are innervated by a single cranial nerve (I).

The amniotic and mammary glands contain molecules gotten from the mother's diet like protein, electrolytes, and glucose. A young infant after delivery shows characteristic taste preferences for sweet and sour taste which may serve as a driver for foods that are calorie and protein dense and an aversion to foods with toxic or poisonous taste (Drewnowski et al. 2001). Also early likes and dislikes are influenced by these innate preferences but can be modified. Repetitive exposure to fresh or disliked foods that occur in a positive, supportive environment may promote the acceptance of and eventually a preference for those foods. Instead, children who are pressured to eat certain foods may also show decreased preference for those foods later on (Keller et al. 2002). With advancement in age, the influence of a number of factors, such as peers and food availability, continues to mold food preferences and eating behaviors (Anliker et al. 1991). Taste can be defined as the sensations that occur when chemicals dissolved in saliva come into contact with taste receptors that are arranged in groups of 50–100 cells called taste buds throughout the oral cavity. These clusters of cells send messages to the brain via three cranial nerves, the facial (7th), glossopharyngeal (9th), and vagal (10th) nerves, which allow the perception of a small number of primary taste qualities.

Taste receptors are located on the tongue but may also occur on the soft palate, pharynx, epiglottis, larynx, and upper portions of the esophagus; some even occur in the stomach. On the tongue, taste receptors occur within taste buds. Taste sensations are produced from the activation of the gustatory system and are limited to the sensations of sweet, sour, bitter, salty, and umami or savory; however, evidence is building up for additional basic tastes, such as fat and calcium (Gorovic et al. 2011). In contrast, thousands of different smells stimulate the olfactory system to create smell sensations. Flavor perception produced from the integration of taste and smell sensory systems is what creates different flavor sensations (Jerzsa-Latta et al. 1990). Mouthfeel is activated by free nerve endings and gives rise to the sensations of astringency, viscosity, dryness, coolness, heat, prickling, and pain.

Inorganic ions, sugars and polysaccharides, amino acids and peptides, toxins, and “xenobiotics” are all subject to nutritional chemoreception followed by adaptive behavior. Cell and molecular biology have made clear today that animals perceive taste and smell through transmembrane protein receptors (taste and olfactory receptors – TR and OR, respectively).

Many factors affect the individual’s ability to detect and identify gustatory sensations which are physical, chemical, biological, and psychological. Of the physical factors, temperature is probably the most significant under wine-tasting conditions. Cooling reduces sensitivity to sugars and bitter alkaloids (Green and Frankmann 1987). Nonetheless, low temperatures appear to enhance the perception of bitterness (and astringency). Another important physicochemical factor affecting taste perception is pH. Through its effect on organic and amino acid ionization and on their salts, pH influences perceived sourness, solubility, shape, and biological activity of proteins, respectively. Modification of gustatory receptor shape could markedly affect taste sensitivity (Buettner and Beauchamp 2010). Chemical changes such as salivary flow affect taste sensation. Salivary enzymes can directly affect the concentration and chemical nature of tastants. Cultural influences, such a family upbringing and social pressures, can override some of the genetic underpinnings of personal preference. Many factors affect olfactory acuity like gender (women are more generally sensitive to odors than men), age, and psychology (Doty 2003).

28.10 Physiological Influences on Food Preferences

Food preferences seem to be partially genetically determined, with high coefficients of heritability for preferences for protein foods, fruit, vegetables, and desserts. The mechanism underlying genetic influences on food preferences may be variation in taste perception and preferences. Recent research has identified several genes related to individual differences in sweet, umami, and bitter taste perception.

The perception of these tastes involves G-coupled protein receptors encoded by the TAS1R and TAS2R taste receptor gene families. In contrast, salty and sour tastes are transduced by ion channels in taste receptor cells (Bartoshuk 1979). Single-nucleotide polymorphisms in these gene families are associated with functional variance in sweet, umami, and bitter perception, the mechanisms underlying the majority of these associations shown below (Fig. 28.1).

28.11 Food Neophobia

Over the course of the first few years of life, young children undergo a transition from a predominantly milk-based diet to one consisting of adult table foods. Young children (especially 2–5 year olds) exhibit heightened levels of food neophobia

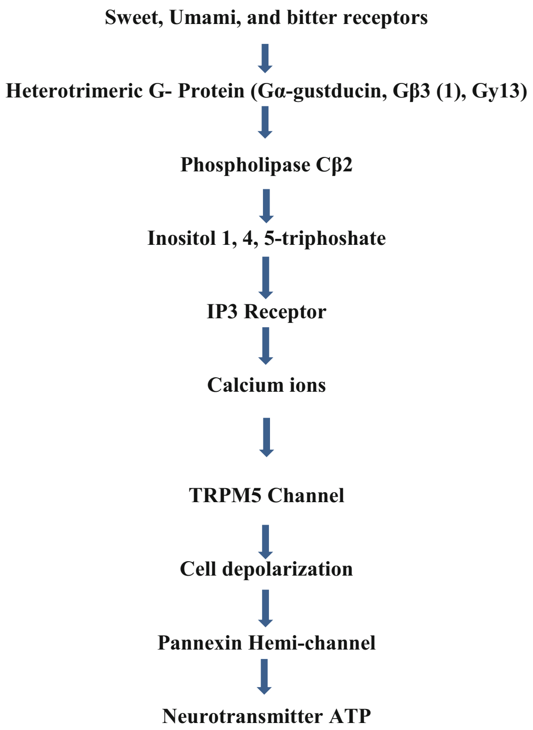

Fig. 28.1 Cellular signaling transduction cascade in taste receptor cell

during this time of rapid dietary change. Food neophobia is defined as an unwillingness to eat new foods and is thought to be an adaptive behavior, ensuring children consume foods that are familiar and safe during a developmental period when children are being exposed to a vast number of new foods (Kim et al. 2005).

28.12 Probiotics: A Nutritional Strategy for Disease Prevention

The positive role of bacteria can be credited to the pioneering work of Metchnikoff in the early 1900s who observed that a large consumption of bacteria contained in fermented milks was used as a way to replace harmful bacteria with useful ones. The term probiotic means “for life” and was created in the 1950s by Kollath and was first coined by Lilly and Stillwell for live bacteria and spores as animal feed supplements that should help limiting the use of antibiotics in animal husbandry (Vasiljevic and Shah 2008).

A growing attention in basic research on the effect of probiotics in human health and the commercial advantages of the probiotic food technology has been observed in the last 30 years.

This increased research has resulted in significant advances in the understanding of the fundamental mechanisms by which probiotics may confer beneficial effects to the host (Fuller 1992).

Research has shown that nutrition plays a critical role in the prevention of chronic diseases, as most of them can be related to diet. Functional food enters the concept of considering food not only necessary for living but also as a source of mental and physical well-being, contributing to the prevention and reducing of risk factors for several diseases, or enhancing certain physiological functions in human system. Nowadays dairy products are excellent media to generate an array of products that fit to current consumer demand for functional food. Fermented dairy products enriched with probiotic bacteria have developed into one of the most successful parts of functional foods. The food industry is especially active in studying probiotics because the gastrointestinal tract is one of the richest zones of biodiversity within the body with at least 450 known species of microorganisms commonly found there.

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al. 2014). “Probiotics” comprise many different types of microbes. Probiotics have recently gained popularity in conjunction with the recognition of the deleterious effects of antibiotic overuse (Mitsuoka and Kaneuchi 1977). Today probiotics are defined as live microorganisms that, when administered, produce some therapeutic or preventive health benefits to the host. Probiotics are gaining more attention in biomedical research based on their health benefits and importance in “functional” foods: those that provide health-enhancing properties beyond their nutrients. In recent times, individuals are becoming more aware of how diet impacts their health and disease risk and seek out foods that meet their specific needs (Sandlers 2003). Probiotics can be considered functional foods because they provide health benefits beyond the traditional nutrition function.

The two major probiotic microbial species are:

Lactobacilli

Lactobacilli are generally described as Gram-positive, non-spore-forming, and non-flagellated rods or coccobacilli, aerotolerant, fastidious, acid-tolerant, and strictly fermentative. There are numerous species of lactobacilli in the gastrointestinal system, because they thrive well at variable pH environments (Oelschlaeger 2010). Some principal lactobacilli species include *Lactobacillus acidophilus*, *L. rhamnosus*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *L. johnsonii*, *L. gasseri*, *L. fermentum*, and *L. crispatus*. The genus *Lactobacillus* is so named due to the ability of this group of bacteria to produce lactic acid and does not relate to the ability to digest lactose (Ahrne et al. 1998). Lactobacilli have complex growth requirements such as low oxygen tension, protein, and its breakdown products; vitamins and growth may occur in a temperature range between 30 and 45 °C. They are often distributed in various ecological niches throughout the gastrointestinal and genital tracts which constitute an important part of indigenous microbiota of man and higher animals (Gomes and Malcata 1999).

Bifidobacterium lactis

Some principal *Bifidobacteria such as B. longum*, *B. bifidum*, *B. infantis*, *B. lactis*, *B. breve*, *B. animalis*, and *B. adolescentis* and other probiotics like *Escherichia coli* Nissle, *Saccharomyces boulardii*, *Enterococcus faecalis*, *Lactococcus lactis*, and *Propionibacteria* can be found in cultured dairy foods like yogurt, fermented vegetables, nutrition bars, some beverages, and other foods fortified with probiotics (Wagner et al. 1997). *Bifidobacteria* are generally considered as a Gram-positive, rod-shaped, non-gas-producing, non-spore-forming, catalase-negative, and anaerobic microorganisms. They are saccharolytic organisms that produce acetic and lactic acid without generation of CO_2. *Bifidobacteria* are microorganisms of paramount significance in the active and complex ecosystem of the intestinal tract of humans. The number of *Bifidobacteria* decreases with increasing age of the host (Tuohy et al. 2003).

28.13 Probiotics and Health Benefit

The past decade witnessed a new era in medical science with increased use of probiotics for health benefits, especially in diarrhea and other disease conditions. Some typical food items such as yogurt, garlic, and cheese contain probiotics in the form of live lactic acid bacteria or prebiotics in the form of fructans, which is a dietary fiber. Cheese contains both probiotic bacteria and the prebiotic dietary fiber inulin. The regular consumption of cheese has been linked with a reduction in the risk of *Campylobacter enteritis*. Additionally, cheese is known to contain substances that can reduce the risk of dental caries. Majority of probiotics are bacteria with the species of *Lactobacillus* and *Bifidobacterium* being the most common type of bacteria used. Before bacteria can be selected as a probiotic, it should be non-pathogenic and non-toxigenic and should retain its viability during storage and use. Also it should have the capacity to survive and metabolize in the gut.

28.13.1 Gastrointestinal Physiology

The microenvironment of the gut system is complex in which the lymphoid organ interfaces with countless of endogenous and exogenous stimuli. The protection against potentially harmful agents by the intestinal mucosa is ensured by saliva, peristalsis movement, mucus, gastric acid, intestinal proteolysis, epithelial cell membranes, intestinal flora, and intercellular junctional complexes together with a well-functioning immunological defense. The immunological regulation takes place in several compartments: aggregations of lymphoid cells in follicles and the Peyer's patches, distributed within the mucosa, and in the intestinal epithelium, as well as in secretory sites. The lamina propria is also endowed with lymphocytes belonging to the B-cell lineage. IgA antibody production is abundant at mucosal surfaces, where secretory IgA is present in dimeric or polymeric form. Secretory IgA is relatively resistant to intraluminal proteolysis and does not activate complement or inflammatory responses.

There are differences between the upper and lower parts of the human gut-associated immune system in the isotype distribution of immunoglobulin-producing cells. IgA1 immunocytes predominate in the small intestine, while IgA2-producing cells are most frequent in the colon, the latter being more resistant to bacterial proteases. The secretory IgA antibodies in the gut are part of the common mucosal immune system including respiratory tract and lacrimal, salivary and mammary glands. Consequently, an immune response initiated in the gut-associated lymphoid tissue can affect immune responses at other mucosal surfaces. The intestine's mucosal surface provides a defense barrier against antigens encountered by the enteric route. As a result of the barrier function, systemic hyporesponsiveness to antigens such as food proteins, oral tolerance, is a hallmark of the intestinal immune system. In this system also a balance is generated and maintained between the host and the normal resident microflora. In addition to antigen degradation and thereby participating in tolerance induction, intestinal colonization acts as an important endogenous stimulus for the maturation of the gut-associated lymphoid tissue (Helgeland et al. 1996). So far, the human gut microflora is still an unexplored organ of host defense, and its impact in health and disease may be stronger than currently known.

Probiotics have been shown to promote intestinal health associated with the intake of antibiotics, reduce relapses of ulcerative colitis, decrease serum cholesterol, alleviate lactose intolerance, inhibit *Helicobacter pylori* growth, and reduce in diarrhea severity (Stark and Lee 1982). The most common alteration of intestinal flora in children occurs with antimicrobial therapy, especially with broad-spectrum antibiotics. Since children receive antibiotics often and are predisposed to gastrointestinal disease in the first few years of life, the use of probiotics in children remains an area of significant interest and research. Positive effects in the prevention of pediatric antibiotic-associated diarrhea and *Clostridium difficile*-associated diarrhea have been identified with *L. rhamnosus* GG by changing the gut microflora (Gorbach 2000).

28.13.2 Lactose Intolerance

Lactase activity in most mammals decreases after weaning, though studies have revealed that in some individuals lactase activity persist even into adulthood causing lactase persistence which varies based on ethnicity, consumption of dairy products across the globe. Studies have shown that in some individuals lacking lactase, reach the large intestine, broken down by microflora into carbon dioxide, methane, and hydrogen, thereby altering the osmotic balance resulting into cramps, flatulence, bloating, and diarrhea. It is well established that fortifying high-lactose milk with lactobacilli and/or *Bifidobacteria* can be tolerated by individuals with lactose intolerance possibly because the fermented products contain microbial β-galactosidase producing lactose hydrolysis in the small intestine. Additionally, probiotic bacteria contain high levels of lactase, which is released within the intestinal lumen when these bacteria are lysed by bile secretions. Lactase then acts on the ingested lactose decreasing maldigestion symptoms (Turpin et al. 2010). Also consumption of fermented milk is seen to lower the level of hydrogen in the breath when compared to the hydrogen level for subjects fed with unfermented milk (Kim and Gilliland 1983). Hydrogen in breath is seen as a marker for bacterial metabolism of lactose in the large bowel. A lower hydrogen level indicates that lactose has been metabolized prior to entering the large intestine.

28.13.3 Lowering of Cholesterol

Various mechanisms have been proposed for the probiotics reducing effects of cholesterol. These include deconjugation of bile acids by bile salt hydrolase enzymes of probiotics, assimilation of cholesterol by probiotics, coprecipitation of cholesterol with deconjugated bile, cholesterol binding to cell walls of probiotics, incorporation of cholesterol into the cellular membranes of probiotics during growth, conversion of cholesterol into coprostanol, and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics (Ammor et al. 2006).

28.13.4 Allergic and Inflammatory Disorders

The therapeutic effect of probiotics in the treatment of allergic and inflammatory reaction is a new area of study. Studies have suggested the possible role of stimulating the gut immune system with probiotics as a means of redirecting the immune system and reducing both the development and severity of allergic disease in children. The occurrence of large numbers of T_H2 lymphocytes in the gut is linked with heightened production of IgE antibodies against a variety of environmental antigens (Dai and Walker 1999). A number of gut microflora appear to be able to encourage the differentiation of lymphocytes into T_H1 and T_H3 types, promoting a more healthy balance of lymphocytes in the gut and perhaps reducing the tendency toward allergic reaction (Mack et al. 2003).

T_H2 prevalence prevails in utero and in early infancy is a key observation related to the possible mechanism of the development of infantile allergic conditions. When intestinal inflammation and microbial imbalance occur, permeability increases, and large antigen molecules and intestinal bacteria can migrate across the mucous membrane. The gut microflora appears to influence gut permeability, and some probiotics have been confirmed via in vitro and in vivo methods to significantly reduce gut permeability, preventing macromolecule transfer. Enhancement of local and antigen-specific IgA response has been shown with *L. rhamnosus* GG in response to food antigens (Negretti et al. 1997).

28.14 Antipathogenic Activity

Many probiotics have been demonstrated to produce antipathogenic compounds ranging from small molecules to bioactive antimicrobial peptides. The lowering of pH by acids like lactic and acetic has bactericidal and bacteriostatic effects. The production of H_2O_2 by probiotic strains may result in an antimicrobial effect due to the oxidation of sulfhydryl groups causing denaturing of a number of enzymes and the peroxidation of membrane lipids, thus increasing membrane permeability. Probiotics may also produce antimicrobial peptides like defensins and bacteriocins; the antimicrobial action of these compounds involves increased permeability of the cytoplasmic membrane of the target cells, which leads to the release of small cytoplasmic particles, to depolarization of the membrane potential, and eventually to cell death by apoptosis (Simova et al. 2009).

28.14.1 Immune System

Studies have shown that probiotics support the immune system by decreasing upper respiratory tract infection in adults and reduction in cold and flu-like symptoms in children. Also the innate immune system via toll-like receptors recognizes a large group of chemical structures in pathogens and lipoteichoic acids which enables them to recognize foreign objects which trigger a cascade of immunological defense mechanisms. Probiotics can influence the immune system (innate and acquired) by products like metabolites, cell-wall components (lipopolysaccharides and peptidoglycans), and DNA. Probiotic products are recognized by host cells sensitive for these because they are equipped with recognition receptors (Ohashi and Ushida 2009).

Probiotics have been shown to have immunomodulatory properties through the inhibition of bacterial translocation, stimulation of phagocytes/macrophages and natural killer cells, increased proliferation in organs of the immune system (Peyer’s patches spleen), and increased release of cytokines (Ooi and Liong 2010). The gastrointestinal immune system consists of two main components: organized lymphoid follicles (Payer’s patches and mesenteric lymph nodes and a large number of immunocompetent cells – the organized tissues) serve as a potential site for the induction of immune responses to new antigens, whereas the intestinal mucus serves as the effector site.

Probiotics are thus suggested to confer protection against enteropathogens by:

1. Stimulating cytokine production
2. Enhancing the phagocytic capacity of polymorphonuclear cells and macrophages
3. Augmenting natural killer cell activity
4. Enhancing specific antibody responses to pathogens

28.14.2 Probiotic and Disease Conditions

28.14.2.1 Cancer

Recently, the use of dietary intervention in the treatment and prevention of cancer has received massive attention from scientists, nutritionists, and industries. Indeed foods with prebiotics and probiotics are in the frontline of cancer management and prevention of chronic diseases like cancer, high blood pressure, obesity, and diabetes. Yogurt and other fermented milk products have been supported as cancer preventing, especially for colon cancer. Colon cancer has been linked with high-fat diet due to its stimulating ability toward bile acid in the colon affecting the metabolism of the bacteria flora. Also studies have shown that indigenous microflora in the intestine could produce enzymes such as glucuronidase, nitroreductase, and azoreductase which can convert pro-carcinogens into carcinogens. This has necessitated research on probiotics in cancer prevention. Some other mechanisms proposed in cancer preventive effects of probiotics are preventing the growth of bacteria that produce enzymes responsible for converting pro-carcinogens into carcinogens, production of tumor necrosis factor by macrophages, and inhibition of tumor growth by metabolites of the lactobacilli stimulating the immune system (Dicks and Botes 2009).

Probiotics is known to produce a wide range of metabolites that results into health benefits to the host. These metabolites are glutamine, arginine, bacteriocins, short-chain fatty acids (SCFAs), and hydrogen peroxide which are protective to the intestine. Butyrate, acetate, and propionate are the most important primary SCFAs produced by the probiotics in the intestine during fermentation of prebiotics. Among the organic acids produced by probiotic *Bifidobacteria* and lactobacilli, butyric acid showed the highest inhibition of mutagens compared to acetic acid, while lactic and pyruvic acids failed to inhibit at appreciable levels.

28.15 Exclusion of Pathogenic Microorganisms by Probiotics

Infections are known to be linked with cancer development and have been reported that 17.8% of global burden of cancer are due to infections even though the mechanism of infection induced cancer pathogenesis is not fully understood. In the developing countries, the rate of cancer caused by infections is even higher (about 26.3%), thereby placing these countries on global cancer burden. About 15.6% of global incidence of cancer is attributed to infection with bacterium *Helicobacter pylori* and viruses such as hepatitis B, human papilloma viruses, hepatitis C, human T cell lymphotropic virus I (HTLV1), HIV virus, and Epstein-Barr virus. Epstein-Barr virus has been shown to cause nasopharyngeal carcinoma, Burkitt's lymphoma, and many other types of lymphomas, while HTLV1 is seen to cause adult T-cell leukemia. Human papilloma virus causes bladder carcinoma, and parasites like liver fluke cause cancer of the liver (Fujiwara et al. 2001).

Probiotics such as *L. rhamnosus* are found to cause the elimination of herpes simplex virus type I by activating macrophages, also in the gut, and it helps in the eradication of *H. pylori*, thereby mitigating the frequency of epigastric pain, diarrhea, and vomiting/nausea. The mechanism involved in this action may be due to immunomodulatory properties of probiotics such as increased immune response by elevated levels of sIgA and anti-inflammatory cytokines, production of antiviral compounds that aids in restricting the pathogenesis (Aso et al. 1995). Consequently, eradication of pathogens using probiotics would help in the prevention of cancer, thereby reducing the burden of infection associated to cancer in humans.

28.16 Probiotics in Nonpregnant Women

Vaginal infections represent one of the most common reasons for gynecological consultation. It is estimated that approximately seven women out of ten will experience at least one episode of vulvovaginal candidiasis (VVC) in their lives. Studies have shown that bacterial vaginosis (BV) is another highly prevalent vaginal disorder associated with an increased risk for pelvic inflammatory disease, sexually transmitted infections, HIV transmission, and preterm delivery (Haahr et al. 2017). Bacterial vaginosis is characterized by a reduction or depletion of lactobacilli and overgrowth of *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Prevotella* species, and other pathogenic anaerobic bacteria *Lactobacillus* species produce lactic and acetic acid and hydrogen peroxide, maintain the vaginal pH around 4.5 or less, hamper growth of pathogenic bacteria and *Candida albicans*, and are thus considered protective against VVC and BV. Accordingly, the putative beneficial effect of *Lactobacillus* species-containing probiotics in restoring and maintaining the physiologic vaginal microbiota fostered their use for the treatment of both vaginal disorders (Olsen et al. 2018).

28.17 Probiotics in Pregnant Women

It has been suggested that probiotics could play a role in the prevention of preterm birth. Preterm birth rates vary across different countries, ranging from 6.2% in Europe to 10.6% in USA and 11.9% Africa (WHO 2000). The etiology of preterm birth is multifactorial, but it has been estimated that about one third of cases is due to intrauterine inflammation caused by ascending vaginal infections. In particular, a preexisting bacterial vaginosis (BV) appears to be strongly associated with premature birth (Sherrard et al. 2011). Therefore, here the putative role of probiotics might be associated with their potential ability to displace and kill pathogens. The hypothesized mechanisms include the development of anti-inflammatory cytokines and the reduction of the vaginal pH, so that the vaginal environment becomes again favorable to the growth of healthy bacteria (Kolacek et al. 2017). Moreover, the use of probiotics in pregnancy could improve maternal glucidic metabolism through the modification of gut microbial composition and function, as well as the improvement of insulin sensitivity.

Despite increasing marketing and sales of probiotics, the results originated by clinical trials are inconsistent and generally of sub-optimal quality (Griffin 2015). Furthermore, a substantial proportion of these trials have been sponsored by parties with a commercial interest in the outcome. In addition, there was considerable heterogeneity in published studies in terms of strain/s of probiotic adopted, route of administration (oral or vaginal), and duration of treatment which compounded the efficacy. The effects of probiotics seem to be strain-specific and dose-dependent, and the lack of a standardized manufacturing process could affect microbial survival, growth, and viability (Arnold 2013). Though the primary aim of probiotics is the re-establishment of a physiological vaginal microbiome, however, there is currently no consensus regarding their use for the treatment of vaginal infections and their sequelae. Thus, further better-quality data are needed to define the real effect size of probiotic use in different obstetrical and gynecological conditions.

28.18 Mental Health

It is well established that infusion of intestinal long-chain fatty acids such as linolenic and linoleic acids modulated food preference as well as total calorie intake via the vagal nerve and midbrain hypothalamic neural pathways. Also, the pharmabiotics such as gamma aminobutyric acid (GABA), acetylcholine, serotonin, catecholamines, etc. produced by probiotics and other commensal gut microbiota may modulate neural signaling with the enteric nervous system when they release into intestinal lumen (Bercik et al. 2011). Acetylcholine, the principal vagal neurotransmitter, can significantly attenuate the release of cytokines TNF, interlukin-1beta, Il-6, and IL-18 even though the exact mechanism is yet to be elucidated; additionally several studies also showed TNF blocking in anti-cancer therapy. Since several beneficial aspects of probiotics on health are evident, probiotics can be also used to improve the physical and mental health status of cancer survivors. Psychobiotics, a class of probiotics, produces a health benefit in patients suffering from psychiatric illness. These bacteria are capable of producing and transferring neuroactive substances such as GABA and serotonin which act on the gut brain axis (Dinan et al. 2013).

28.19 Mechanism of Action of Probiotics

The intestinal tract resists the colonization of both the harmful and harmless bacteria invasion to take a permanent residence in the gut. Ultimately the gut resists these bacteria by an increase in acidic environment of the stomach, the peristalsis movements, bile acid and salt on the duodenum, immunological responses, and the mucus layer of the intestinal epithelium. *L. rhamnosus* GG has been shown to attach and adhere to the intestinal mucus and epithelial cells, thereby generating immunomodulatory effects (Saavedra et al. 1994). Also probiotics like *L. rhamnosus* GG and others are known to produce hydrogen peroxide, biosurfactants, upregulation of mucin-encoding genes which stimulate the production of mucus to form a protective gut barrier, and bacteriocins that help in their colonization and survival. Also probiotics can act to lower intestinal pH by stimulating lactic acid production that favors growth of more beneficial organisms and inhibiting the growth of potential pathogens. *Lactobacillus rhamnosus* GG has been shown to increase the total levels of anaerobic bacteria, particularly *Bifidobacteria*, *Clostridia*, and *Bacteroides*. Probiotics effectively adapt to intestinal microflora and do not displace the indigenous bacteria. To become a good probiotic, bacteria must be ingested in live form, be able to colonize in the gut to maintain sufficient viable microorganisms that survive the host's digestive process and environment, reproduce to the extent that they can be identified in the stool, and be able to attach and adhere to the gut epithelium (Apostolou et al. 2001).

Currently used criteria for the study of probiotic strains (in vitro test)

1. Antimicrobial activity against potentially pathogenic bacteria
2. Resistance to gastric acidity
3. Bile acid resistance
4. Bile salt hydrolase activity
5. Resistance to spermicides (for vaginal use)
6. Adherence to mucus or human epithelial cells and cell lines
7. Ability to reduce pathogen adhesion to surfaces

28.20 Adverse Effects

Probiotics available as food ingredients or dietary supplements that contain microorganisms have been extensively used in food processing industries for several years, with a long history of safety and no serious adverse effects on metabolic processes. Though, when considering the safety of probiotics, potential adverse effects may include systemic infections, altered metabolism, gene transfer, and excessive immune stimulation in susceptible individuals (Marteau 2002).

Most commercially available strains of *Lactobacillus* and *Bifidobacterium* species are generally regarded as safe by the Food and Drug Administration and may be especially helpful in the treatment of pediatric diarrheal illnesses. However, the clinical benefit of probiotic therapy depends on numerous factors, such as type of bacteria, dosing regimen, delivery method, and other underlying host factors. Recommendation of a specific probiotic for any condition requires thoughtful analysis of these issues. Therefore the following must be recognized:

1. Genus, species, and strain designation. Strain designation should not mislead consumers about the functionality of the strain.
2. Minimum viable numbers of each probiotic strain at the end of the shelf-life.
3. The suggested serving size must deliver the effective dose of probiotics related to the health claim.
4. Health claim(s).
5. Proper storage conditions.
6. Corporate contact details for consumer information.

28.21 Prebiotics

Prebiotics are engaged in promoting both beneficial bacteria which are already established in the colon and externally administered probiotic bacteria (Ridley et al. 2001). Prebiotics are "a selectively fermented ingredient that allows specific changes, both in the composition or activity in the gastrointestinal microflora that confers beneficial effects on the host well-being and health" (Olano-Martin et al. 2001).

Currently, the known prebiotics are carbohydrates, there are many different carbohydrates marketed worldwide as prebiotics, and the only three that are well supported are:

1. Fructans (inulin and fructo-oligosaccharides)
2. Galacto-oligosaccharides
3. The synthetic disaccharide (lactulose)

28.22 Fructans

A fructan is composed of fructose polymers which are generally linked to the moiety of a terminal glucose. Fructans are resistant to digestive enzymes in the gastrointestinal tract of humans, pass through the upper portion of the human GI tract, reach the colon, and are fermented by colonic microflora producing SCFA (Brown et al. 1997).

Inulin is a natural occurring carbohydrate found in garlic, onions, and wheat. Nair et al. (2010) reported the functional aspects of inulin and revealed that several studies have demonstrated inulin to promote optimal digestive health, positively influence lipid metabolism, decrease the risk of osteoporosis by increasing calcium absorption, as well as reduce the risk of breast cancer, colon cancer, and tumor growth (Silvi et al. 1999).

Fructo-oligosaccharides (FOS) on the other hand are made of a mixture of oligosaccharides consisting of glucose connected to fructose units by β-(1, 2) linkage with a degree of polymerization between 1 and 5. They occur in several varieties of plant. In the body especially the colon, FOS are completely fermented to lactate mostly, SCFA (acetate, butyrate, and propionate) and gas. The FOSs stimulates bifidobacterial growth and suppresses the growth of potentially harmful species in the colon. Other physiological effects of FOS include decrease in fecal pH, increase in fecal or colonic organic acids, decrease in fecal bacterial enzymatic activities, modification in fecal neutral sterols, enhance magnesium absorption, and enhancement of both colon butyrate concentrations and local immune system effectors, thereby reducing the development of colon cancer (Jacobasch et al. 2006).

28.23 Galacto-oligosaccharides

Galacto-oligosaccharide (GOS) is a collective term for a group of carbohydrates composed of oligo-galactose with some lactose and glucose, produced commercially from lactose by β-galactosidase. Due to the stability of GOS at high temperature and in acidic environments, they are majorly used in many food processes such as infant formulas, dairy products, soups, sauces, breakfast cereals, ice creams, snack bars, beverages, animal feeds, bakery products, and as sugar replacements to increase texture and mouthfeel of foods and act as bulking agents. Also, the mixture of 90% GOS and 10% inulin is used in infant formulas to simulate human milk (Dongowski et al. 2005).

28.24 Lactulose

Lactulose is a synthetic disaccharide used as a laxative. It has been demonstrated to increase lactobacilli and *Bifidobacteria* plus significantly decreasing *Bacteroides* in mixed continuous fecal culture. But in spite of the promising results as a prebiotics, lactulose is not yet widely used as a prebiotic, although it remains a recognized product in the medical and pharmaceutical industry (Le Blay et al. 1999). Other emerging and novel prebiotics are xylo-oligosaccharides (XOS), resistant starch, and pectic oligosaccharides.

Xylo-oligosaccharides (XOS) which are chains of xylose molecules linked by β1–4 bonds which are produced enzymatically by hydrolysis of xylan from birchwood. They have proven to be very selective for *Bifidobacteria*, and the prebiotic potential has also been confirmed in many animal studies (Topping et al. 2003).

Resistant starch is a fraction of starch that escapes digestion in the upper GI tract and that reaches the colon to be fermented by the colonic microbiota. Very few animal studies on the probiotic properties of resistant starch have been carried out and in broad terms show an increase in *Bifidobacteria* and SCFA in rats (Lecerf et al. 2012).

Pectin is a complex galacturonic acid-rich polysaccharide which occurs naturally in the cell walls of higher plants and acts as a cement-like material for the cellulosic components of the plant cell wall. The major polysaccharides forming pectin include homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II) which are believed to covalently link together (Santos et al. 2006). Enzymatic or physical methods can be used to produce pectic oligosaccharides. Enzymatic hydrolysis of citrus and apple pectins in membrane reactors produces oligosaccharides of 3–4 kDa molecular weights. A nitric acid hydrolysis of citrus peel produces low molecular weight arabinose-based oligosaccharides. Both materials have been evaluated in fecal batch cultures and have both been demonstrated to promote growth of *Bifidobacteria* (Hsu et al. 2004).

28.25 Probiotics and Prebiotics as Functional Food

Probiotics and prebiotics simultaneously present in a product are called synbiotics. Such a combination aids survival of the administered probiotics and facilitates its inoculation into the colon. Furthermore, the prebiotics induce growth and increase activity of positive endogenic intestinal microflora (Tomasik and Tomasik 2003). It was experimentally shown that synbiotics protect the organism from carcinogens significantly better than do either probiotics or prebiotics separately (Gallaher and Khil 1999). Several foodstuffs with probiotics and prebiotics are commercially available (Bekers et al. 1999).

28.26 Future Target: Using Genetically Modified Customized Probiotics

Genetic manipulation offers opportunity to enhance the current probiotic properties of an organism (Steidler 2003). Clarification of mechanisms of actions of a probiotic could enable the manipulation of organisms to produce specific and targeted probiotics. Studies have revealed that consumer resistance to genetically modified organisms is such that GMO probiotic foods are unlikely in the near future and potential clinical applications to ameliorate chronic diseases may be more readily accepted. Braat et al. (2006) also created a biologically contained *L. lactis* to produce human IL-10 and treated Crohn's disease patients with this GM *L. lactis* in a phase-1 placebo-uncontrolled trial. A reduction in disease activity was detected with minor side effects, and suppression of the organism was achieved through its dependency on thymidine for growth and IL-10 production.

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Caffeine: Nutraceutical and Health Benefit of Caffeine-Containing Commodities and Products

29

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Abstract

The ever-increasing population of mankind are globally combatting with several health challenges couple with a high level of synthetic drugs demands the search for a green solution that will be sustainable. Therefore, caffeine has been identified as a sustainable daily consumable that might be tailored to deliver its active component for the management of the various highlighted health challenges combating mankind through the daily and/or healthy meal. Caffeine (1,3,7-trimethylxanthine) is a soluble natural alkaloid present in plants which constitutes the main active biologically compound present in several food and beverages containing caffeine usually consumed by several individuals. The significance of caffeine-containing food and drinks has been documented over the years as antimicrobial agents; neuroprotective, antioxidant, and cardiovascular agents; and antidiabetics. Hence, this review intends to highlight the health

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and nutritional benefits of caffeine which has been highlighted as a vital nutritional ingredient which is present in some food products. Also, some previously in vitro and in vivo experiment carried out with caffeine and caffeine-containing products were well elucidated which have great attributes to release its controlled dose which plays an active role in the maintenance of the body cells, system, and their normal functioning.

Keywords

Antimicrobial · Caffeine · Cardiovascular · Health benefit · Neuroprotective · Nutraceutical

29.1 Introduction

Caffeine (1,3,7-trimethylxanthine) has been identified as a major component available in various products rich in chocolate content and beverages known for having a sweet and well-flavored taste. Caffeine is a natural alkaloid with an addictive property which tends to make the consumer addictive to its continuous use (Nawrot et al. 2003). The presence of caffeine in plant products such as coffee beans, tea leaves, cocoa beans, cola nuts, energy drinks, and/or beverages. It has been observed in recent times that its consumption has increased significantly among athletes and nonathletes which could be attributed to its pleasant taste and addictive property, bearing in mind the purpose of its consumption (Nawrot et al. 2003).

One of the major reasons for the high consumption of caffeine-related products could be linked to health benefits which might be attributed to the presence of some phytochemical constituents and arrays of numerous antioxidants present in their various caffeine products. Although some side effects has been documented when caffeine is consumed on a very high level. Sometimes, when it is consumed as a mild stimulant, caffeine could increase the levels of alertness to avoid drowsiness an indication of mental alertness. Owing to the high demands of several products containing caffeine as the active compound and some other related products such as caffeinated energy drinks, industries which produce such products have significantly increase over the years with several brand names, while others are yet to be established with the main effect on children and adolescent as a higher percentage of persons who are at risk with the adverse effect of caffeine (Verster and Koenig 2017).

Moreover, over time, it has been observed that most of the synthetic drugs used for the treatment of various diseases have developed a high level of resistance to treatment. Therefore, there is a need to search for a sustainable solution in the form of food and drink that could serve as a neutraceutical and healthy maintenance food and drinks. Over the years, several scientists have documented health and food benefits of foods containing caffeine, their effectiveness, and challenges encountered during the production and formulation of this caffeine-containing products.

In view of the aforementioned, this study intends to highlight food, health, and nutritional benefits of consuming caffeine-containing products and beverages. Moreover, some future recommendation was stated during this review that could help toward the improvement of production and enhancement of caffeine-containing products.

29.2 Antimicrobial Activity

Dash and Gummadi (2008) assessed the antimicrobial of (1,3,7-trimethylxanthine) against the morphology, growth, and viability of non-caffeine-degrading bacterial strains and caffeine- degrading *Pseudomonas* sp. This was carried out using caffeine medium, while the minimal medium containing caffeine and without was amended at the log phase of growth. It was discovered that the caffeine-degrading *Pseudomonas* sp. produced the highest cell dry weight of 1.1 g L^{-1} after amending with caffeine during the log phase of growth, but there is no alteration in the morphology of the bacterial cell. Moreover, it was observed that there was a decrease in the number of *E. coli* DH5α most especially at the log phase. Also, cell distortion and breakage were observed on *Bacillus subtilis and other* Gram-negative bacterial species when exposed to caffeine. Furthermore, it was observed that the transformation of *E. coli* DH5α with the plasmid isolated from *Pseudomonas* sp. that possess the capability to degrade caffeine was discovered to become tolerant to caffeine. Their study shows that the growth and viability of *E. coli* DH5α and other bacterial strains were greatly reduced upon addition of caffeine at the log phase of growth. *E. coli* DH5α strain formed long filamentous cells on caffeine exposure, and lyses occurred for other Gram-negative bacterial species and *Bacillus subtilis*. *E. coli* DH5α transformed with a plasmid from caffeine-degrading *Pseudomonas* sp. was found to be tolerant to caffeine. This study shows the proneness of bacterial strains that could not degrade caffeine and will go a long way in providing better knowledge on evolution and how xenobiotic-degrading strains survive in nature.

Ibrahim et al. (2006) evaluate the potential of caffeine, 1,3,7-trimethylxanthine, and their capability to control various strains of *Escherichia coli* O157:H7 containing cider, E0019, 944, F4546, and H1730. The various strains were cultured overnight in broth containing brain heart infusion. The various concentrations of caffeine from 0.00% to 2.00% were tested against all the inoculum containing 2 log CFU/ml. The cultural medium was incubated for 24 h at 37 °C. The growth of the inoculum was assessed at a different interval by measuring the turbidity at 610 nm with the aids of a spectrophotometer. The result obtained exhibited that the inclusion of caffeine in the culture medium led to a drastic reduction of *E. coli* O157:H7. Moreover, the highest concentration that showed the highest activity was 0.50% when caffeine was added to the cultural growth medium. Their study showed that caffeine could serve as an antimicrobial agent and could be used as a food additive to ensure and guarantee the biosafety of food products which are consumable.

Olajubu (2017) evaluated the anti-hemolytic and antimicrobial effectiveness of coffee and tea commonly consumed in Nigeria. The antimicrobial effectiveness was performed using disc diffusion techniques against animal and man pathogens, while the colorimetric method was utilized for the anti-hemolytic assay. The result obtained showed that the extract obtained from tea and coffee showed an enhanced inhibitory effect against *Streptococcus pneumoniae*, *Shigella dysenteriae*, *Staphylococcus aureus*, *and Salmonella pullorum*, *while a slight inhibitory effect was detected against Escherichia coli*. Moreover, it was observed that the extract obtained from coffee exhibits bactericidal effect against all the tested isolates at 6 h, but the tea extract showed activity between 18 and 24 h. Also, it was observed that the extracts obtained from tea prevented the hemolytic capability of alpha toxins, but no activity was observed from coffee. Their study shows that coffee should be taken as a treatment of some bacterial infection since they do possess side effects. This will also go a long way by preventing the high level of resistance developed by synthetic drugs used in the treatment of various bacterial infections.

Nonthakaew et al. (2015) wrote on the antimicrobial efficacy of coffee and tea. They stated that concentration of caffeine-containing 62.5 to gt; 2000 $\mu g{\cdot}ml^{-1}$ could prevent the growth of pathogenic bacteria, while 5000 $\mu g{\cdot}ml^{-1}$ will inhibit the growth of mold. Their study showed that the consumption of coffee and tea possesses caffeine as the major constituent, and they could be used as food additives in various food products.

Arora et al. (2009) tested the antimicrobial activity of coffee and tea against some pathogenic microorganisms in comparison with some commonly employed antibiotics.

The result obtained showed that the extract obtained from coffee and tea exhibited some degree of antimicrobial effect against the tested pathogen even in the presence of sugar and milk. Zakir et al. (2015) assessed the numerous types of tea available in Pakistan against some human pathogenic bacteria like *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeuroginosa* as well as plant pathogenic bacteria like *Agrobacterium tumefaciens and Erwinia carotovora and Candida albicans* using disc diffusion susceptibility techniques. Their result shows that *P. aeruginosa showed the highest level of sensitivity when exposed to* three different tea varieties. Moreover, it was discovered that black tea extract shows less antimicrobial activity in comparison with the lemongrass and green tea, while all the three extracts utilized during their study demonstrated enhanced antimicrobial activity against all the tested plant pathogenic bacteria.

Pruthviraj et al. (2011) evaluated the antimicrobial effect of the pure solvent and soluble solvent of methanol, acetonitrile, ethanol, methanol, water extract of leaf buds, and leaves from coffee and green tea. The active compound extracted using all the plant materials was caffeine (3,7-dihydro-1, 3,7-trimethyl-1H-purine-2,6-dione). This was extracted using thin-layer chromatography (TLC) plates using standard caffeine as a positive control. The active compound was tested against the following bacterial isolates including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, and *Proteus mirabilis*. It was observed that concentration of 2 mg/ml exhibits some level of antimicrobial activity against all tested bacteria, except for *P. mirabilis*. Also, it was observed the maximum effect of the active compound was observed against *P. aeruginosa* with the aids of modified agar diffusion techniques.

The minimal inhibitory concentration was performed with the aids of a broth microdilution technique in 96 multi-well microtiter plates. Also, the minimal inhibitory concentration obtained when the active compound from coffee was tested ranges from 65.5 to 250.0 μg/ml, while 65.5 to 500.0 μg/ml for green tea caffeine.

Duangjaia et al. (2016) tested the effectiveness of bioactive components available inside the coffee pulp that prepared in three different ways. The following biological activities were tested which include polyphenol contents, antibacterial, nutritional values, and antioxidant activity, respectively. The active component present in the pulp of the coffee was determined by liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS). Moreover, it was discovered that the active component exhibits a potent biological activities on all the tested parameters and demonstrate strong antibacterial activity against the following bacterial isolates which were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermidis*, respectively.

Patay et al. (2016) wrote a comprehensive review on the pharmacological significant of three most significant coffee species which includes *Coffea liberica Hiern*, *Coffea arabica L.*, and *Coffea robusta L. Linden*, while emphasis was laid on the *Coffea benghalensis Roxb. Ex Schult.* a typical example of a wild type of coffee. The authors laid emphasis on the medical studies, phytochemical, and ethnomedicinal, respectively. Moreover, the typical emphasis was laid on the significant of Bengal coffee which has been acclaimed to possess antimicrobial activity against *S. aureus*, *Proteus vulgaris*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*, and *Klebsiella pneumonia*. The enhanced antimicrobial properties might be linked to the presence of chlorogenic acid and Maillard reaction which are liberated as a result of roasting of the seed of *C. canephora*. *C. canephora* has been reported to possess antimicrobial activity against *Streptococcus mutans* which has been acclaimed to cause tooth decay in humans.

29.3 Antioxidant Activity

1,3,7-trimethylxanthine commonly referred to as caffeine is a purine alkaloid found in numerous daily beverages with nutraceutical benefit. Devasagayam et al. (1996) studied the inhibition effect of caffeine as an antioxidant on lipid peroxidation induced by reactive oxygen species. Their finding reveals that at millimolar concentration, caffeine is capable of inhibiting the effect of lipid peroxidation initiating radicals such as hydroxyl radical (^{-}OH), peroxyl radical (ROO^{-}), and singlet oxygen ($^{1}O_2$) capable of causing in vivo membrane damage. The investigator, therefore, suggests that caffeine activity in scavenging reactive oxygen species is higher than ascorbic acid but similar to glutathione (Devasagayam et al. 1996).

Meanwhile, León-Carmona and Annia Galano (2011) reported that caffeine scavenges these radicals through radical adduct formation mechanism of action. In an article published by Messina et al. (2015), it was reported that coffee has a varying degrees of caffeine concentration which is dependent on the type as well as the method of preparation. Dudley et al. (2017) also reported that health-care workers should be mindful that caffeine content varies in a different product due to their sources. The health benefit of this caffeine on the biological system cannot be overemphasized. Caffeine has been of nutraceutical benefit in lowering the risk of liver cirrhosis, colon cancer, type 2 diabetes, and gallstone to people who take it on a regular basis as a functional food.

Wachamo (2017) also discussed on the benefit of coffee and decaffeinated drinks in disease prevention and management such as reduce the risk of liver, prostate, and colorectal cancer, Parkinson's disease, type 2 diabetes, dementia, and stroke. It also boosts physical performance, burns fat, protects our mind, brightens our mood, fights depression, and minimizes suicidal risk by 50% (Wachamo 2017). In all, coffee consumers have less risk of heart disease, with strongly integrated DNA due to the presence of the active constituent caffeine.

Afify et al. (2011) compared the antioxidant activity of aqueous extracts of different caffeine products. In their research, they used the cold and hot extract, and it was reported that the hot water extract showed a higher antioxidant activity using DPPH and ABTS methods and reducing power method at 50 and 100 μg/ml.

Demirtas et al. (2012) studied the effect of caffeine on the oxidant-antioxidant mechanism in rat liver, and it was observed that administration of caffeine at 30 and 100 mg/kg doses for 14 days significantly increases the activity of the enzymatic antioxidant (SOD, catalase, GPx, and GST) while decreasing malondialdehyde concentration. In an attempt to determine the antioxidant property of coffee components, Liang and Kitts (2014) reported that brewed coffee contains a lot of active components inclusive of caffeine with an antioxidant characteristic capable of scavenging free radicals, donating hydrogen and electrons, acting as metal ion prooxidant chelators, and providing reducing power activity. This can be viewed in the ability of caffeine to trigger tissues and antioxidants like SOD, GSH, and catalase during gene expression with the ability to protect against inducing oxidative stress (Liang and Kitts 2014).

Da Silva et al. (2017) reported on the mechanism and biological effects of caffeine on substrate metabolism homeostasis. The investigators revealed that the level of serum insulin and glycemia was elevated with the intake of caffeine suggesting that it has the ability to alter the blood glucose level in diabetic men. They also report that long-term intake of caffeine appears to be effective in controlling glucose and adiponectin level and thus beneficial in diabetes mellitus individual (Da Silva et al. 2017). In an experimental study to determine the in vitro effect of caffeine, Gulcln (2008) observed that caffeine demonstrated an effective prooxidant potential in linoleic acid emulsion assay. Therefore, it was recommended that caffeine should be restricted to only lipid-containing food to extend the shelf life and nutritional quality of food and pharmaceutical products.

Cakir et al. (2017) investigated the protective effect of low-dose caffeine on psychological stress rats and cognitive function. It was observed that pretreatment and chronic administration of caffeine significantly decreased the elevated myeloperoxidase activities, while chronic administration of caffeine through inhibition reaction decreased the increased noticed in malondialdehyde, lucigenin, and nitric oxide levels caused by acute stress, but the reverse was observed for malondialdehyde and nitric oxide with acute caffeine (Cakir et al. 2017). Moreover, the acute administration of caffeine also decreased SOD activity and improve glutathione and luminol level, while the histological damaged caused by exposure to stress was ameliorated with chronic caffeine (Cakir et al. 2017). Jayakeerthana (2016) found that diets that are supplemented with the combine green tea constituents like caffeine and catechin reduced weight gain through the burning off some calories; this process is termed thermogenesis.

29.4 Neuroprotective Effect of Caffeine

Chen et al. (2001) investigated on the neuroprotective effect of caffeine on experimental models that were induced with Parkinson's disease with the understanding that caffeine is a universal psychrotrophic dietary product with a high rate of consumption, which is associated with decreased distress of developing Parkinson's disease. Having this in mind, the authors' investigation was to probe how caffeine and A_{2A} adenosine receptor could provide a neuroprotective effect on experimental models which were induced with Parkinson's disease. Deduced from their experiment, the authors observed that caffeine exhibited a neuroprotective effect on the pathophysiological feedback of dopaminergic nigrostriatal neurons of the experimental animals used. With a look at the neurotoxin 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine (MPTP) induced on their experimental animals, the authors validated that the animals had biochemical and anatomical lesions associated with a dopaminergic nigrostriatal system which is a representation of diagnostic features of Parkinson's disease. Following the introduction of MPTP, the authors observed a reduction in striatal dopamine levels which after the administration of 10 mg/kg dose of caffeine administered 10 min before MPTP dose had a significant effect in reducing a decline in dopamine levels, thus preserving the functional and anatomical biomarkers of nigrostriatal neurons. According to the authors, the ability of caffeine to exert a neuroprotective action against MPTP-induced PD shows that caffeine consumption is connected with neural basis on how to control the spread of neurodegenerative disease including the threat associated with being knockdown by PD. The author's investigation revealed that $A_{2A}R$ is a credible receptor target revealing the neuroprotective significance of caffeine on the toxicity caused by MTPT which is an indicator that $A_{2A}R$ has curative potential in ameliorating PD. From the author's observation, it could be deduced that specific A_{2A} combatant possesses more ability than a nonspecific combatant such as caffeine; this results from the ability of A_1R barricade to aggravate dopaminergic toxicity which could impair the helpfulness of A_{2A} blockade. Deduced from their study, it could be drawn that caffeine has a neuroprotective in ameliorating the diagnostic features associated with Parkinson's disease.

Ritchie et al. (2007) investigated the neuroprotective effect of caffeine which has a significant effect in increasing vigilance, attention, the elevation of one's mood, and arousal among others. Deduced from their investigation, the authors observed that the consumption of caffeine is associated with reducing the rate of neurodegeneration where they observed, experimenting their ideas on a small clinical sample which shows that regular consumption of caffeine is associated with reduced risk of Alzheimer's disease (AD). Their further clinical trial on a large subject recorded that regular intake of caffeine in aged individuals revealed that the individuals had high ability of cognitive performance which was more in women. During their investigation, a total of 100 elderly subjects were used for the clinical trial period which is linked to the fact that neurodegenerative diseases are common among the aged. The author's aim was to observe the effect of caffeine use on cognitive function, and they observed that intake of caffeine is remarkably linked with abroad compass of variables which are linked with cognitive decline. The author's experiment also proved that consumption of caffeine in women had a remarkable cognitive potential after accustoming themselves to several multiple factors presenting cognitive decline while making use of two models that are disparate: a logistic regression model for incidence of cognitive disability using categorical cognitive variables and a random effect model for mean differences over a period of time using continuous cognitive variables. The authors observed that neuroprotective effect of caffeine was significant in female gender only and was remarkable in verbal skill area; this could result from the pharmacodynamics metabolism of caffeine which was more in the females than in the males making the females have a more responsive therapeutic effect to caffeine than in the males. The authors also attribute their finding to the fact that elderly females are prone to cholinergic properties of caffeine than their male counterpart. The authors discovered that there was no synergy amid consumption of caffeine and anticholinergic use, but with steroid levels, they discovered that previous research carried out in postmenopausal women, consumption of caffeine, and estrone or sex hormone-binding globulin levels has a positive correlation. Their study did not reveal any mutual outcome between reduction in cognitive ability and the use of steroids; however, they noticed that females who have the habit of consuming more than three units of caffeine per day were with a high result of neuroprotection than females who received two to three units of caffeine.

Furthermore, their investigation revealed a no remarkable neuroprotective effect of caffeine on females which received less than two units of caffeine a day which shows that caffeine has a dose-dependent effect as the higher the dose, the greater the neuroprotective effect. The limitation the authors had was their inability to measure the neuroprotective effect of tea over caffeine as the females used had a history of high tea consumption than caffeine. However, deduced from their study, it could be revealed that the intake of caffeine is linked with verbal recovery than visuospatial task explaining that verbal resolving task has the ability to assist the subjects to recollect nonverbal stimuli. However, it could be deduced from their study which had reveal that females who consume caffeine at high volume had a remarkably reduced decline verbal cognitive ability and visuospatial memory over females who consume caffeine at a low rate or those who do not consume caffeine at all. During their study, the author's investigated the effect of caffeine intake on their subject of over 80 years, and they could deduce that consumption of caffeine at high volume has the ability to ameliorate age-related cognitive decline. In the female folks, the author's observed that caffeine could lessen the drop in verbal cognitive ability in women but has no ability in ameliorating episodes of dementia. The author's observed that caffeine had no protective potential during the onset of dementia, which was accrued to a short time frame of caffeine intake. Deduced from their study, it could be observed that caffeine is associated with neuroprotective effect, and for efficiency, it should be taken on a regular basis; however, the beneficial effect of caffeine is evident in females than in males.

Ross and Petrovitch (2001) investigated on the neuroprotective effect of nicotine against Parkinson's disease (PD) a neurodegenerative disease which is progressive in nature showing features of bradykinesia, rigidity with cogwheeling, rest tremor, and posture. In other to ameliorate the effect of PD, the authors investigated the curative effect of nicotine which after administration the authors observed that nicotine had the ability to reduce dopaminergic cell loss. While accessing their experimental models having partial unilateral mesodiencephalic transection, the authors observed that immersion of nicotine through subcutaneous injection osmotic pumps for a long period of time has the potential effect in averting lesion-induced decrease in the number of nigral dopamine neurons without any effect on the disparate mass of either neurons or nonneuronal cells. The authors experimented on how presentation to tobacco smoke prior to lesioning of dopaminergic neurons with MTPT could decrease. According to the authors, their findings and other study were not in consonance with each other, and it was deduced that the differences among each of these studies could have resulted from the different dose of nicotine used which varies from one research to the other. Using subcutaneous mode of administering nicotine which was done before and after lesion of nigra using 6-hydroxydopamine, the authors observed that the administration of 6-hydroxydopamine had the ability to change striatal dopamine loss in comparison with other studies; they observed that nicotine administered over a long period of time in ameliorating 6-hydroxydopamine treatment administered to rats was dose dependent as a low dose of nicotine was effective in treating neuronal degeneration than high dose. While trying to understand the mechanism behind the neuroprotective effect of nicotine on nigral dopaminergic neurons, the author's investigation reveals that pretreatment with nicotine arrested glutamate-induced neurotoxicity. The authors observed that free radical which possesses toxic effect is responsible for neuronal injury and death which is caused by an iron-mediated catalyst such as hydrogen peroxide found in Fenton reaction. These effects are supposed to be annulled by antioxidants; however, in the presence of oxidative stress, the authors validated that these free radicals are produced in excess of the detoxifying ability of the natural antioxidants found in the organism; deduced from this, nicotine is an antioxidant which has the potential of inhibiting Fenton reaction in an in vivo test. The authors further investigated the effect of smoking on MAO levels of the brain, and it was deduced that smoking had the ability of decreasing free radical production through decrease in MAO levels of the brain. While using humans to experiment on this, they observed that in the brain of the smokers subject using positron emission tomography, a result of 40% reduction in the MAO B levels was seen than in nonsmokers and individuals who have quit smoking; understanding the mechanism responsible for cigarette was unknown. They further evaluated that nicotine is responsible for causing an elevation in mRNA levels for fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor; these factors cause a significant increase in neurotrophins in striatum and ventral midbrain and quicken dopaminergic neuron existence in vivo. Experimenting on mice induced with Parkinson's disease and treated with MPTP, the authors observed that intracerebral treatment with FGF-2 has the ability to instigate the retrieval of dopaminergic function. The author's search revealed that autopsy cases reveal that human who are affected with PD have a significant decrease in FGF-2 levels in substantia nigra of the brain; however, they observed that nicotine has the ability to elevate the secretion of neurotrophins which was caused by the activation of nAChR; this could result from the fact that neurotrophins can be blocked by nicotinic receptor antagonist. Aside from this mechanism, the authors observed a second mechanism showing the neuroprotective ability of nicotine which could be deduced from their study that the neuroprotective mechanism of nicotine was related by nAChR during the upregulation of cerebral blood flow; comparing this finding with others, the authors observed that an author's idea prove that in experimental animals, cholinergic neurons found at the basal forebrain have a great ability in impelling cerebral blood flow, while another study revealed that intravenous administration of nicotine to experimental animals remarkably elevated the cortical cerebral blood flow without any effect from blood pressure which was obstructed by nicotinic receptor antagonist.

Moreover, the authors observed that with the inclusion to the substantia nigra, nicotine had the potential to induce elevation of cerebral glucose usage in large areas of the brain which was observed using ultrasonic Doppler in humans that cigarette smoking has the potency of causing raised levels of cerebral blood flow; however, they also observed that administration of nicotine to humans who smoke tend to have a remarkable raised levels of regional cerebral blood flow to some regions such as cortical and subcortical regions; to observe this, the authors made use of positron emission tomography. The author's research into previous work on caffeine was able to deduce that coffee intake was linked with neuroprotective effect as a large number of subjects who are not consumers of coffee had PD than the controls who are regular consumers of coffee. While the authors verified the component of coffee, they deduce that the following active ingredients were found in coffee; caffeine, niacin, milk, and sugar; they validated that of these components, caffeine was the major component associated with ameliorating Parkinson's disease, while the others were not. The authors also validated that coffee consumption is linked with a potential effect of reducing the susceptibility of developing PD in the near future, and the main component responsible for this action was caffeine. Deduced from this study, caffeine is associated with the prevention of neurodegenerative disease when consumed before the onset of the disease which is a proof that caffeine has a neuroprotective ability.

Dall'lgna et al. (2003) investigated the neuroprotective effect of caffeine and adenosine on neurotoxicity that was caused by β-amyloid. The authors validated that adenosine a neuromodulator is found in the nervous system having the potential via activation of inhibitory A1 receptor to avoid neuronal restlessness. According to the authors, this function of adenosine gave several scientists the urge to make use of A1 receptor ligands as a probable neuroprotective negotiator. They confirm the presence of adenosine has an implicit behavior to activate A2A receptor, and at a high proportion, A2A is commonly found in the basal ganglia with low density in other parts of the brain which is linked with dopamine D2 receptors in the basal ganglia to enhance the improvement of A2A receptor antagonist acting as an anti-parkinsonian drug which has both the effect of specific amelioration and the ability to restrain the progression of neurodegeneration in animals induced with Parkinson's disease. The author's investigation proved that several studies which made use of caffeine for the purpose of ameliorating Alzheimer's disease led to their study which meddled both caffeine and adenosine to investigate how they can prevent neurodegeneration observation from their study revealed that caffeine is endowed with great potential to ameliorate neurotoxicity induced by β-amyloid in cerebellar neurons of experimental animals used and the neuroprotective ability of the caffeine was based on the restrain on adenosine A2A rather than adenosine A1 receptors. According to the authors, it is believed from previous studies that the restrain of the A2A receptor is a generally acceptable way by scientist showing the neuroprotective mechanism of the A2A receptor. The authors believed that neuroprotective effect observed in the neurons is extremely explicative owing from a repetitive mode of action as the act of neuroprotection aids the blockade of adenosine A2A receptor which has a direct effect of preventing neuronal death independent of some elements such as astrocytes, microglia, or vascular. The deduction from their study showed that adenosine A2A receptor blockade emulates the neuroprotective potential of caffeine in ameliorating neurotoxicity caused by β-amyloid which indicate firmly that the blockade of adenosine A2A receptors is the target for the observed neuroprotective effects of consumption of caffeine in neurodegenerative disease mainly Alzheimer's disease. Deduced from their study, it could be observed that caffeine, as well as adenosine, has a neuroprotective ability ameliorating the progression and onset of neurodegenerative disease.

Munoz and Fujioka (2018) investigated the neuroprotective ability of caffeine over Parkinson's disease. The authors investigated on several neuroprotective factors such as smoking, urate, and consumption of caffeine looking into the neuroprotective effect of caffeine, which is also found as constituent of tea and soda; the authors observed in men that consuming four cups of coffee per day had a dramatic effect in reducing the risk of Parkinson's disease, and in women, the author's observed no neuroprotective effect, while comparing coffee with decaffeinated coffee, the author's observed that decaffeinated coffee had no neuroprotective effect. The authors investigated the neuroprotective effect of caffeine over 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which was used to induce Parkinsonism; their experiment proved that caffeine had a protective effect over MPTP. The authors also validated that in a trial study, caffeine had the ability to enhance the motor function of subjects affected with Parkinson's disease but has no effect in improving sleepiness experienced during the daytime. Owing to this, the author's further investigation proved that caffeine act as an antagonist to adenosine 2A receptor (A2A-R) which is found mainly in the striatum and explicitly at the medium spiny neurons having a coexpressing D2 dopamine receptor which is an important link in the indirect motor pathway. Through excitotoxic injury, the authors validated that the blockade of adenosine receptors has the ability to protect dopaminergic neurons found in the substantia nigra. However, the authors observed that istradefylline during a randomized trial acted as an A2A-R antagonist with the purpose of decreasing off time and improving the motor presentation of Parkinson's disease. The author's further investigation was to ascertain the effect of caffeine and metabolites which are 11 in numbers in the serum of patients with PD and healthy patients. The result of their study showed that PD patients were with significant decrease in serum levels of all metabolites; this change in the serum levels of PD patients was seen by the authors as appropriate technique in distinguishing PD patients from control while looking at the differences under the receiver operating characteristic curve that measures 0.98 signifying it as a reliable prognostic test method; furthermore, they observed that patients who have motor vagaries had lower levels of serum than in patients without motor vagaries; understanding the causes of this, the authors observed that lower levels of caffeine are associated with an extreme stage of PD which is an indicator that reduction in caffeine metabolites happens right from the onset of PD. They observed that differences observed in caffeine levels of PD patients and control were not associated with the amount of caffeinated beverages taken but could not ascertain the mechanism through which caffeine could ameliorate PD; several factors were considered and looked into such as intestinal absorption, treatment received, and metabolism rate. All these factors according to the authors have no effect on how caffeine could help in the therapeutic process of PD. The author's further investigation produced the desired outcome that patients who are fast metabolizers of caffeine have a genetic or metabolic component that is different from each other, and these qualities make the patients more likely affected to the causes of PD; hence, reduced serum caffeine levels and low consumption of caffeinated beverages are indicators of exposure to PD. Deduced from their study, the authors highlighted that caffeine has the neuroprotective ability if consumed.

Postuma et al. (2017) investigated on how caffeine could be a significant therapy for Parkinson's disease which was driven by the findings of previous research that caffeine could ameliorate PD. During their investigations, the authors validated that motor improvement in patients with PD was not linked with the consumption of caffeine; however, caffeine intake was only accrued to causing alertness, neutralize elevation in dyskinesia and reduction of cognition which was observed using a cognitive test. However, the authors observed that previous study has associated the use of caffeine in improving motor function in PD patients; the result from their study reveals that caffeine has the characteristic features of tachyphylactic indicating that caffeine has temporary benefit which in a short time can be depleted which reveals from their findings that caffeine is not a good therapeutic tool for ameliorating Parkinsonism. Furthermore, the author's experiment prove that during the day, the consumption of caffeine is associated with temporary daytime alertness which they reveal was in consonance with another which demonstrated that consumption of caffeine is linked with drowsiness which led to the author's remark that the effect of caffeine is best seen during the day adverting sleepiness while stimulating alertness of the patients, but this effect tends to diminish with a long time subjection to caffeine. To this effect, the authors regarding the unharmed nature of caffeine advise patients and populace to consume caffeine in an intermittent dose; however, the consumption of caffeine can be repeated if a derived enhancement performance is noticed. Their validation further revealed that consumption of caffeine is not associated with dyskinesia which previous authors in their findings have shown that caffeine is linked with reduction of dyskinesia; however, the authors observed that exposure time is a factor that must be looked into as caffeine has the ability to prevent dyskinesia on the basis that caffeine must be used before dopamine denervation. Giving the reason for this effect, the author's proved that their findings is due to the confusing ability in estimating markers of disease improvement, and these markers have the susceptibility in affecting disease risk using patients who have had PD with a history of consuming caffeine as a neuroprotective factor; the authors validated that the occurrence of the disease may be due to either a distinct pathophysiologically effect from other forms of the disease associated with high-risk profile or the disease has a distinct anatomical structure. Deduced from their study, it could be observed that the consumption of caffeine had no protective effect or benefit against Parkinsonism in PD.

29.5 Hepatoprotective Effect of Caffeine

Costentin et al. (2011) investigated the hepatoprotective effect of caffeine owing to previous findings of several researchers that caffeine has some hepatoprotective ability. Deduced from their investigation, it was observed that ingestion of caffeine has a reciprocal link with the severity of necroinflammation lesions when patients are faced with cases of untreated severe hepatitis C, but activity grade □A2 in patients was reciprocal to the amount of caffeine that was consumed per day. The author's further investigation using multivariate analysis proves that patients with regular ingestion of caffeine on a daily basis of at least three (3) cups of coffee per day were models who were independent indicators of a reduced threat of controlled to noticeable activity grade as well as age and steatosis grade or level which are all predictive factors showing that caffeine is associated with hepatoprotective ability. The authors further experimented on patients with severe hepatitis C, and their results prove that ingestion of caffeine was solely linked with low-grade activity and did not include fibrosis or ALT levels comparing their findings with Ruhl and Everhart (2005), whose research reveals that elevated levels of caffeine ingestion were linked with reduced ALT levels, while Modi et al. (2010) investigation only proves the effect of caffeine ingestion on fibrosis without taking into consideration ALT activity levels. The author's further investigation and comparison with other studies revealed that they could not find any correlation uniting histological activity grade with abusive consumption of alcohol and tobacco smoking which led to the investigation to ascertain the startling consequence of caffeine with respect to factors such as alcohol and tobacco smoking.

During their study, it was observed that the susceptible effect of alcohol consumption on necroinflammation is relatively associated with low regularity of alcohol abuse, while in tobacco consumption, the author's observation revealed that there was a relationship between heavy tobacco smoking and consumption of caffeine. Validating the relationship amid Metavir activity grade and tobacco, the authors observed that patients who were present with low consumption of caffeine had an association of Metavir activity grade $\geq A_2$ and not in patients with high caffeine consumption and heavy smokers which is an indicator that caffeine at high dose has hepatoprotective. Using a multivariate analysis, the author's observed that ingestion of coffee has a therapeutic benefit of ameliorating hepatic fibrosis as well as lowering the threats associated with the disease which was a data they use in comparing their investigation; however, during their investigation, their results revealed no link amid fibrosis stage and ingestion of caffeine; they also observed that necroinflammatory grade was directly associated with fibrosis stage and contrarily linked with ingestion of caffeine which is an indicator that caffeine reduces fibrosis progression via decrease in necroinflammatory liver impairment. During their investigation, the authors found no clue to the mechanism through which caffeine uses to exhibit its hepatoprotective effect. But in relationship with other studies, they observed that caffeine and the components of coffee which includes kahweol and cafestol are endowed with antioxidant properties which provide hepatoprotective properties to the liver. However, it could be deduced from the author's observation that caffeine is present with some hepatoprotective ability.

Salomone et al. (2017) investigated on the molecular mechanism that necessitates the hepatoprotective effect of caffeine. Deduced from their study, the authors observed using in vivo and in vitro experiment to determine the molecular effect of caffeine on liver steatogenesis in comparison with other studies, and they found out that in a genetically obese model, chlorogenic acid (CGA) had no connection with the molecular pathway, and based on this, there was no significant effect found during their experiment which was not in consonance with the study carried out by Rodriguez de Sotillo and Hadley (2002). Comparing their findings with that from a nonobese model, the authors observed that oral application of green coffee bean extract with caffeine with CGA in the experimental animals used revealed a significant reduction in the levels of hepatic triglycerides in the CGA groups. Their further investigation revealed that restriction and knockdown of the enzyme AMP-activated protein kinase (AMPK) have the ability to revoke the beneficial effect of CGA which is an indicator that CGA is efficient in raising the levels of glucose and lipid metabolism through activation of AMPK; according to the authors, their findings were in line with the research carried out by Murase et al. (2011), whose findings revealed that coffee polyphenolic fraction having CGA as a part of it with other compounds and fed to their experimental animal which also received high-fat diet (HFD) had a complete change in the metabolic turmoils which was linked to HFD-induced obesity. The authors observed that coffee polyphenols (CPP) when consumed have the ability to oppose an increase in body weight gain and accumulation of fat in the visceral and liver steatogenesis, and this is achieved via improving the body metabolic rate which cannot be associated with food consumption, locomotor activity, as well as fecal lipid excretion. Furthermore, they observed that ingestion of CPP is associated with reduction of lipogenic enzyme expression that includes fatty acid synthase (FAS), acetyl-CoA carboxylase-1 (ACC1), and stearoyl-CoA desaturase-1 (SCD 1) and the transcription factor, sterol regulatory element-binding protein 1-c (SREBP1-c), which is found in the liver and adipose tissue. However, it could be deduced from their research that CPP is endowed with the potential to restrain lipogenic enzyme expression and Hepa 1-6 cells which reveal that CPP has the ability to influence regulators of lipid homeostasis in hepatocytes. Comparing their study with several studies, they found out Vitaglione et al. (2010) study has shown that ingestion of decaffeinated coffee has the tendency to decrease both liver steatosis inflammation and fibrosis in rats fed with HFD which was in line with the research carried out by Salomone et al. (2017) as Vitaglione et al. (2010) investigation suggested that caffeine is not necessary for the anti-fibrotic outcome of coffee, and those coffee polyphenols and melanoidins which are the major components of coffee are important factors which contribute to the hepatoprotective ability of coffee. The author's investigation proves that coffee polyphenol ingestion outlines the whole effect of coffee in neutralizing oxidative stress, inflammatory cytokine thi down in imbalance, and fibrogenesis in a perfect way. To verify preventive and therapeutic effects of CGA, the authors observed that in the experimental animals fed with HFD, there was a high margin showing that administration of CGA for the preventive purpose had the ability to obstruct the progress of fatty liver without any significant effect on body weight. However, CGA administration was found to cause a change HFD-induced hepatic steatosis and insulin resistance through reducing hepatic expression of peroxisome proliferator-activated receptor gamma (Pparγ), cluster of differentiation (Cd36), fatty acid-binding protein 4 (Fabp4), and Mgat1 genes; furthermore, their research also observed that administration of CGA thins down irritation in the liver and white adipose tissue which is accompanied by the following effects: reduction of mRNA levels of macrophage marker genes which includes gene makers such as EGF-like module-containing mucin-like hormone receptor-like 1 (F4/80), cluster of differentiation (Cd68), integrin Cd11b, integrin Cd11c, tumor necrosis factor-alpha (Tnfα), monocyte chemotactic protein 1 (Mcp-1), and C-c chemokine receptor type 2(Ccr2) encoding inflammatory proteins. Deduced from their study, the authors observed that ingestion of decaffeinated coffee on experimental animals fed with HFD revealed that ingestion of coffee had the potential of reducing liver impairment through reduction of oxidative stress which is made visible via decrease in isoprostanes and 8-deoxyguanosine as well as regulating the expression of cell chaperones which includes glucose-regulated protein (GRP-78), which necessitate protein folding and protein deglycase (DJ-1), which regulates autophagy. The author's analysis from the previous study observed that caffeine is an effective stimulator of hepatic autophagic instability which includes downregulation of mammalian target of rapamycin signaling with changes in hepatic amino acids and sphingolipid levels. While accessing their experimental animals which were fed with HFD, the authors observed that ingestion of caffeine in these animals had a remarkable decrease in hepatosteatosis and consequently elevate autophagy and lipid uptake in lysosomes. The authors analyzing the relationship amid coffee consumption with fibrosis and cirrhosis evaluated that hepatitis C patients extend either with fibrosis or cirrhosis. However, the beginning of coffee consumption was inversely connected with the progression of liver disease while using a randomized control trial; the authors observed that ingestion of four cups of coffee for a period of 30 days had a significant effect by decreasing 8-hydroxydeoxyguanosine as well as collagen levels. The authors investigating on the spread and control of hepatic disease researched on the connection amid consumption of coffee and cancer of the liver which they observed using both meta-analyses of cohort and case control studies. However, findings from their research revealed that the chances of being infected with HCC were reduced by 40% for patients who ingest coffee. Deduced from their study, it could be observed that ingestion of coffee is associated with a hepatoprotective effect.

Furtado et al. (2012) with the understanding that coffee is considered as a worldwide beverage that has a high rate of consumption, the author's investigation were to demonstrate how coffee and caffeine could protect the liver against any impairment induce by harmful substances that have the potency of causing harm to the liver. Looking at coffee, the authors affirm of its beneficial effect on human health but warned that daily consumption of coffee should be at a minimal rate of at least three cups per day which is a good quantity that the body system can tolerate without causing any harmful effect to the body. Their investigation revealed that traditional and decaffeinated coffee beverages or ingestion of caffeine had a hepatoprotective ability which was induced by continuous administration of thioacetamide (TAA); this hepatoprotective effect was seen in the biochemical and morphological alterations caused by TAA. Deduced from their study, the authors observed that continuous use of TAA is associated with a negative effect of causing liver damage, fibrosis, and cirrhosis linked with hepatocyte death and activation of Kupffer cells (KCs) and hepatic stellate cells (HSCs). Their further investigation revealed that administration of TAA had the ability to inflict damages to the liver cell with special features such as high levels of ALT and GSSG, PCNA and cleaved caspase-3 labeling indexes, inflammation and fibrosis with some collagen deposition, MMP-2 activation, and increased TGF-b1 protein expression; however, their further investigation revealed that with the administration of traditional and decaffeinated coffee and caffeine, the histological and biochemical analysis revealed cell proliferation and apoptosis indexes were associated with TGF-b1 protein expression; hence, it is a good indicator that coffee and caffeine are associated with hepatoprotective ability caused by hepatotoxicant. Deduced from their study, they validated that TGF-b1 is a profibrogenic cytokine that is gotten through inflammation of KCs and HSC cells which have several effects on the turnover of ECM, hepatocellular proliferation and apoptosis, liver regeneration, inflammation, and immunosuppression; deduced from this, the authors observed that a significant reduction in TGF-b1 protein expression in the experimental models receiving traditional and decaffeinated coffee and caffeine was traced to be connected with a decrease in collagen deposition and fibrosis. The authors further observed that caffeine has the ability to the downmodulate expression of TGF-b in connective tissue growth factor (CTGF) in hepatocytes through stirring up the degradation of SMAD 2 which could act as a TGF-b effector, inhibition of SMAD3 phosphorylation, and upregulation of peroxisome proliferator-activated receptor-gamma (PPAR-c).

Moreover, it was further observed that in the experimental group that was administered with decaffeinated coffee, there was a decrease in hepatic TGF-b1 expression which is an indicator that caffeine is not the only hepatoprotective compound present in coffee beverages, but it is responsible for protecting the liver against mechanical injury as reduction in fibrosis and inflammation were observed in the experimental group that was administered with traditional coffee and caffeine. They further linked the hepatoprotective ability of caffeine to A (2A) receptor antagonist nonselective adenosine this is because adenosine acts as an active endogenous regulator of inflammation and has the ability to repair worn-out tissue. Deduced from their study, the authors observed that degradation of ECM necessitates the maintenance of homeostasis taking place in tissues but in a condition where there is no equilibrium amid MMPs, and its inhibitors could make it an active component responsible for inflicting liver damage and fibrosis development/retrogradation influenced by hepatotoxicants. Deduced from this study, traditional and decaffeinated coffee or caffeine had the ability to decrease, elevate the pattern of total, and activate MMP-2. In comparison with other studies that recorded that elevation of MMP-2 mRNA expression in the course of the inflammatory process caused by CCl4, the authors observed from their study that decrease in MMP-S activity in the liver of experimental groups which was administered with traditional coffee was associated with amelioration of inflammatory and fibrotic processes, while elevation of total and active forms of MMP-2 activity was observed in experimental groups which were administered with decaffeinated coffee or caffeine. They observed that the experimental group had temporal variation in fibrogenesis instead of inadequate fibrinolysis, based on the treatment received as these treatments have the ability to cause a decrease in TGF-b1 protein expression and collagen deposition. It was also observed that caffeine has the potential to increase essential enzymes associated with the hepatic microsomal system making reference to CYP1A2 found in humans and CYP2C found in experimental animals like mice and rats, which illustrate the ability of coffee and caffeine to act with the breakdown of drugs and hepatotoxicants to alter the balanced found between activation and excretion of substances which shows that for caffeine to be broken down by the liver, the enzyme cytochrome P450 causes the metabolism action producing dimethylxanthines mainly by CYP1A2 enzymes, and since 90% of caffeine metabolism is done by CYP1A2 enzymes, it shows that caffeine has no effect in TAA phase I biotransformation as this effect is mainly the responsibility of CYP2E2. Looking at some compounds which include coffee and caffeine, diterpene cafestol, and kahweol, the authors observed that they are responsible for the increase in a broad-spectrum which elevate phase II detoxification enzymes such as sulfotransferase, UDP-glucuronosyltransferase, and glutathione transferase and peroxidase of these enzymes; the authors observed that glutathione is the most substantial low-molecular- weight tripeptide thiol found in living cells which is present in decreased GSH, and oxidized GSSG states to this,their GSH and GSSG ratio is a determinant factor used in ascertaining the biomarker of cellular toxicity. Hence, their study shows that the therapeutic effect of traditional and decaffeinated coffee and caffeine has the ability to function as an antioxidant agent through preventing hepatic TAA caused by GSH depletion, which is a renowned marker of the oxidative stress at the cytosolic level. Owing to this, the authors observed that reduction of oxidative stress must not be accrued to coffee beverages alone as it could be linked to its antioxidant properties intervened by Nrf2-ARE pathway and incitement of phase II enzymes related to detoxification and antioxidant defenses. The authors observed that GST-P expression is an essential marker for hepatic preneoplastic and neoplastic lesions experienced mainly in male adult Wistar rats. However, TAA treatment in a short and medium time regime has the ability to influence the development of PNL in the liver cells of the male rats that is not effected which the authors observed that a remarkable decrease in the numerical value of GST-P positive PNL in the experimental group administered with traditional coffee drink prescribe a hepatoprotective effect of coffee against liver carcinogenesis. Deduced from their study, it could be observed that traditional and decaffeinated coffee with 0.1% of the caffeine has hepatoprotective effect by protecting the liver cells from TAA which has the ability to cause liver impairment. However, it could be deduced from the study that experimental groups which receive traditional coffee and 0.1% of caffeine had a more hepatoprotective effect which is an indication that caffeine has as an active protective effect on the liver cells.

29.6 Conclusion and Future Direction

This chapter has highlighted various health and nutritional benefits of caffeine and caffeine products. Moreover, several insights on the practical application of caffeine as pharmacologically active substance and their wide application as antimicrobial, antioxidant, neuroprotective, and cardioprotective properties. Moreover, there is a need to validate the mode of action through which caffeine demonstrate its biological activities. There is also a need to standardize, optimize, and quantify the amount of active component present in daily beverages and nutraceutical foods containing caffeine as the active components. This will go a long way to alleviate all the highlighted negative hazards that have been highlighted by various scientists whenever an overdose of caffeine consumed by different individuals.

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Nutraceutical Potential of Major Edible Oilseeds of India

30

Ritika Malaviya and Neelam Yadav

Abstract

Oilseeds are one of the important crops of Indian economy. Major edible oilseed crops of India are soybean, groundnut, mustard, sesame and sunflower. Oilseeds are known to be a concentrated source of energy and nutrition. The incorporation of oilseeds in food provides distinct flavour and aroma besides making them nutritious. Nutraceuticals are those substances of food that provide physiological benefits beyond basic nutrition. Oilseeds are considered to be a storehouse of such nutraceuticals and play a potent role in the prevention and management of lifestyle- and immune-related diseases. Therefore, besides providing edible oil, they also provide polyphenols, flavonoids, isoflavones, lignans, phytosterols, amino acids and peptides which have biological activity in living beings and can direct health towards wellness. Therefore, the present work is intended to deal with discussion of such bioactive components present in major oilseed crop of India.

Keywords

Isoflavones · Lignans · Nutraceuticals · Polyphenols · Oilseeds

30.1 Introduction

Oilseeds are among the important crops of Indian agricultural economy apart from cereals. India produces various kinds of oilseeds, occupying 12–15% of oilseed area of the world and which have an important role in the agronomy of the country. India's climate is ideal for growing all the major and minor oilseed crops. Nine types of oilseed crops are grown in the country, out of which seven are major edible

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oilseed crops (soybean, groundnut, rapeseed/mustard, sunflower, safflower, sesame, niger) and rest two produce non-edible oil (castor, linseed/flaxseed) (Jha et al. 2012).

Oilseeds are one of the best natural sources of energy and nutrition. They are basically produced for extracting cooking oil which makes food more palatable. Some of the oilseeds, like sesame, mustard and groundnut, can also be incorporated in various Indian cuisines for their distinct flavour and aroma. This distinction of flavour and aroma of oilseeds and their respective oil owes to presence of different secondary metabolites present in them.

Nutraceuticals are those bioactive substances or secondary metabolites in plant and its seeds which are responsible for providing therapeutic benefits, besides nutritional value, and they also provide specific characteristic to seeds. The term nutraceuticals was devised by Stephen DeFelice in 1989, who was the founder and chairman of an American organization, 'Foundation for Innovation in Medicine'. It comprises two words 'nutrient' and 'pharmaceutical' which clearly explains nutrients related to medicinal benefits. It is a wide-ranging term and includes all nutrients, herbal extracts, concentrates, dietary supplement and several bioactive components which are being identified to possess various health benefits. Nutraceuticals have noteworthy role in human nutrition which in recent times has become one of the important areas of scientific exploration (Ganapathy and Bhunia 2016). Oilseeds, therefore, not only provide energy-rich nutrition but also have nutraceutical properties and functional role.

30.2 Soybean

Soybean (*Glycine max*) is an important leguminous and oleaginous seed. The soybean plant has been probably originated in China during the Shang dynasty. It is widely cultivated as commercial crop in over 35 countries of the world. The major producers of soybean are the United States, Brazil, Argentina and India.

Soybean is a nutritious seed and possesses high concentration of oil (18–25%) and protein (38–50%). The soy protein contains acceptable amounts of essential amino acids. Soybean protein is almost equivalent to animal protein quality but with lesser saturated fats. Soy protein can serve as primary protein source by people having lactose intolerance and can also be given in severe gastroenteritis of infants (Tidke et al. 2015). Initially, dairy food products were precursors for bioactive peptides. However, other food protein-rich sources were also utilized for the same purpose. Soybean, being a vegetarian protein source, has also been used to isolate and characterize its bioactive peptides. The major storage protein components of soybean are β-conglycinin and glycinin and account for 65–80% of total seed protein. Many enzymes are also found in it like lipoxygenase, chalcone synthase, catalase and urease. Soy proteins are classified on unit basis of rate of sedimentation during centrifugation, expressed as Svedberg (S) unit. They are 2S, 7S, 11S and 15S. From these, 11S and 15S are pure protein made of polymers of glycinin, and the other two are made of several proteins. Molecular weight (MW) of β-conglycinin is about 180 kDa, and it is composed of three subunits α (63 kDa), ά (67 kDa) and β

(48 kDa), whereas glycinin is composed of five major subunits (G1, G2, G3, G4 and G5), and its MW ranges between 320 and 375 kDa. Each subunit comprises two polypeptide chains. One of them is acidic (40 kDa) and other basic (about 20 kDa) joined together by intra chain disulphide bond. Bowman-Birk inhibitor (BBI), one of the trypsin inhibitors, is also found in 2S soy protein component. BBI is known to suppress carcinogenesis in animals and is also effective against human prostate cancer cells (Wang and Mejia 2005).

Soybean oil is characterized by the presence of a large amount of polyunsaturated fatty acids (PUFA) and also essential fatty acids belonging to ω-6 and ω-3 family. These fatty acids are important for the regulation of a number of metabolic pathways and serve essential physiological function. Soy oil also contains phospholipids (1–3%), phosphatidylcholine (35%), phosphatidylethanolamine (25%), phosphatidylinositol (15%) and phosphatidic acid (5–10%) (Tidke et al. 2015). The unsaponifiable matter of plant-derived oil is composed of sterols. Such group of steroid alcohols, having structural similarity with cholesterol that naturally occurs in plants, are known as phytosterols. They are generally isolated during vegetable oil production such as soybean oil. Soybean oil deodorizer distillate (SODD) is a by-product of soybean oil processing industry. SODD is rich in phytosterols and is a source of commercial phytosterol manufacture. β-Sitosterol, stigmasterol, campesterol and brassicasterol are the main phytosterols of soybean. Phytosterols are known to lower intestinal absorption of cholesterol, thereby reducing low density lipoprotein (LDL or bad cholesterol) and also risk of atherosclerosis. Phytosterols from soybean can be utilized to convert them to therapeutic steroid hormones by microbial transformation (Wang et al. 2011).

Soybean is a better source of B vitamins than most cereals. However, it lacks vitamins like B_{12} and ascorbic acid. It is one of the excellent sources of tocopherols, which also serve as a natural antioxidant, and also folic acid (2500 μg/kg on dry matter basis) which is an important micronutrient required for physiological nucleic acid synthesis and regulation of gene expression, cell division, neurotransmitter synthesis and amino acid metabolism. Minerals like calcium, phosphorus, potassium, magnesium and iron are also found in sufficient amounts in soybean.

Secondary metabolites of soybean include isoflavone (0.1–0.4%), phytosterols (1–1.5 mg/g), saponins (0.1–0.3%), phytic acids, etc. Daidzein and genistein are two important isoflavones of soybean and play an important role as phytoestrogens which can mimic natural endogenous oestrogen. Certain clinical studies on isoflavones have reported considerable reduction in risk of breast cancer. Isoflavones are also known to reduce post-menopausal symptoms of women and can also regulate lipid profile by their antioxidant effect. They help in atherosclerosis by blocking the activation of inflammatory cells which adhere to vascular endothelium causing blockage in arteries. They also help to prevent osteoporosis and improve bone health. Soy protein and folic acid have synergistic effect for conserving calcium in body and preventing bone loss (Tidke et al. 2015).

30.3 Groundnut

Groundnut, also known as peanut, earthnut or monkey nut, is one the most important oilseed crops and is grown on 26.4 million ha over the 100 countries of the world. The major production areas are Asia and Africa contributing 56% and 40% of global area with total global production around 68% and 25%, respectively (Ntare et al. 2008). Domestication of groundnut has probably first took in the valleys of the Parana and Paraguay River in the Gran Chaco area of South America (Hammons and Smartt 1994). India ranks second in groundnut production. The highest groundnut-producing states are Gujarat (2.5 million tons), Tamil Nadu (1 million tons) and Andhra Pradesh (1 million tons). *Arachis hypogaea* is the most cultivated species of groundnut. Other two subspecies are *Arachis fastigiata* and *Arachis vulgaris* (Ntare et al. 2008). Groundnut is also known as 'key of oilseeds' and is one of the most important food and cash crops of India. It is also referred as 'wondernut' and 'poor man cashewnut' (Misra 2017). In India, groundnut is generally utilized for oil extraction followed by industrial production of groundnut butter, confectionery, roasted groundnuts, snack products, savoury, groundnut flour, soups and desserts. Other unpopular groundnut products are groundnut milk, fermented groundnut product, cheese analogues and groundnut beverages (Arya et al. 2016).

Nutritional profile of groundnut has shown that fat, protein and fibre are the major nutritional components of it. Groundnut fat is basically composed of 50% monounsaturated fats (MUFA), 33% paraformaldehyde and 14% saturated fatty acids. The high level of MUFA helps to lower total cholesterol and LDL by 11% and 14%, respectively. The fatty acid combination of groundnut oil also helps to maintain high density lipoprotein (HDL) with reduction in excess triglycerides (Arya et al. 2016). Protein content of groundnut ranges within 15.4–30.2% as a result of various genotypes and environmental conditions. Groundnut flour obtained after defatting is most commonly used for fortification purpose and protein content nearly around 50%. Groundnut protein also possesses good physiochemical properties like emulsifying activity and stability, foaming capacity, water retention and solubility capacity. Therefore, groundnut or groundnut protein has scope of its utilization in protein-rich formulated products. Protein isolates and concentrates are also prepared from it and contain higher protein percentage depending upon the method used. Relative protein value and true digestibility of groundnut flour are better than soybean protein isolate and soy flour, respectively.

Groundnut and its products (kernel, flour, isolate, concentrate) contain all the amino acids. However, certain amino acids like threonine, methionine, tryptophan and cysteine are present in limited amounts (0.9–2.6 g/100 g) (Singh and Singh 1991). Arginine, a semi-essential amino acid, is found in highest amount in groundnuts. Although the body produces its own supply, at times, its supplementation is required like in the case of wounds or illness. In such cases, arginine helps to stimulate the thymus gland to produce T cells, thereby improving the immune system. Some recent studies have also found the potential of arginine for treatment of AIDS, cancer and other diseases which are linked to suppress the immune system. It has an important role in regulating hormones and blood sugar and the promotion of male fertility. It also assists the liver for its function of detoxification. It is also claimed to be a protective nutrient for the gastrointestinal tract. Thus, arginine is an important nutraceutical of groundnut having vast pharmacological potential.

Groundnut contains around 16.13% carbohydrate having starch and sucrose as major sugars with minor quantities of reducing sugars. It also contains a good amount of dietary fibre (8.5%). Glycemic index (GI) and glycemic load (GL) of groundnut are 14 and 1, respectively, which is relatively low and therefore helps to stabilize rising blood sugar level after meals. Owing to the low GI of groundnut, the American Diabetes Association has put groundnut as a super food for diabetes.

Groundnut supplies nearly all important B vitamins. It is one of the best sources of niacin (12.066 mg/100 g), folic acid (240 μg/100 g), thiamine (0.640 mg), riboflavin (0.135 mg/100 g), pyridoxine (0.348 mg/100 g), pantothenic acid (1.767 mg/100 g) and also vitamin E (8.55 mg/100 g). Groundnut also provides good source of important nutrients like phosphorus, magnesium, manganese, copper and iron (Arya et al. 2016).

Researches have explored many types of phenolics and flavonoids in groundnut and seed coat or skin. Almost 24 kinds of polyphenolic compounds have been identified in methanolic and chloroform extracts of groundnut skin. Some of the major identified compounds were 1,3,5-benzenetriol, cis-vaccenic acid, 9,12-octadecadienoic acid, catechol, phenol, 4-butoxy, 1,3-benzenediol, 4-propyl and oleic acid (Sivanath et al. 2017).

Groundnut also contains a special polyphenolic compound called resveratrol (stilbenes). It is a fat-soluble compound, found in *cis* and *trans* form bound to glucose molecule. This compound has been found to have protective role against cancer, heart disease, degenerative nerve disease, tumour and inflammation. This compound helps to improve blood flow by brain and reduces risk of stroke. The amazing benefits of this nutraceuticals of groundnut have prompted researches to form product having maximum amount of useful nutraceuticals.

Phytosterols like β-sitosterol, campesterol and stigmasterol are found in groundnut which contribute to a healthy heart and prevent cancer of the lung, stomach, ovarian, prostrate, colon and breast (Arya et al. 2016). Groundnut oil cake can also be used for preparation of nutraceutical formulation for sustainable drug (ketoprofen) release for veterinary drug delivery purpose (Agrawal et al. 2015).

30.4 Mustard

Mustard belongs to Cruciferae family and genus *Brassica*. This oilseed crop was originated in China and then moved to North-east India, and from there it extended to Afghanistan. India is the centre of origin of brown mustard (*Brassica juncea*). Other species of mustard popular over the world are *Brassica rapa*, *Brassica napus*, *Brassica albus* and *Brassica nigra*. Mustard crop grows well in tropical and temperate zones of the world.

Mustard seed contains 30–46% oil and is popular cooking oil in Northern and Eastern Asia. Kachi ghani and refined mustard oil are its two forms that are popular

Table 30.1 Fatty acid composition of mustard oil quantified by gas-liquid chromatography (Sarwar et al. 2014)

S. no.	Fatty acids	Commercial oil (%)	Kachi ghani oil (%)	Commercial oil after removal of erucic acid (%)
1.	Palmitic acid	1.84	1.99	1.34
2.	Oleic acid	16.66	17.04	16.55
3.	Stearic acid	3.43	3.60	3.29
4.	Arachidic acid	2.03	2.46	2.03
5.	Erucic acid	41.80	51.98	20.14

among the people. The former is mostly preferred for its characteristic colour and flavour and also used for preservation of pickles and chutney. The oil of mustard is characterized by fatty acids like erucic, oleic, linoleic, arachidic and α-linolenic acid. Their saturated fatty acids include palmitic and stearic acid (Table 30.1). From the total fatty acid content, erucic acid is predominantly found in Indian mustard cultivar. However, the erucic acid is considered antinutritional component because it is reported to cause lipidosis in children and myocardial fibrosis in monkeys. Certain steps can eliminate the higher quantities of erucic acid. Various extraction procedures like saponification, acidification and isolation of erucic acid can help to reduce its major content (Sarwar et al. 2014).

Mustard seed is also used as condiment since thousands of years by humans for various culinary purposes. Traditional mustard products include mustard flour, ground mustard and prepared mustard. Mustard flour is used for salad dressing, mayonnaise, barbeque sauces, pickles and processed meats. Ground mustard is primarily used in meat industry as an emulsifier, water binder and bulking agent which is due to presence of mucilaginous material in mustard bran. Smooth mustard paste is also made from ground mustard and mustard flour together with salt, sugar, spices or vinegar. Brown mustard flour possesses a volatile oil which causes pungency, whereas yellow mustard flour contains non-volatile compounds and is responsible for the sharp taste. Isothiocyanates are responsible for spiciness of mustard. These compounds are released from the seeds by the hydrolysis action of myrosinase on glucosinolates, when seeds are crushed and are exposed to some liquids. The isothiocyanates possess some biological activity like antimicrobial and anticancerous activity and so it is a potent nutraceutical of mustard.

Mustard seed contains 23–30% of protein, 12–18% of carbohydrate and 4% of mineral matter. Mustard bran contains 15% dietary fibre. Mustard protein contains higher lysine, methionine and cysteine (Cui 1997). Since mustard contains glucosinolates as well as myrosinase, they can be the cause of pungency in protein concentrates when prepared from it. However, enzymatic treatment followed by steeping in 4% NaCl at pH 5 can effectively reduce glucosinolates and phytate (Niazi et al. 1989).

Other bioactive components of mustard are dithiolthiones and sinapic acid isomers. The former are responsible for antimutagenic and anticarcinogenic properties, and the latter are predominant phenolics of mustard responsible for antioxidant properties (Cui 1997).

30.5 Sesame

Sesame is another important oilseed crop of India next to previously mentioned oilseed crop. It is also known by several vernacular names like til (Hindi), hu ma (Chinese), goma (Japanese), gergelim (Portuguese) and anjonjoli (Spanish) (Anilakumar et al. 2010). There are almost 16 genera and 60 species of sesame belonging to Pedaliaceae family. The cultivated species of sesame are *Sesamum indicum*, *S. indicum* L syn and *S. orientale* L, and the rest are wild species. Some of the well-known wild species of sesame that are found in the Indian Peninsula are *S. malabaricum*, *S. mulayanam*, *S. laciniatum*, *S. prostratum*, *S. alatum*, *S. radiatum*, *S. occidentale*, *S. trifoliatum* and *S. schenckii* (Bhatnagar and Krishna 2009). The oilseed plant has been originated from the dry bush savannah of tropical Africa and extended from there to India and China, where it is still extensively cultivated (Tunde-Akintunde et al. 2012). Major producing nations of sesame seed are India, Myanmar, China and Sudan who together contribute 68% of total world production. Sesame seed generally occurs as black and white varieties. However, some intermediate colour varieties of it are also available like yellow, red, brown and grey. Sesame is also referred as 'queen of oilseed crops' because of the existence of a plethora of several kinds of nutrients having immense nutraceutical properties. They are oil or fat, protein, carbohydrates, minerals, lignans and other micronutrients (Nagendra Prasad et al. 2012).

The edible oil in sesame seed approximately ranges within 41.6–62.5% depending upon various species and cultivars around the world (Asghar et al. 2014). The sesame oil is considered sacred, emollient, demulcent and moisturizing oil. It possesses antibacterial properties and therefore it is preferred oil for practicing oil pulling. This causes damage to the cell wall of microorganisms and eventually kills them. Oil possesses moderate antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus.* This practice with sesame oil reduces severity of dental caries, gingivitis, periodontitis and bad breadth (Shanbhag 2017). It is one best oil well suited for infants because it protects tender baby skin from rashes caused by the acidity of the body and it is well absorbed by the skin and tissues and enters into microcirculation of the body. It helps to control dry scalp dandruff and so it is also good oil for hair massage (Anilakumar et al. 2010).

The oil contains around 85% of unsaturated fatty acids (Asghar et al. 2014). Fatty acid profile of sesame includes oleic acid > linoleic acid > palmitic acid > stearic acid > arachidic acid > hexadecenoic acid > myristic acid (Bhatnagar and Krishna 2009). The unsaponifiable fraction of oil includes phytosterols and phytoestrogens. Sesame is supposed to contain the highest total phytosterol content (400–413 mg/100 g). These phytosterols of sesame oil are claimed to reduce the total cholesterol

Table 30.2 Lignan composition of different varieties of sesame seeds (Bhatnagar and Krishna 2009)

S. no.	Sesame seed variety	Sesamin (mg/100 g)	Sesamolin (mg/100 g)	Sesaminol (mg/100 g)	Sesamolinol (mg/100 g)	Total lignans (mg/100 g)
1.	White	177	100	342	55	674
2.	Black	104	97	252	48	501
3.	Yellow	142	99	283	48	572
4.	Whitish	126	90	366	59	641
5.	Brown	208	78	149	34	469
6.	Grey	543	272	82	–	897

from the body, improve the immune system and protect against cardiovascular diseases and certain cancers (Shanbhag 2017).

Phytoestrogens are weak oestrogens found in plant sources and comprise several classes of chemical compounds. The most important ones are isoflavones chiefly found in soybeans, and others are lignans found in sesame (Landete 2012). There are about 16 types of lignans that are isolated from sesame. Most of them are derived from the oil portion of sesame or aglycone lignan. They are sesamin, sesamolin, sesamol, sesaminol, sesamolinol, pinoresinol, matairesinol, lariciresinol and episesamin. Sesaminoltriglucosides, sesaminoldiglucosides and sesaminolmonoglucosides are glycosides obtained from the fat-free part of sesame or sesame cake. Table 30.2 presents composition of some lignans from different coloured varieties of sesame. *Sesamum alatum*, a sesame species having winged seeds, is an exception which lacks sesamin and sesamolin but contains novel furfuran lignan 2-episeralatin. Three additional lignans have also been reported in perosperm of *Sesamum indicum* viz. saminol, episesaminone-9-*O*-β-D-sophoroside and semamolactol. Sesamin plays a significant role in metabolism of lipid and glucose. It is helpful in regulating hypertension and anti-inflammatory conditions and also has antioxidant activity (Dar and Arumugam 2013). The mammalian lignans (enterodiol and enterolactone), produced as a result of bacterial action on ingested plant lignans (e.g. sesamin), are known to have estrogenic and anti-estrogenic effect, that is why these lignans are called as phytoestrogens (Landete 2012). Sesaminol is known to inhibit membrane lipid peroxidation. Sesamol possesses antioxidant activity even higher than sesamin and sesamolin. It also has anti-estrogenic or estrogenic activity in a hormone-dependent manner for breast cancer cells. These phytoestrogens are better substitute for hormone replacement therapy for women having post-menopausal symptoms (Dar and Arumugam 2013). Sesame oil is highly stable because of the presence of such lignans having antioxidant activity, and its antioxidant extracts are even beneficial for protecting other vegetable oils like soybean, sunflower and safflower oil (Suja et al. 2004).

Sesame seed has a delicate nutty taste and is consumed as whole, crushed or paste in preparing various cuisines. Generally, sesame contains protein range within 25–35% (Asghar et al. 2014). The 95% of sesame protein fraction contains 13S globulin which is simple, hydrophobic, salt soluble and very susceptible to heat denaturation. Sesame protein is a good source of sulphur containing amino acids (Anilakumar et al. 2010). Sesame protein isolate is much soluble at both acidic and alkaline pH and has higher emulsifying and foaming properties than other proteins (Khalid et al. 2003).

Sesame seed is a great source of minerals like calcium, phosphorus, magnesium, copper, iron and zinc which are important content of the human body composition and has important role in metabolic functions of the body. Thiamine, niacin and tocopherols are some important vitamins of the seed (Anilakumar et al. 2010). Tocopherols being oil soluble are important constituent of sesame oil. Various bioactive tocopherols homologues of sesame are α-tocopherol, δ-tocopherol, γ-tocopherol, tocotrienols and naphthoquinones (Suja et al. 2004). They function as an antioxidant and prevent ageing by acting as first-line defense against lipid peroxidation and also have anti-inflammatory role.

Composition of different carbohydrates of sesame includes 3.2% glucose, 2.6% fructose and 0.2% sucrose, while the remaining quantity is dietary fibres (Anilakumar et al. 2010). Raw sesame contains 19.33% of total dietary fibre on dry matter basis (Elleuch et al. 2007). The sesame fibres have antidiabetic, antitumor, antiulcer, anticarcinogenic and cardioprotective role (Nagendra Prasad et al. 2012).

Sesame is a valuable source of certain phytochemicals which are fruitful in the management of diseases related to oxidative stress and some degenerative diseases. Ethyl protocatechuate is a phytochemical from sesame seed coat and possesses antioxidant activity. It has a protective role against human LDL oxidative modification by copper ions. It also contains 39–49% of phospholipids. These phospholipids are also important nutraceuticals because they help in cognitive health and promote better learning and memory power. They also play synergist role with phenolic and strengthen their antioxidant capacity. Phenolic compounds and flavonoids are also found abundantly in sesame and its seed coat. They are well-known to provide antioxidant activity, but studies have also proven their other bioactivities like anti-inflammatory, antimicrobial, anticancer and antiallergenic. Lectins are another nutraceuticals of sesame and are useful in cancer prevention. They also serve as tool in the study of cell biology, biochemistry and immunology (Asghar et al. 2014).

30.6 Sunflower

Sunflower (*Helianthus annuus* L.) is an annual plant cultivated for seeds which possess nearly 37% oil and so is a valuable oilseed crop. Sunflower is an annual plant having beautiful yellow colour inflorescence classified as capitulum or head. The plant has probably originated in America and is now cultivated across the world (Anjum et al. 2012). Basically two types of sunflower seeds are recognized, i.e. oilseed and non-oilseed types. The former is primarily utilized for oil extraction and the latter for bakery, confectionery and avian feed (Grompone 2011).

The seed is also endowed with other nutrients like protein (18.72%), crude fibre (28.30%), minerals (3.49%) and carbohydrates (6.11%). Fatty acid profile of sunflower revealed the presence of palmitic acid (3–35.5%), palmitoleic acid (0–8.6%), stearic acid (1.4–30.3%), oleic acid (7.7–90.7) and linoleic acid (1.8–74.5) (Anjum et al. 2012). Various biotechnological breeding strategies like mutagenesis have led to formulation of genetically modified sunflower hybrid seeds. Such hybrid cultivars of sunflower produce oil having different fatty acid compositions than regular seeds. The production of high oleic sunflower oilseed (HOSO) cultivar was initiated in the United States in 1984. The developed HOSO sunflower seeds produce oil containing oleic acid which ranges between 75.0% and 90.7% and is sold at a higher price than regular sunflower seeds. The National Sunflower Association of America promoted sunflower oil having intermediate oleic acid (43.1–71.8%) content in 1995, and such seeds were marketed as 'NuSun' (Grompone 2011). Sunflower seed has also good potential to be used with wheat flour as flour and protein concentrates for value addition of different nutrient of the formulated products (Anjum et al. 2012).

Other useful components of sunflower oil are tocopherols or vitamin E. An average of 669.1 mg/kg of tocopherol is found in oil. They are made up of 92.4% α-tocopherol, 5.6% β-tocopherol and 2.0% γ-tocopherol (Anjum et al. 2012). Sunflower oil as obtained from regular, mid-oleic and HOSO contains total tocopherol ranging between 440 and 1520 mg/kg, 509 and 741 mg/kg and 450 and 1120 mg/kg, respectively. Among this, α-tocopherol is present in highest range in regular seeds accounting for approximately 91–97% of total tocopherol content (Grompone 2011).

Sunflower seeds are also bestowed with various kinds of phenolic compounds. Total phenolic content of sunflower seeds as given by various workers ranges between 2938.8 mg/100 g and 4175.9 mg/100 g (Anjum et al. 2012). Methanolic extracts of sunflower seeds contained non-esterified phenolic acids, caffeoylquinic acid, isomers of coumaroylquinic acid (3-*O*-*p*-coumaroylquinic and 4-*O*-*p*-coumaroylquinic acids), isomers of dicaffeoylquinic acids (1,3-di-*O*-caffeoyl-dimethoxycinnamoylquinic acid) and certain compounds belonging to flavonoid class. Various antioxidant assays like ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl) radical cation decolourization and reducing power on sunflower seed extracts have shown positive correlation between each other and also with their phenolic contents (Karamać et al. 2012).

The residue of sunflower oil extraction, i.e. sunflower meal, is also a valuable source of protein (40–50%) depending on extraction procedures. Besides having high protein, the meal is also rich in phenolic compounds (4200 mg/100 g) of total mass, having chlorogenic acid as principal component. The high phenolic content greatly affects protein quality of sunflower seeds because protein binds to phenolics and so decreases its digestibility. Further, polyphenol oxidase activity and conventional alkaline extraction of protein result in oxidation of polyphenol causing undesirable browning along with formation of highly reactive compounds which can form covalent bond with protein. Nevertheless, beneficial antioxidant effects of useful phenolic compounds are far more than counteracting effects on protein (Weisz et al. 2009).

Such phenolic profile and tocopherols content clearly indicate appreciable nutraceutical potential of sunflower seeds as an effective antioxidant against damage causing free radicals.

30.7 Conclusion

Oilseeds have numerous nutraceuticals and have immense potential to be beneficial for protection and treatment of various diseases of modern times caused due to oxidative stress, suppressed immune system and other degenerative diseases. The rich phytochemistry of oilseeds is mainly responsible for imparting such effects. There will always be a need of further research and development to combat future challenges of exploring more nutraceuticals in oilseed so that food would be our medicine rather than synthetically derived drugs.

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31 Elimination of Fungal Leaf *Mosaic* Disease of *Bottle Gourd* (*Lagenaria siceraria*) Using Fungo-Phage Therapy: A Possible Approach for Food Security with Plant Protection

Ved Kumar Mishra, Prashant Ankur Jain, and Raghvendra Raman Mishra

Abstract

Plant protection using fungal phage has always been a point of research and discussion to minimise the chemical intervention. The ergonomics of phage production is relatively simple, and its cost-effectiveness makes it an efficient tool to address the epidemics of plant kingdom. Prevalent environmental aspects do interfere with the phage efficacy, also being significant in the alteration of other biological control agents, such as susceptibility of the target organism. Dynamicity of phage-based plant protection process requires continuous tuning of the phage preparation to combat pathogenic bacteria effectively. The implication of lytic cycle abbreviates the process since the invading lytic phage destroys bacterial cell post virion replication and navigates for new hosts to infect. Host specificity is most significant aspect pertaining to bacteriophagic plant protection.

Keywords

Bottle gourd · Fungal phage · Plant protection · Lytic cycle · Bioinformatics techniques · Leaf curl and mosaic disease

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31.1 Introduction

Lagenaria siceraria (bottle gourd) hails from Cucurbitaceae family and has been appreciated for its curative attributes since ages. It has been reported that gourds can be used to treat mental disorders being the assembler of highest choline level and other metabolites and precursor invaluable for synthesis of neurotransmitters leading to healthy brain function (Aramburu et al. 2007). Bottle gourd requires warm and sultry condition, cultivated through the stretches of Asia especially in India and Pakistan. Summer-rainy seasons and prevalent hot and humid conditions are best suitable for bottle gourd production in northern India (Aramburu et al. 2007; Naylor et al. 2004). Its annual availability makes it a good choice health food, also known as herbal drug. It is being classified into two varieties, namely, bitter and sweet. Sweet variety is used as daily edibles for curries, while bitter variant has been utilized in medicines. Population outbursts pose a serious challenge in meeting the demands of adequate supplies of nutrient-rich sources. Orphan crops (aren't traded internationally and hence are not attended with research and development) such as bottle gourd play a vital role in addressing such issues in developing countries compatible with socio-economic conditions and agro-ecology (Stadler et al. 2009). Orphan crops' tolerance to abiotic stresses such as moisture scarcity makes it worth for food security (Vasudeva and Lal 1943). Millet, sorghum, tef and cassava are important orphan crops in African agriculture (Ejeta 2010). Dominant staple crop of sweet potato provides the diet surplus for poor households (Mishra et al. 2016).

Expanding the arable area can lead to an increase in crop yield through the utilization of ameliorated cultivable and irrigation methods, seeds, fertilizer, herbicides and fungicide. Agricultural intensification will contribute to 80% of future increment in yield and productivity in developing countries. Henceforth, researchers have emphasized procuring improved cultivars, producing higher yields tolerant to altering climatic conditions and the suboptimal soil (Food and Agricultural Organization 2002; Mantri et al. 2004).

31.2 Leaf Curl

Curling of leaves characterizes leaf curl (plant disease). The causative agent is a fungus genus *Taphrina*, particularly pertaining to genus *Begomovirus,* family Geminiviridae. Reduction in the amount of leaves and fruit production is marked by peach leaf curl reported in nectarine and peach trees. Severity of the symptoms depends upon early incidence of infection (Bhargava and Bhargava 1977). Leaf curl is distinctively noticeable, and the diseased leaves can usually be identified soon, post the proliferation from the bud, through their reddish appearance and distorted shape which increases as the leaf develops. These leaves are noticed for thick and rubbery nature as compared to normal leaves. Alteration in the colors of the leaves from green to red and purple and eventually a whitish bloom takes over each leaf, consequently affecting the fruit development from diseased blossoms. Early shedding of infected leaves leads to growth of second flush of leaves, rarely diseased transmitted through whitefly transmission and sequence identity of putative coat protein (CP) and replication initiator protein (Rep) genes (Lazarowitz 1992; Samretwanich et al. 2000; Vasudeva and Samraj 1948). Cucumber mosaic disease, causatives of viral diseases of many important plants worldwide, has been isolated from pumpkin (*Cucurbita pepo* L.) plant leaves. Diseased plants had light green mottled foliage (Jain et al. 2015).

31.3 Mosaic Disease

Mottled leaves characterizing the mosaic disease also feature light and dark yellowish green spots or streaks. It is noticed infecting cucumbers, tomatoes, potatoes and squash. Wrinkled or curled leaves, stunted growth and reduced yields mottled appearance of infected fruit are some other characterizing attributes. Mosaic disease spends the winters on perennial weeds and infested by insects such as aphids, leafhoppers, whiteflies and cucumber beetles feeding on them. Infested plant cuttings/divisions also will also transmit the disease (Jain et al. 2015).

31.4 The Phage

Phage or bacterial disease is other recognizing name for the same, infecting bacteria and single-celled prokaryotes. Phages were discovered in 1915 by Frederick W. Twort at Great Britain and in 1917 by Félix d'Hérelle at France. Phage infects bacterium adhering to it and injecting its genetic material into the cell phage following lytic (virulent) or lysogenic (temperate) pathway (Young et al. 2002). Lytic phages seize over the replication machinery of the cell to replicate phage components, eventually lysing the host cell, releasing new phage particles (Guttman and Kutter n.d.; Sridhar Rao 2006). (Fig. 31.1).

On the other hand, lysogenic phages incorporating their nucleic acid into the chromosome of the host cell lead to it consequent replication with it as a unit without destruction of host cell. Under certain conditions, lysogenic phages can be induced to follow a lytic cycle. In pseudolysogeny, a fungal phage invading host cell neither captivates cell replication machinery nor integrates into the host genome. It has been observed when a host cell encounters unfavourable growth (Campbell 1996; Ptashne 1993; Zaman et al. 1991).

31.5 Purpose of Chapter

Since the advent of the common interest group in 2002, the Ministry of Agriculture emphasized on promotion of orphan crops that resulted in an increase in number of interested farmers in orphan crop production. The investigations studied the influence of common interest group approach on orphan crop productivity among smallhold farmers. The purpose of this study was to determine the influence of

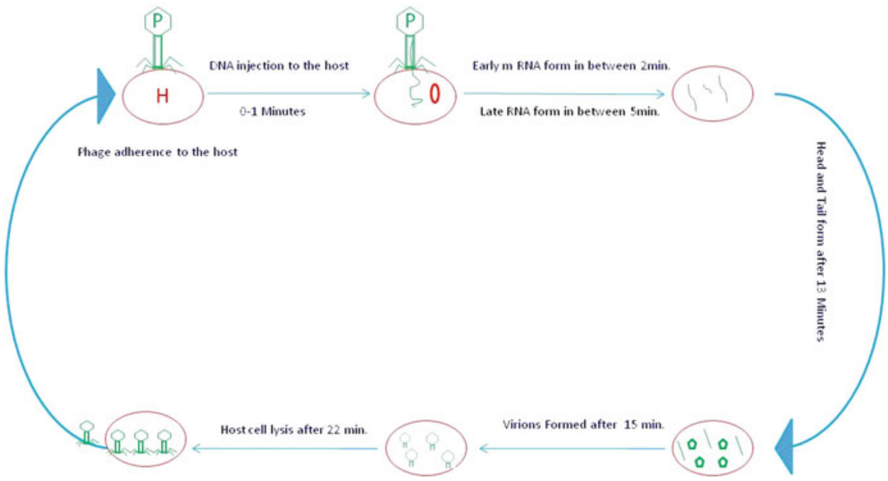


Fig. 31.1 Representation of life cycle of a bacteriophage. *P* is phage and *H* is host. (Mishra and Nath 2013)

common interest group approach on *Lagenaria siceraria* crop productivity among smallholder farmers of bank of Ganga River (adjacent area of Gangetic belt of Allahabad and Varanasi region to search out fungal phages specific against leaf curl diseases and mosaic diseases, identifying the yields, identification of the host and pathogenic bacterial interaction with disease (leaf curl and mosaic diseases). A consequent construction of its replication associated regulatory pathways in *Lagenaria siceraria* encouraging ways to develop newer antiviral therapy methodology using fungal phage specific against leaf curl diseases and mosaic diseases eventually leading to the procurement of novel products of such orphan crops.

31.6 Plan Proposal

Procurements of Materials Leaf, shoot and fruits will be collected at designated time interval and finally at the end of season of fruiting along with roots. Experimental techniques which will be using in this study are as follows.

(i) *Bioinformatics Techniques*. Bioinformatics techniques study the genomics of the plant and search out the sequences for the construction of replication associated regulatory pathways in *Lagenaria siceraria*.

Genome Re-sequencing Data Genome re-sequencing data will be done by Rubin et al. (2010) using standard procedure.

UCSC Genome Bioinformatics UCSC Genome will be done by Barnes, (2010) using standard procedure.

Gene Ontology (GO) database consulted for functions of candidate gene from ensemble.

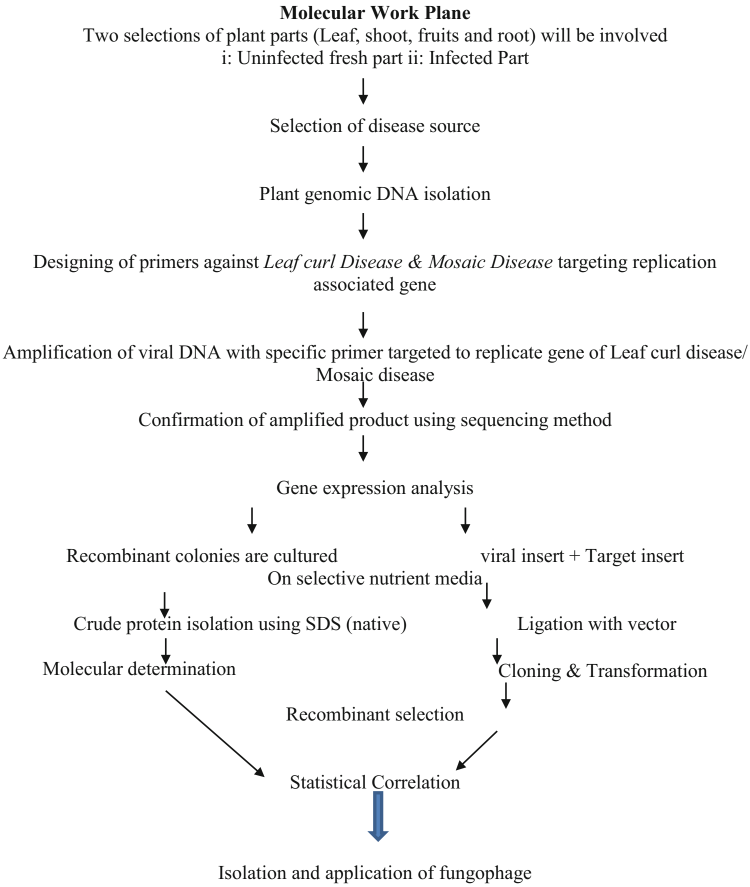
Mapping of gene will be done by **KEGG biological pathways**.

(ii) *Molecular and Proteomic Study*. *Leaf curl disease* and *mosaic disease* are pandemics of crops having replication sense ORFs and coat protein. Their replication-associated protein (Rep-protein) is encoded by its specific ORF located in its DNA strands. The Rep is the protein essential for replication and localized within nucleus and has specific endonuclease ligation activity for positive strand. Rep-protein can repress its own promoter and can stimulate the expression of the proliferating cell nuclear antigen. Keeping these facts in our mind, the viral DNA and its expression analysis will be on two different methods, viz. infected part and from purified viral particle as per given work plan.

Statistical Analysis The data obtained from the study will be analyzed by using analysis of variance technique **(ANOVA)** (Fig. 31.2).

31.7 Conclusion

We will study the topic for the social and economic impacts pertaining to common interest group. Each group of selected crops would approach extending its service in the country. Short- and longer-term future goals of biotechnology for crop improvement for the developing world are increasing continuously. The implication of aforementioned technology engineered for plant protection will lead to natural avenues, minimizing the chemical application for the same. This will serve as a model technological innovation for the farmers. The use of comparative genomics will divulge out the significant and positive influence on crop production.



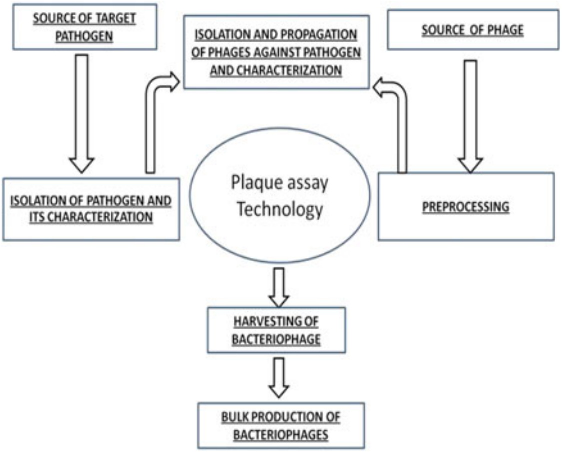


Fig. 31.2 A flowchart of isolation and propagation of bacteriophages (Kishor et al. 2016) against applied for fungal pathogen. (Nath and Mishra 2013)

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β-Sitosterol: Predominant Phytosterol of Therapeutic Potential

32

Ena Gupta

Abstract

In plant cell membranes, there is a group of naturally occurring compounds referred to as phytosterols (plant sterol and stanol esters). Phytosterols are structurally similar to cholesterol, occurring in plants and vary in absence or presence of a double bond in carbon side chain. These phytosterols does not produce undesirable side effects and are generally recognized as safe (GRAS). There are around more than 200 sterols, and allied compounds have been identified. The plants exclusively made the most predominant phytosterol that is β-sitosterol, a white waxy powder in its pure form. A deoxyxylulose and mevalonate pathway promotes its biological synthesis. It is majorly found in plant kingdom (nuts and seeds, fruits, fresh vegetables, and higher concentration in unrefined plant oils such as flaxseed, olive, canola, corn, and sesame oil). Some clinical and preclinical studies suggest that β-sitosterol provides many significant health benefits. It lowers the level of bad cholesterol (LDL) and reduces the risk of coronary artery disease, heart attack, and atherosclerosis, preventing many types of cancers along with supporting body's natural recovery process. This review article is aimed at the chemistry of β-sitosterol, biosynthetic pathways, and their metabolism along with wide-range pharmacological and therapeutic applications.

Keywords

Phytosterols · β-sitosterol · Therapeutic applications

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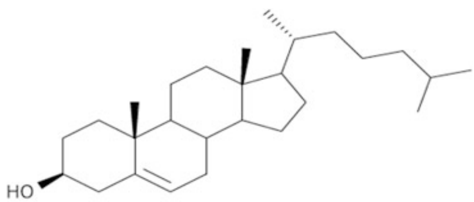
32.1 Introduction

In plants various types of chemicals are present that are nonnutritive but possess disease-preventive properties. The major chemicals which are produced by plants through primary and secondary metabolism are termed as "phytochemicals." It is a Greek word named *phyton*, which means "plant." There are more than thousands of phytochemicals, and plants produce these chemicals for growth or to protect themselves against pathogens or competitors. In 1994 the term phytochemicals was first introduced, and quickly it became a topic of interest among scientists and researchers. One of the important classes of bioorganic molecules is phytosterols (a subgroup of the steroids). Plant-derived compounds (sterols and stanols) are commonly known as phytosterols having structural similarity to cholesterol, and it is widely spread among plants, animals, and fungi group (Fig. 32.1). The third most important class of lipid are sterols which are considered as membrane reinforcers as they maintain the domain structure of cell membranes and also regulate the important biological processes (Ribeiro et al. 2007; Yin et al. 2018). The major sterols present in vertebrates are cholesterol (CHO) mainly found in animal's cell membrane. In developmental signalling it serves as secondary messenger and also affects the cell membrane's fluidity (Akhisa and Kokke 1991).

In plant species the embryonic growth is due to the presence of most important constituents of the sterol profiles such as two 24-ethyl sterols, sitosterol (SIT), campesterol, and stigmasterol (STI). In plants majorly two classes of plant sterols have been identified (Fig. 32.2): (I) campesterol, β-sitosterol, and stigmasterol are the most abundant sterols found in plant and human diet, and there is a presence of double bond in the sterol ring of these plant sterols. (II) campestanol and sitostanol are some important stanols that comprise of 10% of the total dietary phytosterols, and these plant stanols do not have a double bond in the sterol ring; These saturated sterols show unique health benefits by promoting embryonic growth of plants and involved in forming liquid-ordered (*lo*) lipid domains (lipid rafts) important for biological processes like polarized growth of root hair and pollen tube, cytoskeleton reorganization, secondary messenger in signal transduction, cellular sorting, asymmetric distribution of membrane components, and infectious diseases. This review article is aimed at the chemistry of β-sitosterol, biosynthetic pathways, and their metabolism along with wide-range pharmacological and therapeutic applications.

Fig. 32.1 Chemical structure of cholesterol



Cholesterol

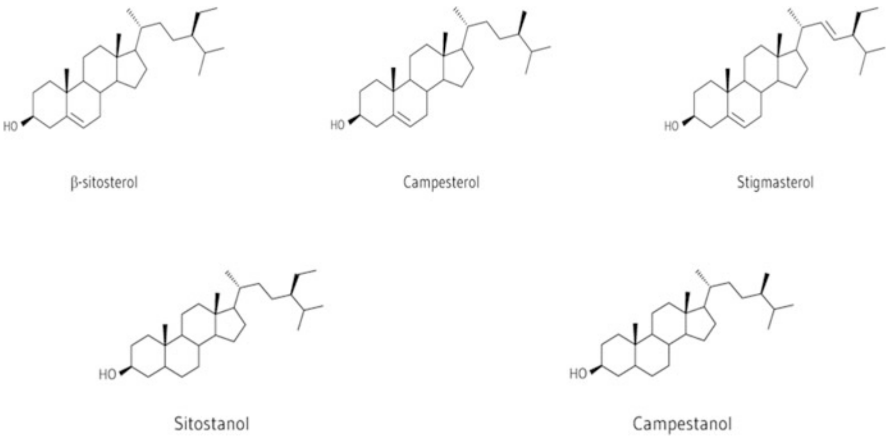


Fig. 32.2 Chemical structure of plant-derived sterols and stanols

Table 32.1 High phytosterol-rich foods (mg/100 g) (Kritchevsky 1997)

Food sources	Total phytosterol content (mg/100 g)
Oils	
Rice bran	1055
Corn	952
Wheat germ	553
Nuts	
Cashew	158
Almond	143
Pecan	108
Legumes	
Pea	135
Kidney bean	127
Broad bean	124
Vegetables	
Beet root	25
Brussels sprout	24
Cauliflower	18
Fruits	
Orange	24
Banana	16
Apple	12

These phytosterol-rich metabolites constitute health-promoting functions in natural foods and being constantly marketed for decades worldwide. Significantly high phytosterol content was found in plant oils or products made from them, whereas in fruits and vegetables, it is present in lesser extent as depicted in Table 32.1 where the sum of phytosterol content is due to presence of campesterol, stigmasterol, and beta-sitosterol. In plants phytosterols are mostly present in free forms (in plants, vegetable oils, corn oil, rice bran oil, wheat germ oil) and esterified forms (whole wheat, wheat germ oil, vegetable oils, corn oil, Promise activ® spreads (sterol esters), Benecol® spreads (stanol esters), whereas in some foods, it is in the glycosylated form that has an attached glucose molecule like bran, seeds, legumes, nuts, whole grains, wheat germ, vegetables, fruits, and unrefined plant-derived lecithin (Moreau et al. (2002)). Many clinical studies assessed the beneficial effects of number of food products enriched in plant sterols/stanols found on the market, such as spreads, margarines, yogurts, and milk (Gylling and Simonen 2015).

32.2 Structure

β-sitosterol is an optically active steroidal compound. Optical activity is reflected in its chemical name (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol. Like mammalian sterols, phytosterols possess same tetracyclic core structure, for example, cholesterol, but there is a difference in the nature of the substitution at C_{24} and absence or presence of a double bond at C_{22}. Plants contain mixture of phytosterols; the most predominant sterols include β-sitosterol ($C_{29}H_{50}O$, molecular weight 414.71 g/mol, unsaturated sterol with one double bond in sterol ring structure), campesterol (structurally similar to β-sitosterol but instead of an ethyl group, it has a methyl substituent at C_{24} position), and stigmasterol (is an unsaturated sterol, having one double bond in the side chain and one double bond in the sterol ring structure); another important saturated sterol is stigmastanol with sterol ring structure and side chain, whereas the major phytosterol present in most plant foods is β-sitosterol, and the second abundant is campesterol comprising 95% of isolated phytosterols, and there is a significant contribution of other sterols specifically in seeds and nuts (Phillips et al. 2005); examples are shown in Fig. 32.2.

32.3 Synthesis

Until now β-sitosterol has not been totally synthesized; its production is through pure stigmasterol by two ways. Firstly, β-sitosterol was produced together with fully saturated stigmastanol and different levels of stigmasterol by hydrogenation of side-chain Δ_{22-23} alkene, whereas in the second approach, this selective hydrogenation is followed by protection of Δ_{5-6} alkene to produce cyclopropylcarbinyl ether. Hydrogenation of the Δ_{22-23} double bond should be followed in this process along with solvolyzation of the cyclopropane for the production of Δ_{5-6} alkene and C3 alcohol. The second method synthesizes high purity β-sitosterol. In reality, semi-synthesis of β-sitosterol is still a challenge due to production of the methyl ether by products whose elimination is quite difficult (Hang and Dussault 2010; Khripach et al. 2005).

32.4 Biosynthesis

During membrane biogenesis biosynthesis of sterols and particular lipids occurs through 13C-labeling patterns. Research studies suggest that in the synthesis of β-sitosterol, both deoxyxylulose and mevalonate pathways are involved (De-Eknamkul and Potduang 2003). Most accurate mechanism involved for the formation of β-sitosterol comes from cycloartenol which varies according to the organism used. The beginning of cycloartenol biosynthesis is by joining of one molecule of isopentenyl diphosphate (IPP) and two molecules of dimethylallyl diphosphate (DMAPP) to produce farnesyl diphosphate (FPP). Later, tail-to-tail attachment of two molecules of FPP forms a triterpene (squalene). Finally through a cyclization, cycloartenol is produced by reaction of squalene with an intermediate 2,3-oxidosqualene.

32.5 Acceptable Daily Intake (ADI)

β-sitosterol is the most common plant sterol having minimal potential for adverse effects in the organism, and it has been considered as natural, effective, and safe nutritional supplement. A report was published by international organizations such as USFDA, EFSA, and JECFA on phytosterols as food additives or supplements without emphasizing specifically on any individual compound. British Dietetic Association (2012) recommended the daily dietary intake of phytosterols (200–400 mg/day) and plant stanols (30–50 mg/day) from natural sources (Ras et al. 2014). The Joint FAO/WHO Expert Committee on Food Additives (*JECFA*) in 2009 recommended the approximate calculated values from phytosterol mixtures, not specific information on β-sitosterol separately; the acceptable daily intake (ADI) is 40 mg/kg_BW/day; no-observed-adverse-effect level (NOAEL) is 4200 mg/kg_BW/day; margin of safety (MOS) is 210 mg/kg_BW/day; and for the systemic and cosmetic products is 8.3 mg/kg_BW/day, respectively (Schneider et al. 2009). The scientific opinions of European Food Safety Authority (EFSA) were published on phytosterols but not solely on β-sitosterol (Raport 2008).

32.6 Therapeutic Applications

β-sitosterol has been shown to possess multiple roles and plays an important part in biomedical world as it is a natural bioactive compound found in cells and membranes of all oil-producing plants, trees, grains, vegetables, fruits, and seeds. It is known to possess potential health benefits by being nontoxic, safe, and valuable nutritional supplement with diverse therapeutic applications against different communicable and noncommunicable diseases.

32.6.1 Antimicrobial Activity

Higher plants are resistant to various bacteria due to presence of antibacterial substances in their plant tissues. Antibacterial and antifungal property was shown by β-sitosterol obtained from different plants without causing any toxicity in brine shrimp lethality assay (Kiprono et al. 2000). Antitrypanosomal and mosquito larvicidal activities were shown by plant extracts or formulation containing β-sitosterol (Rahuman et al. 2008; Nweze 2011). In the bioactive compound (β-sitosterol-3-*O*-glucoside), a derivative of β-sitosterol shows wide-spectrum antimicrobial activity and was isolated from *Lannea kerstingii* Engl. and *K. Krause* (Anacardiaceae) using dry vacuum liquid chromatography. Antimicrobial activity of β-sitosterol-3-*O*-glucoside (200 µg/ml) was determined and found active against *S. aureus*, *S. typhi*, *E. coli*, *K. pneumonia*, *B. subtilis*, and *P. mirabilis* with zone of inhibition ranging from 24 mm to 34 mm. The compound was equally active against the fungi *C. tropicalis* and *C. albicans* with MIC ranging from 25 µg/ml to 50 µg/ml, while minimum bactericidal and minimum fungicidal concentration (MBC/MFC) ranged from 50 µg/ml to 200 µg/ml (Njinga et al. 2016). In another study bioassay-guided fractionation was used to isolate β-sitosterol from *Vitex agnus-castus* (Verbenaceae), commonly called (chasteberry), and was found to inhibit the growth of pathogenic bacteria *S. epidermidis*, *S. aureus*, *B.subtilis*, *E. faecalis*, and *E.coli* (Arokiyaraj et al. 2011).

Antimicrobial activity of β-sitosterol derivative (β-sitosterol-D-glucopyranoside) obtained from *Desmostachya bipinnata* (L.) Stapf was identified in combination with antibiotics; results of time kill curve shows that most of the pathogens are killed within 5–10 h with (MIC 6–50 µg/ml) working synergistically with most antibiotics, mainly with ciprofloxacin (Subramaniam et al. 2014).

32.6.2 Antioxidant Activity

Research studies suggest that β-sitosterol has been shown to possess antioxidant property as it can modulate human estrogen receptor/PI3-kinase-dependent pathway and antioxidant enzymes. It works as a potent ROS scavenger by maintaining the glutathione peroxidase (GSH) and GSH/total glutathione ratio (Vivancos and Moreno 2005). β-sitosterol isolated from *Vitex agnus-castus* has been shown to possess antioxidant property by preventing the free radicals generation at 50 and 100 µg/ml (58% and 67%) (Arokiyaraj et al. 2011). β-sitosterol is recommended as chemopreventive drug for colon carcinogenesis as it has been shown to be effective against colon carcinogenesis in rats induced by 1,2-dimethylhydrazine and elevates enzymatic and nonenzymatic antioxidant by preventing lipid peroxidation (Baskar et al. 2012). From the medicinal herb, *Solanum surattense*, β-sitosterol (BS) was isolated for studying the antioxidant potential using an experimental rat model with diabetes-induced oxidative damage. Results showed that different doses of BS (10, 15, and 20 mg/kg, p.o.) decrease the thiobarbituric acid-reactive substances (TBARS) with increase in pancreatic antioxidant levels (Gupta et al. 2011). The antioxidant activity of the methanolic extract of *Eulophia campestris* Wall was studied by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method; obtained results showed the moderate to potent antioxidant activity with the ED50 value of 1.593 μg/ml. The high antioxidant activity of the plant might be due to the presence of high alkaloids content (Rao et al. 2013).

32.6.3 Neuroprotection

Plants containing β-sitosterol show anxiolytic, antinociceptive, and tranquillizing effects in rats, although such results were not found in case of humans (Santos et al. 1995; López-Rubalcava et al. 2006). Till now no such studies have been performed related to the brain region or the pathways affected by β-sitosterol. Findings suggest that β-sitosterol has similar effect like diazepam; however, the exact mechanism of action behind this effect has not been studied yet (Aguirre-Hernandez et al. 2007). Incorporation of β-sitosterol into cell membrane can prevent lipid peroxidation and glucose oxidase-induced oxidative stress which is helpful in curing neurodegenerative disorders like Alzheimer's disease (Shi et al. 2013). Research studies suggest that β-sitosterol or its extract increases the proliferation of neural stem cell, although it is recommended to perform additional studies on its possible applications in the area of tissue engineering (Hamedi et al. 2015).

32.6.4 Angiogenic Effect

The angiogenic component (β-sitosterol) was isolated from the *Aloe vera* gel, and its effect was examined upon damaged blood vessels of the Mongolian gerbil, through a chick embryo chorioallantoic membrane assay; it was established that intraperitoneal administration of beta-sitosterol for a period of 19 days at a dosage of 500 microg/kg/day significantly enhances the formation or motion recovery of new vessel in gerbil brains damaged by ischemia/reperfusion Choi et al. (2003). Research findings suggest that β-sitosterol plays a potential role in healing chronic wound and in the formation of new blood vessels, but till now not a single experimental study was reported on the exact mechanism of wound healing. According to Moon et al. (1999), β-sitosterol in the presence of heparin stimulated the motility of human umbilical vein endothelial cells in an in vitro wound migration assay and stimulated neovascularization in the mouse Matrigel plug assay.

32.6.5 Cardioprotective

Research studies suggest that phytosterols and their derivatives (β-sitosterol) reduce the cholesterol levels in the blood and also inhibit the dietary cholesterol absorption and biliary cholesterol. The correct mechanism for this interference in cholesterol absorption is not clear, but it has been recognized that it might be due to three different possibilities such as co-crystallization in the so-called mixed micelles in the small intestine and competitive solubilization or combination with other statins and its effect at absorption site (hydrolysis by esterases and lipases). β-sitosterol have been approved and recommended by US Food and Drug Administration (FDA) for the treatment of hypercholesterolemia or hyperlipidemia and prevention of different cardiovascular diseases (Hu 2003; Retelny et al. 2008).

β-sitosterol and its hydrogenated product (sitostanol) was compared for their hypocholesterolemic activity in young male rats. Results demonstrated that sitostanol significantly lowers cholesterol more efficiently than sitosterol, but their effects were similar on liver concentration of triglyceride and cholesterol. It was concluded from the study that hydrogenation of plant sterols improves their hypocholesterolemic activity without affecting their safety (Sugano et al. 1977).

Another study shows that β-sitosterol (6 g/day) was added to a group of 30 patients (16 men, 14 women) for the treatment of hypercholesterolemia followed by lovastatin for 12 weeks; it was found that in β-sitosterol group, the LDL cholesterol decreases by 35.3–37.1%, whereas there was decrease in total cholesterol by 27.3–29.2% (Richter et al. 1996).

32.6.6 Glucoregulation

Gupta et al. (2011) studied the antioxidant and antidiabetic potential of β-sitosterol in streptozotocin-induced diabetic rats; administration of β-sitosterol protects the pancreatic tissue and enhances the pancreatic insulin and antioxidant level followed by reduction in blood glucose, nitric oxide (NO), and HbA1c levels. It ameliorates diabetic complications (arthritis) by preventing development of diabetes. However, oral administration of β-sitosterol increases glucose-induced insulin secretion and also increases fasting plasma insulin levels while decreasing fasting glycemia, and these effects of β-sitosterol are comparable to standard antidiabetic drug (glibenclamide) Ivorra et al. (1997). Phytosterol derivatives (stigmasterol and sitosterol-3-*O*-β-D-glucopyranoside) extracted from leaves of *Pseuderanthemum palatiferum* were orally administered to diabetic rats at a doses of 0.25 and 0.50 mg/kg for 21 days, it was found that fasting blood glucose (FBS) levels were decreased significantly ($p < 0.05$) with a constant increase in serum insulin, and the highest hypoglycemic effect was shown by sitosterol-3-*O*-β-D-glucopyranoside at a dose of 0.50 mg/kg (Padee et al. 2010). β-sitosterol stimulates adipogenesis in differentiating preadipocytes which increase glucose uptake in adipocytes. In contrast it induces adipocytes lipolysis which was not attenuated by insulin; it downregulates GLUT4 gene (Akt and PI3K) expression like insulin. β-sitosterol shows it's potential in weight management and diabetes due to its unique characteristics like regulating glucose uptake, lipolysis in adipocytes and adipogenesis (Chai et al. 2011).

32.6.7 Fertility

Research finding shows contradictory results for β-sitosterol on the reproductive system. Studies on rat model shows sex hormone levels such as estradiol in females and testosterone in males were increased due to intake of β-sitosterol (Ryokkynen et al. 2005). The bioactive compound β-sitosterol extracted from roots of *Barleria prionitis* was used to determine the antifertility potential in the male albino rats; dose-dependent treatment of β-sitosterol shows marked alterations in the male reproductive organs by suppressing spermatogenesis and fertility, which reflects the potential of β-sitosterol in development of cheap, nontoxic herbal male-contraceptive drug (Singh and Gupta 2016). High-dose level (5 mg/kg per day per rat subcutaneously) of β-sitosterol shows more pronounced antifertility effect than long-term low-dose treatment (0.5 mg/kg per day per rat subcutaneously) in male albino rats. Accessory sex tissues, weight of testis, and sperm concentrations were reduced after time-dependent high-dose treatment with β-sitosterol. Following withdrawal of treatment for 30 days, weights of accessory sex tissues can be restored to normal conditions (Malini and Vanithakumari 1991).

32.6.8 Carcinogenicity

β-sitosterol is an important constituent of human diet; it interacts with different cellular pathways and targets to show anticancer properties (Novotny et al. 2017). This compound shows no genotoxic effects and inhibits mutagenicity by preventing chromosomal breaks. Naturally known phytoesterogen β-sitosterol was isolated from *Cyrtandra cupulata* Ridl. (Gesneriaceae) through bioactivity-guided purification produced growth inhibitory effect on MCF-7 cells which are positive breast cancer cell line with estrogen receptors in a dose-dependent manner. Addition of β-sitosterol to the cell line dose dependently showed an increase in 1.53-fold of DEVDase activity, signifying elevated caspase activity which results in caspase-induced apoptosis (Chai et al. 2008). In addition, β-sitosterol also inhibits the increase of MDA-MB-231 human breast cancer cells and tumor growth formation; it also increases the apoptosis in cell culture which shows its antiproliferative nature in the prevention of breast cancer (Awad et al. 2000). β-sitosterol and taraxasterol reduce the development of breast and colon cancer; both of these compounds affect the development of different levels of tumors, for example, inhibition of tumor cells invasion and metastasis along with producing inhibitory effects on creation, promotion, and induction of cancerous cells. β-sitosterol supplementation decreases E2-induced MCF-7 tumor growth along with circulating 17β-estradiol (E2) levels in ovariectomized athymic nude mice. Thus, results suggest that positive effects are seen in women with breast cancer who are supplemented with high dietary phytosterols (Ju et al. 2004). In another study cytotoxicity of important phytosterols (β-sitosterol and its glycoside daucosterol) was examined against cancers cell lines by MTT assay. Results revealed that daucosterol inhibits the K-562 cell line (leukemia), whereas β-sitosterol was more active against HT-29 cell line (colon carcinoma) (Manayi et al. (2013)). Recent findings have shown that there is a reduced risk of prostate cancer especially in men consuming huge amounts of plant products rich in phytosterols. It was established that β-sitosterol increases the apoptosis of prostate cancer cells by producing a cellular signalling molecule ceramide, which regulates the differentiation, proliferation, apoptosis of cells, and programmed cell death (Von Holtz et al. 1998).

32.6.9 Immunomodulation

β-sitosterol works as an active immune modulator by targeting definite T-helper (Th) lymphocytes and improving the activity of natural killer (NK) cells and T lymphocytes. However, a small dose of β-sitosterol and its glycoside daucosterol promotes in vitro proliferative activity of T lymphocytes, after being stimulated by lower concentrations of phytohaemagglutinin (PHA). A significant increase in the expression of HLA-Dr antigens and CD25 expression on T lymphocytes was observed caused by essential sterolin formulation (ESF) which promotes the growth in the secretion of gamma interferon and IL-2 (Bouic 2001). In AIDS there was a slight decrease in the apoptosis of CD4 lymphocytes. It was found that in AIDS, stable CD4 cell counts were maintained by β-sitosterol, and slight decrease in the apoptosis of CD4 lymphocytes was observed, thus slowing HIV. In infected cells viral replication rates are slowing down due to significant decrease in IL-6 levels which overall decrease the viral load in HIV cases (Bouic 1997).

32.7 β-Sitosterol Herbal Dietary Supplement-Drug Interactions

β-sitosterol is one of the most dominant phytosterol consumed (200–400 mg daily) in human diets, found in large quantities in nonpolar fractions of plants and marines. β-sitosterol should be consumed in caution, or doses may be adjusted when using with supplements or herbs that may lower blood sugar levels. β-sitosterol should not be consumed with the drugs that increase the bleeding risk such as antiplatelet drug like clopidogrel (Plavix®), anticoagulants (blood thinners) like warfarin (Coumadin®) or heparin, and nonsteroidal anti-inflammatory drugs like ibuprofen (Motrin®, Advil®) or naproxen (Naprosyn®, Aleve®) and aspirin. Beta-sitosterol may also interact with agents (herbs and supplements) that affect the immune system or the heart, antiarthritic agents, anticancer agents, alpha1-blockers, cholesterol-lowering agents, cyclooxygenase inhibitors, diosgenin, cholestyramine, dalcetrapib, ezetimibe, beta-lactoglobulin tryptic hydrolysate (LTH), rifampin, lifibrol, and statins. It may also interact with lycopene, sterols, lutein, olestra, and vitamins A, D, and E (Saeidnia et al. 2014).

32.8 Conclusion

β-sitosterol commonly known as "plant sterol ester" are majorly found in plants and possess diverse applications in different fields such as in medicinal world and global food industry. There is a vital role of chemistry, biochemistry, and biotechnology in understanding the structure, biosynthesis, and behavior of β-sitosterol. This compound is structurally similar to cholesterol and has huge impact on human physiology. It is a well-known natural sterol with reported potential therapeutic mode of applications in cancer, diabetes, cardiovascular, immunomodulatory, neurological, and reproductive system. It is considered as the remarkable drug of the future with higher efficacy. There is a requirement to cover a broad spectrum of scientific applications for understanding the potential benefits presented by this remarkable phytosterol.

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Odontonutraceuticals: Phytochemicals for Oral Health Care

33

Shikha Pandhi and Arvind

Abstract

The use of plants for the management of various diseases had been documented for decades. Alike various health problems, oral diseases had also emerged as a severe health concern among people. Due to the growing incidence of oral infections, amplified resistance by bacteria to antibiotics, unfavorable effects caused by the chemical-based remedies currently used in dentistry, and also the financial considerations serve as the key cause for the expansion of newer prevention and treatment options that are safe, reasonably priced, and efficient in this regard. Odontonutraceuticals is a new term introduced to identify those phytochemicals that contribute to the prevention and management of oral diseases. Plant polyphenols are secondary metabolites that exert preventive action against infectious and degenerative diseases and may also help in preventing oral diseases. Poor oral bioavailability and easy modification by environmental is a major constraint associated. Nano-delivery systems have been developed to overcome the delivery challenges of polyphenols. The main objective of this chapter is to provide a comprehensive introduction about the function of polyphenols in oral health care and also about the utilization of nanocarriers as a delivery system to improve their bioavailability and stability.

Keywords

Antimicrobial · Bioavailability · Nanocarriers · Phytochemicals · Polyphenols

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33.1 Introduction

Oral infections are among the most common health problem associated with a major fragment of the population. The occurrence of these infections is mainly rampant in children more, but adults are also affected by these infections (Petersen et al. 2005). Increasing occurrences of oral infections, fast resistance development by bacteria to antibiotics, ill-effects associated with chemical-based formulations, and financial considerations had encouraged the need for the development of an alternative technique that is reasonably cheaper and more effectual and possesses lesser health effects. The use of plants for the management of various health diseases had been known for decades. Exploration of different phytochemicals and associated health-promoting effects had encouraged their use as a remedy to treat or prevent many diseases. Alike different health diseases, dental and oral diseases had also become an alarming problem of this century. Poor hygiene, poor nutrition, and smoking pay a chief contribution to occurring dental and oral problems. The use of plants over chemical-based remedies is always more appreciated. Various agents that are used for dental treatment are bisguanide-antiseptics, quaternary ammonium-antiseptics, phenolic antiseptics, and other remedies such as oxygenating agents, and metal ions (Addy 1986). But these agents have many side effects associated with them such as vomiting, diarrhea, and tooth staining. There have been numerous citations concerning the use of traditional plants and the products obtained from them in the maintenance of oral hygiene (Cowan 1999; Kalemba and Kunicka 2003). Odontonutraceuticals is a new term introduced to identify those phytochemicals that contribute to the prevention and management of oral diseases (Varoni and Iriti 2016). Recently, much attention has focused on the possibility that polyphenolics have a putative role in the prevention of chronic diseases (Duthie et al. 2000). Polyphenols (PPs) exert preventive activity against infectious and degenerative diseases and may also help to prevent oral diseases using different mechanisms (Petti and Scully 2009). The present chapter aims to create an interest among people regarding the potential health benefits of natural plant constituents in treating various infections and disorders of the oral cavity and to also provide a comprehensive introduction about the bioavailability of polyphenols, methods for enhancing its stability and bioavailability using novel delivery systems as a carrier.

33.2 Plant Polyphenols

Polyphenols (PPs) are plant secondary metabolites that are mainly characterized by the presence of several phenol groups, i.e., aromatic rings with hydroxyls (Chesson et al. 1997). They are effective in free radicals neutralization (Leopoldini et al. 2006), metal ions chelation (Erdemoglu and Gucer 2005), and binding and precipitation of proteins (Zhu et al. 1997). Polyphenols perform various functions in plants such as pigmentation, promoting plant fertility, pollen germination, and also as a signal molecule in plant-microbe interaction (Duthie et al. 2000). They play an important role in the prevention and management of dental diseases due to their defense mechanism against pathogenic microorganisms.

33.2.1 Absorption and Metabolism of Polyphenols

Polyphenols are small organic molecules with one or more than one phenolic ring structure. To acquire their beneficial effects other than on the gastrointestinal tract, they must be absorbed into the body after ingestion and should be carried out via bloodstream to various target sites where they get absorbed and perform its biological activity. Biological activity has been demonstrated for many of these polyphenols in numerous in vitro systems (Stevenson and Hurst 2007), but it is apparent that the effective concentrations required at least are of the order of magnitude higher than those normally achieved in human plasma (Manach et al. 2005a, b). To achieve sufficient concentrations at their site(s) of action, consumed polyphenols must overcome several barriers such as dissolution in the fluids of the GI tract and survival at low-pH and must also dissolve degradation and metabolism by intestinal enzymes (Sousa et al. 2008).

33.2.2 Bioavailability of Phenolic Compounds

Phenolic bioavailability is low, and the values of urinary excretion of intake range from 0.3% for anthocyanins to 43% for isoflavones (Manach et al. 2005a, b). The assimilation of food phenolics is primarily attributed to their chemical structure, basic structure (i.e., benzene or flavone derivatives), conjugation with other phenolics, molecular size, polymerization, and solubility (Karakaya 2004). The maximum concentration in plasma rarely exceeds 1 mM after the consumption of 10–100 mg of a single phenolic compound (Landete 2012).

33.2.3 Mechanism of Action

Polyphenols are antibacterial and antioxidant agents (Hannig et al. 2009). Polyphenols as antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause mainly age-related degenerative diseases (Petti and Scully 2009). According to a study polyphenol (PP) activity against several forms of cancer, proliferative diseases, inflammation, and neurodegeneration are mainly exerted through the inhibiting and modulating activities against a wide range of receptors, enzymes, and transcription molecules (Williams et al. 2004).

33.3 Antimicrobial Activity of Medicinal Plant for Oral Health and Hygiene

Oral health plays a vital role in maintaining general well-being and enhances the quality of life. Oral microbiota activities are well linked with oral diseases (Jenkinson and Lamont 2005). The growing need for alternative prevention and treatment options, and products for oral diseases that are secure, efficient, and cost-effective have encouraged the utilization of herbal formulations for maintaining oral health care (Tichy and Novak 1998; Badria and Zidan 2004).

33.4 Antimicrobial Effects of Some Plants Against Oral Microbes

33.4.1 Neem (*Azadirachta indica*)

Neem (*Azadirachta indica*) is an evergreen tree. Neem extract had shown significant inhibition of bacterial adhesion to hydroxyapatite, a composite of bone and enamel. Also, it inhibited the synthesis of insoluble glucan that reduces the adherence of streptococci to tooth surfaces (Wolinsky et al. 1996). The maximum zone of inhibition on *Streptococcus mutans* was shown at the concentration of 50% by neem extract (Prashanth et al. 2007). An in vivo study on neem mouthwash showed inhibitory action toward the *Streptococcus mutans* in saliva (Vanka et al. 2001).

33.4.2 Garlic (*Allium sativum*)

Allium sativum, also known as garlic had been used for the management of Periodontitis, a dental infection (Bakri and Dowglas 2005). Garlic extract had shown to possess an inhibitory effect against a wide range of microorganisms including both Gram-positive and Gram-negative organisms, fungi (Amin et al. 2012), as well as certain multidrug-resistant enterotoxin genetic strains of *Escherichia coli* (Ankri and Mirelman 1999). Garlic juice had shown a remarkable inhibitory effect against *Streptococcus mutans* (Xavier and Vijayalakshmi 2007) isolated from human carious teeth (Fani et al. 2007) which is resistant to the majority of the antibacterial agents used such as penicillin, amoxicillin, tetracycline, and erythromycin. Apart from the beneficial effects, it also possesses certain side effects such as objectionable taste, halitosis, and nausea (Groppo et al. 2007).

33.4.3 Paan (*Piper betel*)

Piper betel is the leaf of a vine that belongs to the family *Piperaceae*. Crude aqueous extract of *Piper betel* showed reduced growth, adhering ability, and glucosyltransferase activity against *Streptococcus mutans* (Nalina and Rahim

2007) and also showed profound ultra-structural changes to the morphology of bacteria. Suppression of bacterial growth may result in slower plaque development and reduce its accumulation on the tooth surface. Thus inclusion of *P. betel* extract into different formulations may be considered beneficial for oral plaque control.

33.4.4 Clove (*Syzygium aromaticum*)

Syzygium aromaticum commonly called clove is known for its remedial action in case of toothache. Methanolic extracts of clove can be utilized for the treatment of periodontal diseases due to their preferential activity against Gram-negative anaerobic oral pathogens (Cai and Wu 1996). Kaempferol and myricetin are the principal compounds that had shown significant inhibitory action against periodontal pathogens. The clove and clove bud oil possess potent antimicrobial activity against the five microorganisms, namely, *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus* (bacteria), *Candida albicans*, and *Saccharomyces cerevisiae* (yeast) that makes them suitable for the treatment of dental caries.

33.4.5 *Myristica fragrans*

Myristica fragrans is an evergreen tree native to Indonesia. Ethanol extracts of *Myristica fragrans* had shown to possess good antibacterial property against both Gram-positive and Gram-negative bacteria such as *Streptococcus mutans*, *Streptococcus salivarius*, and *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Fusobacterium nucleatum*, respectively (Jaiswal et al. 2009).

33.4.6 Bakul (*Mimusops elengi*)

Mimusops elengi is a medium-sized evergreen tree. The extract of bark of *Mimusops elengi* had shown to exert antimicrobial activity against the salivary microflora collected from children of 6–12 years of age. The results further confirmed the antimicrobial potential of the acetone plant extract and suggested its utilization for the treatment of oral infectious diseases (Deshpande et al. 2010).

33.4.7 Indian Gooseberry (*Emblica officinalis*)

Emblica officinalis commonly known as Amla contains phenols, tannins, polyphenols, flavonoids, kaempferol, ellagic acid, and gallic acid (Nair and Chanda 2007) that possess the ability to inhibit virulence factors of *Streptococcus mutans* and *Lactobacillus* causing dental caries and was found to exhibit more antibacterial efficiency than chlorhexidine (Hasan et al. 2012).

33.4.8 Miswak (*Salvadora persica*)

Miswak root and bark had been traditionally used as a chewing stick or natural toothbrush to strengthen gums. It serves as a multi-purpose stick that whitens teeth, cleans the mouth, and sweetens breath. Nowadays, Miswak (also known as Peelu) has been commercially used as an active ingredient in many kinds of toothpaste for cleaning teeth, removal of plaque, disinfecting the mouth, and refreshing the breath. Chemicals analysis revealed the presence of 19 natural substances that are beneficial for dental health. The natural constituents present have a bactericidal action against harmful microorganisms in the mouth. The tannic acid present has astringent qualities and protects the gums from the disease.

33.4.9 Acacia (*Acacia catechu*)

Acacia catechu is widely used for ayurvedic treatment of several diseases. Aqueous and ethanol extract of heartwood of *Acacia catechu* had shown its effectiveness as a potent antibacterial agent against dental caries causing microorganisms such as S*treptococcus mutans*, *Streptococcus salivarius*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* (Geetha et al. 2011).

33.4.10 Other Medicinal Plants

Some other medicinal plants that possess antimicrobial property against various oral microbes include *Cichorium intybus*, commonly known chicory having an anti-plaque effect. Nutmeg, tea tree oil, peppermint essential oil, rosemary, cinnamon, and triphala had also shown to exert preventive action against many oral diseases and infections (Salam et al. 2015).

33.5 Some Possible Applications

33.5.1 Medicated Chewing Gums

Medicated chewing gums are solid, single-dose formulations containing gum as a base which is intended to be chewed but not swallowed. The active substances contained are released by chewing and are intended to be beneficial as a remedy for mouth diseases or allowed systemic delivery after absorption through the buccal mucosa. Incorporation of various plant phytochemicals into these chewing gums may serve as an effective measure to cure and prevent oral infections or diseases. Further, they act as a friendly oral mucosal drug delivery system that enhances the stability and control target release of polyphenols also (Bhoi et al. 2014).

33.5.2 Toothpaste Combined with Plant Constituents

Toothpaste combined with biologically active constituents from plants exerts antibacterial action when the right balance of all essential constituents is provided and thus, ensures proper oral health care (Mazumdar et al. 2013). Several recent studies had shown that various plant extracts exhibit significant antibacterial activity against oral bacterial isolates. As a result, some of these antibacterial agents have been incorporated into different products, including toothpaste. A study conducted showed that the ethanol extract of different medicinal plants in combination with commercial toothpaste showed higher antimicrobial activity against oral isolates than other combination groups (Ali et al. 2016)

33.5.3 Herbal Mouthwash

A variety of mouthwashes incorporated with different plant extracts are commercially available and have shown to be effective against various oral problems and infections. Studies indicated that usage of herbal mouthwashes formulated using turmeric (Waghmare et al. 2011), neem (Chatterjee et al. 2011), and triphala (Anupama et al. 2010) had shown a considerable decline in plaque indices scores, gingival indices scores, and gingival bleeding index scores compared to chlorhexidine (Atul et al. 2011).

33.6 Enhancement of Bioavailability by Drug Delivery Systems

Bioavailability of a compound cannot be predicted correctly however its analysis using Lipinski's "rule of five" provides some insight that a compound will have better bioavailability if it contains not more than 5 hydrogen-bond donors, not more than 10 hydrogen-bond acceptors; has a molecular mass not greater than 500 daltons, a partition coefficient log P-value of not greater than 5; and contains less than 10 rotatable bonds (Lipinski et al. 2001). Most of the chemopreventive agents, including polyphenols such as curcumin and green tea polyphenols, do not fall in these specifications, and they exhibit low bioavailability (Gao and Hu 2010). Other factors also limiting the bioavailability are the solubility of the compound, stability to gastric and colonic pH, metabolism by gut microflora, and absorption across the intestinal wall. The bioavailability issue related to the compound of interest at the target site is of the highest importance. A promising approach to overcome this low bioavailability and stability involves the use of nanosized carriers, such as polymeric nanoparticles (NPs), liposomes, dendrimers, and micelles (Mishra et al. 2010; Oerlemans et al. 2010).

Nanoparticles ranging from 10 nm to 1000 nm can be synthesized from lipids, proteins, and carbohydrates. For delivery of the active constituent first, it needs to be dissolved, and then it is entrapped, encapsulated, or attached to an NP matrix. NP systems improve the therapeutic index of encapsulated drugs or active constituents either by protecting them from enzymatic degradation (Khan et al. 2006) or providing controlled target release for extended periods. Liposomes are nanosize artificial vesicles spherical in shape that can be obtained from natural phospholipids and cholesterol. It can encapsulate drugs or active constituents with varying solubility or lipophilicity. Micelles are lipid molecules that can arrange themselves in a spherical form in aqueous solutions. They increase drug bioavailability, and retention as the drug is well protected from possible inactivation by its micellar surroundings (Kwon 2002).

33.7 Conclusion

The present review indicated that there are ample of medicinal plants that have shown to possess preventive action against various oral ailments. The current researches have indicated use of dietary polyphenols as a preventive and therapeutic agent against a variety of diseases due to their antioxidant property. Plant polyphenols are secondary metabolites that are crucial for many plant functions. They put forth a defensive action against infectious and degenerative diseases and may also assist in preventing oral diseases, by way of mechanisms like antioxidant activity and neutralization/modulation of human/bacterial/viral proteins/enzymes. Various medicinal plants have been studied for the avoidance and management of a range of oral diseases and infections. Bioavailability and stability of these substances are of major concern. This review aims at enlightening the potential of various medicinal plants in maintaining oral hygiene and also discusses a range of new delivery systems that can be used as a carrier for these active constituents by enhancing its bioavailability by several folds.

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Pharmacological Potential of Thymol

34

Swati Agarwal, Shraddha Tripathi, and Neetu Mishra

Abstract

Thymol, belongs to a class of naturally presenting phenols with a ten-carbon unit, chemically known as 2-isopropyl-5-methylphenol ($C_{10}H_{14}O$), is a colourless crystalline monoterpene phenol found in the seeds of *Nigella sativa* and oil of thyme. Thymol is an active ingredient of several plants as *Thymus vulgaris*, *Thymbra spicata*, *Thymus ciliates*, *Trachyspermum ammi* and *Monarda fistulosa*. This versatile molecule is incorporated as a useful ingredient in many food products and finds application in agricultural, pharmaceutical, fragrance, cosmetic, flavour and other industries. For centuries, it has been used in traditional system of medicine and has been shown to possess various beneficial therapeutic effects, including antioxidant, anti-inflammatory, antibacterial, antifungal, local anaesthetic, antiseptic and radio- and cardioprotective effects. The noteworthy effects of thymol are largely attributed to its anti-inflammatory (via inhibiting recruitment of cytokines and chemokines), antioxidant (via scavenging of free radicals, enhancing the endogenous enzymatic and non-enzymatic antioxidants and chelation of metal ions) and antihyperlipidemic (via increasing the levels of high-density lipoprotein cholesterol and decreasing the levels of low-density lipoprotein cholesterol in the circulation and membrane stabilization) (via maintaining ionic homeostasis) effects. It is significant to say that thymol is one of the most powerful contenders in the race of phytochemicals of natural origin with polypharmacological properties against an array of maladies.

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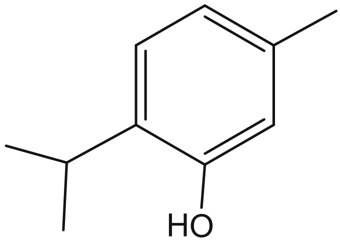
Keywords

Thymol · *Nigella sativa* · Pharmacological activities · Antioxidant · Anti-inflammatory

34.1 Introduction

A larger number of medicinal plants and their purified compounds have been shown beneficial therapeutic potential against various human ailments (Sofowora et al. 2013). The therapeutic significance of the plants has been mentioned in the traditional system of medicine of many countries as they are like to believe to be worthwhile and safe. Numerous plants (e.g. black cumin, thyme, turmeric, olives, oregano, dates, etc.) and their parts are used in culinary preparation or for the treatment of many diseases, since ancient times (Rahmani et al. 2014). Among these plants, Indians and Egyptians have used black cumin (*Nigella sativa*) for the preservation of foods and as odorant and flavouring agent. *Nigella sativa* is an annual flowering plant belongs to family Ranunculaceae, native to south and southwest Asia. *Nigella sativa*, its oils and seeds are mostly used as food additives and herbal medicinal products (Goreja 2003; Ali and Blunden 2003). Furthermore, *Nigella sativa* seeds are reported to possess antihistaminic, antihypertensive, antimicrobial, antitumor, insect repellent and galactogogue effects (Fararh et al. 2002; Khan et al. 2003). *Nigella sativa* seeds contain several bioactive compounds, one of them is thymol (Thy).

Thymol [2-isopropyl-5-methylphenol, ($C_{10}H_{14}O$)], is a colourless crystalline, found in the seeds of *Nigella sativa* and oil of thyme. Thymol is biosynthesized through p-cymene hydroxylation after aromatization of ɣ-terpinene to p-cymene (Poulose and Croteau 1978). Thymol is an active ingredient of several plants as *Thymus vulgaris*, *Thymbra spicata*, *Thymus ciliates*, *Trachyspermum ammi* and *Monarda fistulosa*. Thymol possesses antibacterial (Didri et al. 1994), antifungal (Mahmoud 1994), anti-inflammatory (Aeschbach et al. 1994), antioxidant (Yanishlieva et al. 1999), anti-mutagenic (Zahin et al. 2010), analgesic (Ozen et al. 2011), antimicrobial (Karpanen et al. 2008), anticonvulsant, (Sancheti et al. 2014), wound healing (Riella et al. 2012) and radioprotective (Archana et al. 2011a) properties. Thymol improves digestibility, respiratory problems and menstrual cramps and is also used as active ingredient in food flavourings, ointments, various soaps, shampoos, toothpastes, deodorants and mouthwashes (Shapiro et al. 1994; Manou et al. 1998). Thymol is also used in dentistry due to its antimicrobial properties (Ogaard et al. 1997; Khan et al. 2017).



Thymol (THY)

Fig. 34.1 Chemical structure of thymol

34.2 Physical and Chemical Properties of Thymol

Thymol chemically known as 2-isopropyl-5-methylphenol belongs to a class of naturally presenting phenols with a ten-carbon unit (Fig. 34.1). It is a colourless crystalline substance which has strong flavour, pleasurable aromatic odour and strong disinfecting properties. Its molecular weight is 150.22, density is 0.96 g/ cm^3 at 25 °C, and melting point ranges from 49 °C to 51 °C (Jordan et al. 1991; Lide and Frederikse 1996). It is extremely soluble in alkaline solutions, alcohols and other organic solvents but slightly soluble in water at neutral pH. Thymol absorbs maximum ultraviolet radiation at 274 nm (Wade and Reynolds 1997). It is least soluble in water and less palatable due to its unpleasant taste and smell (Nieddu et al. 2014). Thymol is an important agent of natural origin and has generated interest in the field of scientific research for its therapeutic potential in different diseases. The present review presents an overview of its therapeutic potential for the treatment of different human diseases.

34.3 Pharmacological Potential of Thymol

Numerous in vitro and in vivo studies have been done to investigate the pharmacological potential of thymol.

34.3.1 Antioxidant Activity

Thymol showed antioxidant activity through concentration-dependent manner via superoxide anion, reducing capacity and free radical scavenging activity (Meeran et al. 2015b, 2016). Thymol protects isoproterenol-induced myocardial infarction in rat by its antioxidant and anti-lipid peroxidation properties (Meeran and Prince 2012). Thymol possesses superoxide dismutase activity in vitro by eliminating superoxide radicals (Kruk et al. 2000). Thymol showed antioxidant activity in fibroblast lung cells of V79 Chinese hamster (Undeger et al. 2009). It has been also shown effective antioxidant activity in gamma ray-induced V79 Chinese hamster cell by modulating enzymatic antioxidants activity and decreasing lipid peroxidation (Archana et al. 2011b).

Thymol showed more effective antioxidant activity than carvacrol, its isomer through its more steric hindrance. It showed better antioxidant in triacylglycerols of sunflower oil (TGSO) than triacylglycerols of lard (TGL) at room temperature (Yanishlieva et al. 1999). Thymol supplementation reduced the malondialdehyde levels and improved antioxidant status in broiler chickens (Zidan et al. 2016). Dietary supplementation of thymol-carvacrol (100 mg/kg) in the ratio of 1:1 decreased the risk of intestinal oxidative stress and defects in the intestinal barrier of weaning piglets (Wei et al. 2017). Thymol (24.7 mg/kg) mitigated oxidative stress induced by aflatoxin in male rats via its potent antioxidant potential (El-Nekeety et al. 2011). Thymol (7.5 mg per kg body weight) supplementation has been shown to prevent dyslipidemia, lipid peroxidation, inflammation, glycation, apoptosis and ionic homeostasis malfunction through its potent antioxidant activity (Meeran et al. 2014, 2015c).

34.3.2 Anticancer Activity

Thymol showed chemopreventive or anticancer properties against various types of cancers. The major processes of anticancer activity of thymol includes anti-proliferation, inhibition of angiogenesis and induction of apoptosis, diminution and migration of tumorigenesis through modulating carcinogen-metabolizing enzymes activity. Schematic depiction of the anticancer property of thymol in various types of cancer is presented in Fig. 34.2. Thymol (0.05–1.25 μM) treatment in breast cancer cells fascinated cell cycle in G0/G1 phase by stimulating cytotoxicity (Jaafari et al. 2012). In MCF-7 cells, thymol with LC_{50} of 2.5 μg/ml triggered cytotoxicity (Melo et al. 2014). Thymol ($IC_{50} = 304.81$ μg/ml) induced proliferation and cytotoxicity in MCF-7 breast cancer cell lines (Khadir et al. 2016). Matrix metallopeptidase-9 (MMP9) and MMP2 production were significantly reduced by thymol (30 μM) in the C6 glioma cells. Thymol also diminished protein kinase Cα (PKCα) and extracellular signal-regulated kinases (ERK1/2) phosphorylation (Lee et al. 2016). A report from Dutta et al. (2011) revealed the cytotoxic effect of thymol on HL-60 cells. Association of thymol with induction of cell cycle arrest at G0/G1 phase and apoptotic cell death by genomic DNA fragmentation pattern showed cytotoxic effect. It also showed increase in the production of reactive oxygen species activity, depolarization of mitochondrial membrane potential and increase in the mitochondrial H_2O_2 production. Thus they concluded that thymol-induced apoptosis includes both caspase-dependent and caspase-independent pathways (Dutta et al. 2011).

Thymol (30, 50 and 70 mg/ml) suppressed the phosphatidylinositide 3-kinases/ protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) pathway and induced apoptotic cell death in HL-60 cells. Thymol inhibited cell proliferation in various cancer cell lines like PC-3, HL-60, MCF-7, MDAMB-231and A-549.

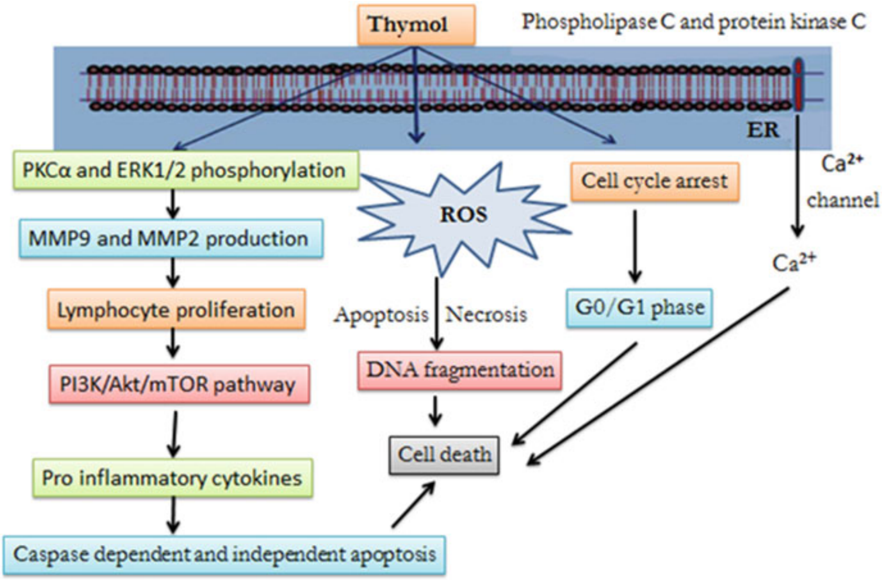


Fig. 34.2 Effect of thymol in various types of cancer (Meeran et al. 2017)

Thymol also reduced downstream and upstream signalling of PI3K/AKT/mTOR pathway (Pathania et al. 2013). Thymol (0–500 μg/ml) showed antiproliferative activity in human monocytic leukaemia cells (Miguel et al. 2015). Thymol-triggered reactive oxygen species mediated apoptosis by mitochondrial pathways in human osteosarcoma cells (Chang et al. 2011). Thymol (100–400 μM) has been shown to change in the morphology of cells by chromatin condensation, cytoplasm shrinkage, membrane blebbing and cleavage of DNA in human gastric AGS cells (Kang et al. 2016). Thymol (400 mg/L) has been shown to reduce cell proliferation in cultured neuroblastoma cells, while thymol (10, 25 and 50 mg/L) improved the total antioxidant capacity (TAC) in rat neurons (Aydın et al. 2017). These studies show that thymol has potent anticancer and antiproliferative activity.

34.3.3 Cardiovascular Activity

Thymol (7.5 mg/kg) showed to inhibit isoproterenol (ISO) (an agent which is used to induced myocardial necrosis) induced oxidative stress in rats. The beneficial effect of thymol was seen by decreasing the levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CDs) and lipid hydroperoxides (LOOHs) in plasma. Thymol also normalized antioxidants like vitamin E, vitamin C and GSH in the plasma by its antioxidant activity (Meeran and Prince 2012). The protective role of thymol in different cardiovascular related disorders is represented in Fig. 34.3 (Meeran et al. 2017). Thymol was shown to alter the activities of lipid biomarker

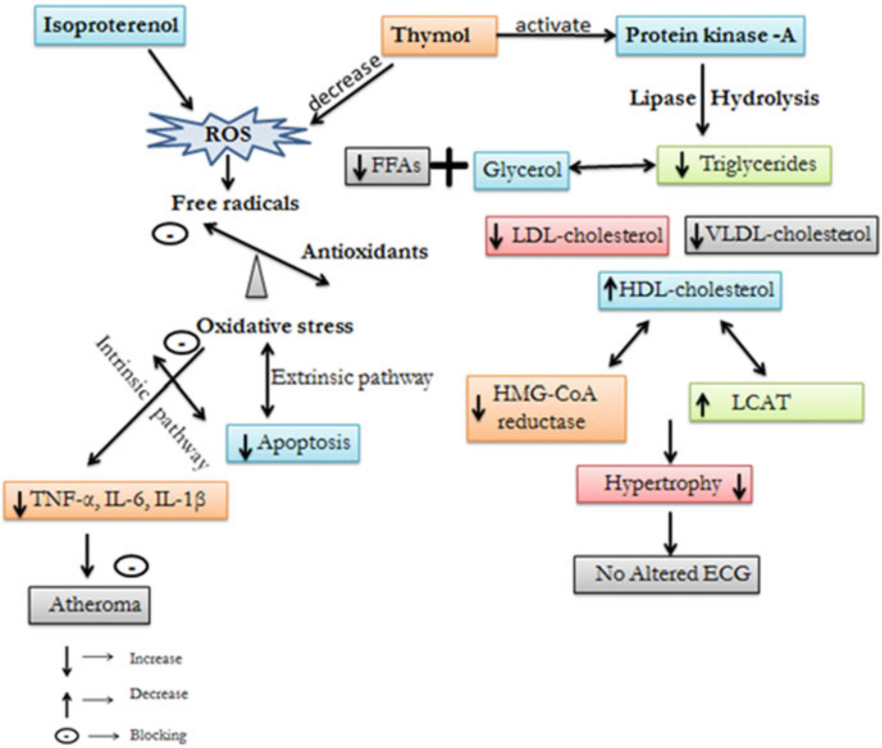


Fig. 34.3 Protective role of thymol in cardiovascular related disorder (Meeran et al. 2017)

enzymes such as lecithin–cholesterol acyltransferase (LCAT) and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) in the liver in ISO-induced myocardial infracted rats (Meeran et al. 2015b).

Thymol attenuated aortic intimal thickening, inflammation and oxidative stress by regulating gene expression in hyperlipidemic rabbits (Yu et al. 2016). Thymol (3 and 6 mg/kg) treatment decreased total cholesterol level, triglycerides (TGs) level, LDL-C level, malondialdehyde (MDA) level, high sensitive C-reactive protein (hsCRP) and aortic intimal thickening while increased HDL-C level and total antioxidant activity in high-fat diet-induced hyperlipidemic rabbits. Moreover, 3 and 6 mg/kg of thymol decreased the expressions of pro-inflammatory cytokines as IL-6, IL-1β, TNF-a, TNF-b, monocyte chemo attractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1) and matrix metallopeptidase-9 (MMP-9) in hyperlipidemic rabbits (Yu et al. 2016).

34.3.4 Antiobesity Effect

Obesity is termed as excessive adiposity and is becoming one of the major health concern and socioeconomic burdens, resulting increase in coronary heart disease, type 2 diabetes, hyperlipidaemia and cancers (Jeusette et al. 2005; Sedova et al. 2004; Lazar 2005; Stunkard et al. 2003). Thymol (30 mg/kg) has been shown to inhibit visceral fats accumulation, enhance insulin sensitivity and leptin levels and improve lipid-lowering action, antioxidant status in HFD-induced obesity in murine models (Haque et al. 2014). Thymol (20 μM) has the potential to normalise fatty acids oxidation, thermogenesis, lipolysis augmentation and reduction (Choi et al. 2016).

34.3.5 Antidiabetic Effect

Thymol supplementation (40 mg/kg body weight) for 5 weeks decreased body weight, blood glucose, HOMA of insulin resistance (HOMA-IR) plasma insulin and glycated haemoglobin (HbA1c) in HFD-induced type 2 diabetic C57BL/6 J mice. It also suppressed hepatic and plasma total cholesterol level, triglycerides (TGs), free fatty acids (FFAs), phospholipids (PLs) and LDL-C and increased HDL-C levels in HFD-induced type 2 diabetic mice. Moreover, thymol treatment increased adiponectin whereas decreased leptin levels in HFD induced mice (Saravanan and Pari 2015). Another study has done by the same group, exposed that thymol (40 mg/kg) administration for 5 weeks reduced blood glucose levels and improved renal function parameters (Saravanan and Pari 2016).

Thymol (0.5–2.0 mg/ml) was shown to protect red blood cells from haemolysis induced by 2,2-azo-bis(2-amidinopropane) dihydrochloride (AAPH) in diabetic patients (Aman et al. 2013). Thymol has been shown to inhibit α-amylase and α-glucosidase enzymes which are responsible for breakdown and intestinal absorption of carbohydrates (Hyun et al. 2014). These findings expose that thymol has potential for treatment of diabetes and its associated complication.

34.3.6 Hepatoprotective Activity

Thymol (30 mg/100 g) was shown to inhibit oxidative stress in hydrocortisone-induced hepatotoxicity in rat model by reducing lipid peroxidation and enhancing antioxidant defence mechanism in the liver (Aboelwafa and Yousef 2015). Thymol (300 mg/kg) was shown to reduce carbon tetrachloride (CCl_4)-induced liver injury in mice (Al-Malki 2010). Thymol (300 mg/kg) administration reduced lipid peroxidation and restored hepatic marker enzymes activity in CCl_4 (20 μl/kg) induced liver cells by its potent antioxidant property (Alam et al. 1999).

Thymol (50 μg/ml) reduced hepatic injury by inhibiting reactive oxygen species overproduction, preventing lipid peroxidation and apoptosis and increasing antioxidant status in tert-butyl hydroperoxide-induced Chang liver cells (Kim et al. 2014).

Thymol (150 mg/kg) was also shown to inhibit paracetamol-induced liver injury in mice by inhibiting the alterations in hepatic marker enzymes activity (Janbaz et al. 2003). Thymol (50–200 mg/kg) increased phase I enzymes [7-ethoxycoumarin O-deethylase (ECOD)] and phase II enzymes [GST and quinone reductase (QR)] activity in mouse liver (Sasaki et al. 2005). These findings revealed that thymol plays an important role against liver injury by virtue of its potent free radical scavenging property.

34.3.7 Gastroprotective Activity

At the present time, the frequency of inflammatory infections in the intestine is rising as a severe problem in human beings. The increased levels of pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-12, TNF-a and IFNɣ) were stated in the inflamed intestinal mucosa of both human and animals (Raddatz et al. 2005; Bukovska et al. 2007). Thymol inhibited 2,4,6- trinitrobenzenesulfonic acid-induced colitis in mice by decreasing the mRNA expressions of pro-inflammatory cytokines and protein expressions of IL-1β and IL-6 (Bukovska et al. 2007). Thymol has the ability to enhance the secretion of salivary amylase in human and bile acid, gastric and pancreatic enzymes such as amylase, lipase and proteases in rats (Platel and Srinivasan 2004). Administration of thymol in broiler chickens also increased the activity of pancreatic amylase, trypsin and maltase (Jang et al. 2007). Thymol (100 mg/kg) has been reported to reduce both acute and chronic ulcer (induced by indomethacin, ethanol and acetic acid) in rats by diminishing infiltration of inflammatory cells and oedema. This property of thymol is due to increased prostaglandins, mucus secretion and ATP-sensitive K^+ channels (Ribeiro et al. 2016).

34.3.8 Contraceptive and Antifertility Activity

Thymol treatment showed antifertility effect in male rats by decreasing testis weight, sperm count and motility and increasing abnormal sperm count (Kumar et al. 2011). Another study also revealed that thymol (100–500 μg/ml) dose dependently declined count of sperm, motility and sperm vitality in human sperm (Chikhoune et al. 2015). These findings suggest that thymol could be tried out as a contraceptive agent in males.

34.4 Conclusion

In recent years, the focus on traditional uses of natural products received much attention as they are believed to be safe for human use. They obviously deserve scrutiny for phytochemical investigation, biological evaluation on experimental animal models, toxicity studies and investigation of molecular mechanism of actions. The present review reveals on all the aspects of the herbal compound and throws the attention to set the mind of the researchers to carry out the work for developing its various formulations, which can ultimately be beneficial for the human as well as animals.

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Trikatu: Transforming Food into Medicines

35

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Abstract

Trikatu, as per Ayurveda's Bhaisajyaratnawali, is a compound herbal formulation containing three bitter herbs mixed together in equal quantities. Dried fruits of *Piper nigrum* (Maricha) and *Piper longum* (Peepli) and dried rhizomes of *Zingiber officinalis* (Sunthi) are used to prepare this miraculous formulation. It is prescribed in Ayurvedic system of medicine for treatment of tastelessness, digestive impairment, diseases of nose and throat like chronic rhinitis/sinusitis, skin diseases, asthma, cough, frequent urination, obesity, and Filariasis. Trikatu is also added in various Ayurvedic formulations with a view to restore the disturbed "tridoshas- vatta, pitta and kapha." It calms down the increased *Vatta* and *Kapha* and increases the *Pitta*. It has pungent (katu) taste, hot (ushna) potency, light (laghu) and dry (ruksha) quality, and digestive (amapachaka) therapeutic effect. Modern pharmacological studies also revealed that Trikatu possesses capability to enhance the bioavailability of various phytoconstituents present in the Ayurvedic and polyherbal formulations. Trikatu when added to other formulations increases the bioavailability of phytoconstituents and helps in achieving therapeutic goals. Apart from traditionally known health benefits, Trikatu also possesses antiviral, expectorant, carminative, hypolipidemic, hypoglycemic, antiemetic, and anti-inflammatory potential. Simply, it is concluded that Trikatu has the potential to transform a normal food material into an effective medication by increasing its absorption.

Keywords

Trikatu · Piper longum · *Piper nigrum* · *Zingiber officinalis* · Ayurvedic · Bioavailability

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35.1 Introduction

Trikatu as the name itself indicate its meaning, “tri” in Sanskrit stands for three and “katu” stands for acrids. The three acrid herbs including Maricha (black pepper), Peepli (long pepper), and Sunthi (ginger) when combined in equal quantities form the miraculous formulation Trikatu. Trikatu is an Ayurvedic formulation mentioned in Ayurveda for a number of ailments. In *Bhaisajyaratnawali* which is an Ayurvedic text, Trikatu is mentioned in shloka number 16 as:

पपि्पली मरचिं शुण्ठी त्रयमेतद्वमिशि्रतिम ।
त्रकिटु त्र्यूषणं व्योषं कटुत्रकिमथोच्यते ।।१।।

The shloka completely defines the procedure for preparation of Trikatu and method of its use along with the indications in which it is to be used.

Individually, Ayurvedic system of medicine prescribes Trikatu for management of disturbed digestion, diseases of nose and throat like chronic rhinitis/sinusitis, asthma, cough, frequent urination, obesity, Filariasis, and skin diseases. Trikatu acts primarily by its effect on the stomach, liver, and pancreas. In the stomach, it increases production of digestive juices thereby stimulating digestion. In the liver, it increases production of bile salts by stimulating gall bladder functioning. Trikatu also have its influence on pancreatic functioning. In a nutshell, Trikatu affects overall digestive system. Along with digestive systems, it also affects respiratory, urinary, immunity, skin, and metabolic systems of our body.

Trikatu is also added in various Ayurvedic formulations with a view to restore the disturbed “tridoshas- vatta, pitta and kapha.” It calms down the increased *Vatta* and *Kapha* and increases the *Pitta*. It has pungent (katu) taste, hot (ushna) potency, light (laghu) and dry (ruksha) quality, and digestive (amapachaka) therapeutic effect.

35.2 Method of Preparation (Ayurvedic Formulary of India (AFI) 2000)

Equal quantities of all the three acrid herbs, dried fruits of *Piper longum* Linn. (long pepper), *Piper nigrum* (black pepper), and dried rhizomes of *Zingiber officinalis*, are finely powdered separately in a mortar pestle or grinder. The fine powders of individual herbs are weighed in equal quantities and mixed together properly. This mixture of powders is then sieved through sieve no. 80 in order to get extra fine powder which has more therapeutic value due to more surface area. The fine powder of Trikatu is then stored in moisture free air tight containers.

35.3 Dosage

Ayurvedic texts prescribe 1–3 g of Trikatu churna to be consumed with honey to mask the bitter taste or warm water for maximum therapeutic benefits. Trikatu is added in many Ayurvedic polyherbal formulations in such a quantity that it will be sufficient to enhance the bioavailability of the main ingredients of that formulation by acting through various mechanisms. When added in formulations, the actual pharmacological activity of Trikatu is not exhibited because its dose is not the therapeutic dose.

35.4 Chemistry of Trikatu

Trikatu contains the three herbs Piper longum, *Piper nigrum*, and *Zingiber officinalis*. The component herbs *Piper longum* and *P. nigrum* contain piperine as the main chemical as well as biological marker along with other constituents in minor quantities. *Zingiber officinalis* contains chemical constituents like gingerols, zingiberene, shogaols, and other chemical components.

35.5 Chemical Composition of *Piper longum* (Zaveri et al. 2010)

Piperine is the major and active constituent of long pepper (*Piper longum*). The piperine content is 3–5% (on dry weight basis) in *P. longum*. The fruit of *P. longum* contains a large number of alkaloids and related compounds, the most abundant of which is piperine, together with methyl piperine, iperonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractomide A, pregumidiene, brachystamide, brachystamide-A, brachystine, pipercide, piperderidine, longamide and tetrahydropiperine, terahydro piperlongumine, dehydropipernonaline piperidine, piperine, terahydropiperlongumine, and trimethoxycinnamoyl-piperidine. Lignans, sesamin, pulvuatilol, fargesin, and others have been isolated from the fruit of *P. longum*. The fruit of *P. longum* contains tridecyl-dihydro-pcoumaarate,eicosanyl-(E)-p-coumarate and Z-12-octadecenoic-glycerol-monoester. The essential oil of the fruit *P. longum* is a complex mixture, the three major components of which are (excluding the volatile piperine) caryophyllene and pentadecane (both about 17.8%) and bisabolene (11%). Others include thujone, terpinolene, zingiberene, p-cymene, p-methoxyacetophenone, and dihydrocarveol.

35.6 Chemical Composition of *Piper nigrum* (Meghwal and Goswami 2012)

Piper nigrum contains lignans, alkaloids, flavonoids, aromatic compounds, and amides. It also contains essential oil up to 3.5%, and this oil constitutes sabinene, pinene, phellandrene, linalool, and limonene. It also has piperine which is a weak basic substance. Chavicine is an isomer of piperine. Piperine and chavicine are not responsible for the aroma of the black pepper, but piperine imparts pungency to the black pepper.

35.7 Chemical Composition of *Zingiber officinalis* (Prasad and Tyagi 2015)

Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds. Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present. The aromatic constituents include zingiberene and bisabolene, while the pungent constituents are known as gingerols and shogaols. Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6-gingerdione, and 10-gingerdione; 4-gingerdiol, 6-gingerdiol, 8-gingerdiol, and 10-gingerdiol; and diarylheptanoids. The characteristic odor and flavor of ginger are due to a mixture of volatile oils like shogaols and gingerols.

35.8 Need of Bioavailability Enhancers

Many herbal drugs and drug extracts despite of their impressive in vitro findings demonstrate less or negligible in vivo activity due to following reasons:

1. Poor lipid solubility
2. Improper molecular size
3. Resulting in poor absorption
4. And hence poor bioavailability

Here the need arises for a herbal solution for combating these bioavailability problems. Trikatu fits best to manage these bioavailability issues with herbal formulations. There are numerous pharmacological findings that support the use of piperine and gingerols to enhance the bioavailability.

35.9 Bioavailability Enhancers

- Bioavailability enhancers are drug facilitators.
- They are molecules which by themselves do not show typical drug activity.

- But when used in combination, they enhance the activity of drug molecule in various ways.

Simply, a bioavailability enhancer is an agent capable of enhancing bioavailability and bioefficacy of a particular drug with which it is combined without any typical pharmacological activity of its own at the dose used.

Piperine is the biomarker of both *Piper longum* and *Piper nigrum*. Piperine acts by a number of mechanisms to enhance the bioavailability.

1. Increases bioavailability of the drug across the membrane
2. Potentiates the drug molecule by conformational interactions
3. Acts as receptors for drug molecule making target cells more receptive to drugs
4. Reduction in HCl secretion and increase in GIT blood supply
5. Inhibition of gastrointestinal transit, gastric emptying time, and intestinal motility
6. Modifications in GIT epithelial cell membrane permeability
7. Chalagogous effects
8. Bioenergetics and thermogenic properties
9. Suppression of *first pass metabolism* and inhibition of drug metabolizing enzymes
10. Stimulation of gamma-glutamyl transpeptidase activity which enhances uptake of amino acids

The effect of simultaneous administration of piperine on plasma concentration of carbamazepine given twice daily in epileptic patients undergoing carbamazepine therapy was evaluated by Pattanaik et al. It was observed that piperine significantly enhanced the bioavailability of carbamazepine. The mechanism of action was possibly by increased absorption and reduced elimination of the carbamazepine (Pattanaik et al. 2009).

Antidepressant effects of curcumin were investigated with co-administration with piperine by Bhutani et al. It was observed that the combination of piperine with curcumin showed significant potentiation of its anti-immobility, neurotransmitter (serotonin and dopamine) enhancing and monoamine oxidase inhibitory effects as compared to curcumin effect when taken alone (Bhutani et al. 2009). Another similar study revealed that there was potentiation of antidepressant activity of curcumin when administered with piperine. (Kulkarni et al. 2009)

While evaluating the effects of tiferron alone and in combination with piperine against beryllium-induced biochemical alterations and oxidative stress, it was found that the combination reversed all the variables significantly towards the control (Nirala et al. 2008).

In a randomized, crossover and placebo controlled study of influence of piperine on the pharmacokinetics of nevirapine (an antiretroviral drug) under fasting conditions, the piperine, and placebo were administered to healthy adult males for 6 days. On the 7th day, piperine and placebo were administered with nevirapine.

Post-dosing blood samples showed enhanced bioavailability of nevirapine with piperine (Kasibhatta and Naidu 2007).

A study of effect of oral curcumin with piperine on the pain and the markers of oxidative stress in patients with tropical pancreatitis for 6 weeks revealed that there was significant reduction of the erythrocyte malondialdehyde levels in combination therapy as compared to placebo treatment with significant increase in glutathione levels (Durgaprasad et al. 2005).

A 1.3 times more plasma bioavailability of epigallocatechin-3-gallate was observed in CF-1 mice when taken with piperine as compared to epigallocatechin-3-gallate alone. The mechanism involved inhibition of glucuronidation and GIT (Lambert et al. 2004).

35.10 Ginger as Bioavailability Enhancer

Ginger is one of the components of Trikatu which also possess significant bioavailability enhancement activity. It has a powerful effect on mucous membrane of gastrointestinal tract. It regulates the intestinal functions to facilitate absorption. Ginger when used in the dose of 10–30 mg/kg body weight acts as bioenhancer. Pharmacological studies shows that it dramatically enhanced the bioavailability of various medicines specially antibiotics like amoxicillin, azithromycin, erythromycin, cephalexin, cefadroxil, cloxacillin, etc. (Qazi et al. 2002).

35.11 Therapeutic Indication (https://www.ayurtimes.com/trikatu-churna/)

Trikatu churna is helpful in following health conditions.

- Constipation with mucous or sticky stool
- Loss of appetite
- Indigestion
- Gas or flatulence
- Bloating
- Abdominal distension
- Irritable bowel syndrome (IBS)
- Common cold (acute phase during running nose)
- Cough with thin white phlegm
- Asthma (chest congestion due to phlegm)
- Weight loss (obesity)
- Body aches with feeling of heaviness in the body
- High cholesterol levels
- Atherosclerosis
- High blood pressure due to hypercholesterolemia
- Gout

35.12 Caution (https://www.ayurtimes.com/trikatu-churna/)

However, Trikatu churna contains herbs and spices, which we use in our daily kitchen, but the excess intake can cause some unwanted effects. In the dosage less than 1 gram per day, it is safe to use.

35.13 Side Effects (https://www.ayurtimes.com/trikatu-churna/)

The most common side effect of Trikatu is heartburn and acidity. The excess dosage may cause following side effects.

- Burning aftertaste
- Heartburn
- Burning sensation in the throat
- Heat sensation in the body
- Mouth ulcer (rare)
- Sweating (rare)
- Redness in eyes or burning sensation in eyes (very rare)

35.14 Contraindications (https://www.ayurtimes.com/trikatu-churna/)

- Acid dyspepsia
- Heartburn
- Burning sensation in any part of the body such as in the throat, abdomen, feet, or hands
- Vomiting
- Red eyes
- Skin diseases with burning sensation as a symptom
- Constipation with dry and hard stool or bleeding in stool
- Bleeding disorders
- High-risk pregnancies
- Threatened abortion

35.15 Conclusion

Trikatu being a herbal formulation will be the best solution for bioavailability related issues with Ayurvedic and herbal formulations. It has got the tremendous potential to increase the bioavailability of nutrients from our daily food chart, making them available in the body all the time they are required transforming a simple food into medicine.

The scientific findings further strengthen the claims of the traditional ancient texts about Trikatu's health benefits.

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Dietary Factors Associated with Cancer

36

Arundhati Rai

Abstract

There is an important piece of evidence to support the concept that most human cancers arise from interaction between the environment (including diet, smoking, etc.) and the genetic material of cells. As seen from the perspective of the experimental biologist, current concepts of the aetiology of human cancer have generally focused on three agents: viruses, radiation and chemicals. Epidemiologists tend to separate out dietary patterns as a specific separate cause, although part of the dietary story overlaps with that of chemicals. At the earliest stages in the cancer process, known individual dietary carcinogens, such as heterocyclic amines, polycyclic aromatic hydrocarbons and N-nitroso compounds, may contribute directly to the body's carcinogenic load. Such compounds are found in cooked foods, cured and spoiled foods and some alcoholic drinks. The extent to which such dietary carcinogens may initiate the cancer process depends on the general quality of diets. This chapter tends to; if diets are high in vegetables and fruits, the availability of a large number of bioactive compounds is increased; these induce detoxification enzymes, which, in turn, reduce the exposure of DNA to carcinogens.

Keywords

Dietary factors · Cancer · Carcinogens · Cooked foods · Bioactive compounds

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36.1 Introduction

Cancer is a complex group of disease with many possible causes such as physical and chemical agents, hormones, infection and inflammation, radiation, heredity and lifestyle factors like alcohol, diet and obesity. In the intermediate stages of carcinogenesis, in which there is growth of the initiated clone of cells, it is probable that energy balance and turnover are critical in maintaining normal cell behaviour or allowing expansion of the abnormal cells. Leanness reduces risk, and obesity increases the likelihood of tumorigenesis, probably through the action of specific hormones and growth factors. This in turn suggests a role for total energy intake and physical activity in the cancer process. In the cancer process, when DNA damage is again central, vegetables and fruits provide folate, a major source of physiological methyl donors reducing the likelihood of DNA hypomethylation and chromosome breakage. In the specific case of the colon, fibre may be fermented by the colonic bacteria producing volatile fatty acids; these may increase the probability that abnormal cells will undergo programmed cell death – apoptosis. Antioxidants are widespread in foods of plant origin and may reduce the generation of oxygen radicals; these radicals are believed to play a role in the later stages of the cancer process during the gross disorganization of DNA. Energy-dense diets that contain substantial amounts of fat may create more reactive metabolites, including lipid peroxides and oxygen radicals (Potter 1997).

Through such chemical changes, the constituents of diet and connected factors, methods of food processing, specific foods and drinks as well as diets as a whole have a part to play in all the stages of the cancer process. Together with this, there is interplay between environmental factors and predisposition, both inherited and acquired, and this may vary at different stages of life (Potter 1997).

36.2 Dietary Modifiers and Biological Responses

Many human cancers that are widely prevalent today can be prevented through modifications of lifestyles of which diet appears to be an important agent (Ramadas and Somepalli 2002). Increasingly genetics are being recognized to have an intimate involvement in directing the cancer process and thus a likely modifier of the response to diet (Milner 2002). More than 500 dietary compounds have been identified as potential modifier of cancer. Compounds encompassing diverse categories as carotenoids, dithiolthiones, flavonoids, glucosinolates, isothiocyanates, allylsulphide and fermentable fibres have been found to influence experimentally induced cancers. Numerous reviews reporting the merits and possible risks of these and other bioactive food components have surfaced in recent years (Abdulla and Gruber 2000; Craig 1997; Diplock et al. 1998; Gill and Cross 2000; Milner 2000; Potter 1997). Increasingly nutrients are being recognized to influence genetic and epigenetic processes that determine cellular metabolism, differentiation and apoptosis (Bradlow et al. 1999; Knowles and Milner 2000; Lipkin et al. 1999; Zhu and Williams 1998). The study of nutritional genomics is beginning to reveal that nutrient excesses and deficiencies can bring about a host of genomic and proteonomic changes.

The following are mechanisms by which nutrients may suppress the cancer process (Milner 2002).

- Inhibit genetic damage caused by endogenous and exogenous agents by:
  - Inhibiting carcinogen uptake
  - Retarding activation
  - Enhancing detoxification
  - Scavenging oxygen radicals
  - Preventing DNA binding
- Influence repair structural/functional genetic defects by:
  - Enhancing endogenous repair
  - Restoring proper methylation
- Eliminate damaged cells or clones by:
  - Inducing apoptosis
  - Promoting differentiation
  - Enhancing immunosurveillance
- Suppress growth and clonal evolution by:
  - Stopping proliferation
  - Retarding angiogenesis
  - Inhibiting invasion

A host of factors can contribute to DNA instability and ultimately to tumour development. Some nutrients such as unsaturated fatty acids and iron may influence endogenous agents including methylating agents and reactive oxygen species leading to DNA damage, while other components such as flavonoids and folate may function to enhance endogenous repair mechanisms. Folate has been found to be essential in maintaining normal DNA synthesis (Duthie et al. 2000). Some dietary components may also retard repairs as has been suggested following alcohol exposure (Asami et al. 2000). Caloric restriction has repeatedly found to retard chemically induced and spontaneous tumours in model systems (Kritchevsky 1999). In addition to influencing immunocompetence, the availability of nutrients can influence the invasiveness of tumours and retard angiogenesis. Clinical studies provide evidence that angiogenesis in solid tumour relates to a poor prognosis and activity of this angiogenesis can be suppressed by resveratrol found in grapes or n-3 fatty acids occurring in fish (Pinto et al. 1999; Rose and Connolly 2000; Subbaramaiah et al. 1999).

36.3 Dietary Carcinogens

The sources of carcinogens in the food supply are attributed to food additives, synthetic pesticides and various environmental contaminants; these chemicals represent less than 1% of carcinogens found in foods. Most known dietary carcinogens either naturally occur in plants or are produced during food preparation (Greenwald 2000).

36.3.1 Contaminants

Many foods and drinks (including water) contain trace residues of chemicals used in agriculture, food manufacture and other industry. Soil has always been fertilized to increase crop yield. Synthetic fertilizer is an indispensable part of modern intensive agriculture. Consequently, the nitrate content of soil and water and thus of foods and drinking water has steadily increased. Chemicals used as food processing aids or in packaging may migrate into these foods as sold and eaten. Water supplies may contain fluoride or chlorine added for reasons of public health and may be contaminated both with organic by-products of chlorine and with residues of other chemicals. Residues of some industrial chemicals accumulate in fatty foods at different stages in the food chain and also accumulate in human fatty tissue. Some of these show hormonal (oestrogenic) activity. A large number of these chemicals are known to be toxic, and some have been determined to be mutagenic or carcinogenic in experimental conditions.

Evidence from three case-control studies suggest that dietary intake of DDT may increase the risk of breast cancer (Potter 1997). Polymeric materials used in packaging are inert, but their monomers, such as vinyl chloride and acrylonitrile, can and do migrate into foods, as do plasticizers such as phthalates. These are mutagenic or carcinogenic in animals. Vinyl chloride has been classified by IARC in group 1 (human carcinogen). Acrylonitrile and acrylamide have been classified by IARC in group 2A (probable human carcinogens). Di-(2- ethylhexyl)-phthalate has been classified by IARC in group 2B (possible human carcinogen). Chloroform, a by-product of chlorinated water, is classified in group 2B (possible human carcinogen) (Potter 1997).

36.3.2 Microbial Contaminants

Certain substances naturally present in food and other substances generated during its preparation have carcinogenic potential. An important source of carcinogen is mycotoxin (Tulpule and Bhat 1978). Mycotoxins are an important class of over 400 naturally occurring toxic compounds produced by fungi (moulds) of which only small number have been shown to be toxic to mammals. Mycotoxin contamination of food stuffs is a major problem in our country. Agriculture commodities such as groundnuts, refined oil, rice and maize are the major commodities which are contaminated with aflatoxins. This problem is being much worse when grains are exposed to unfavourable weather conditions like unseasonal rains, floods and cyclonic conditions. Risks to human health associated with groundnuts contaminated with aflatoxins have been reviewed by Bhat (1987). IARC (1993) concluded that there is sufficient evidence for the carcinogenicity in humans of natural mixtures of aflatoxins and of aflatoxins B_1. Fumonisins mycotoxins have been classified by IARC as possible human carcinogens on the basis of evidence of carcinogenicity in animals and inadequate evidence in humans (IARC 1993). High aflatoxins contamination probably increases the risk of liver cancer (Potter 1997).

36.3.3 Food Additives

Most manufactured foods and drinks contain chemicals added as a deliberate part of the manufacturing process. Colours and flavours are used in manufactured foods to make them more appealing. Preservatives, including antioxidants, are designed to extend food shelf life and/or to protect against microbial contamination. Emulsifiers, stabilizers, solvent and sweeteners are used extensively as are processing aids present as residues in food. As with chemical contaminants, a number of food additives are known to be toxic, and some have been determined to be mutagenic or carcinogenic in experimental conditions. Saccharin is classified by IARC in group 2B (possible human carcinogen) (IARC 1987). Butter yellow – an azo dye once used as a colouring agent in butter – is known to be carcinogenic (Miller and Miller 1953). Nitrates and nitrites are used in cured meats, fish and cheese. There is an extensive literature on the relationship between nitrates, nitrites and N-nitroso compounds and human cancer risk. Salting and pickling commonly involve certain chemicals that are known to combine with protamines in the stomach to produce nitrosamine-powerful carcinogenic agents. This mechanism may account for the continued high incidence of stomach cancer in some areas of Japan and possibly certain other parts of the world; the hypothesis is supported by epidemiological studies in North America and Europe (Miller et al. 1994).

36.3.4 Products of Food Preparation

Cooking food at very high temperatures, especially in flame, generates chemicals that are mutagenic or carcinogenic in experimental conditions. Grilling (broiling) meat, fish or other foods with intense heat over a direct flame results in fat dropping on the hot fire and yielding flames containing a number of polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene and dibenzo[a,h]anthracene. These chemicals adhere to the surface of the food. The more intense the heat, the more PAHs is present (Bogovski 1983). These foods also contain heterocyclic amines (HCAs), which are also potent mutagens and animal carcinogens. A fairly consistent association between grilled (broiled), but not fried fish and meat and stomach cancer suggests that dietary exposure to PAHs may be involved in human gastric carcinogenesis (Potter 1997). In addition, burnt and browned material can be highly mutagenic. Caramelized sugars contain variety of DNA damaging agents.

36.4 Dietary Anticarcinogens

Experimental research has identified hundreds of food-derived compounds as having inhibitory effects on carcinogens. Epidemiological studies emphasizing overall dietary intake or consumption of specific foods provide consistent evidence that population consuming higher levels of fruits and vegetables are at decreased risk of various cancers relative to populations with lesser intakes. Many chemicals that are naturally present in plants, but are not nutrients, have been shown in the laboratory to have potentially anti-carcinogenic properties (Potter and Steinmetz 1996). Classes of these compounds include carotenoids and flavonoids, which can act as antioxidants; isothiocyanates, which can increase the activity of phase-2 detoxification enzymes; sulphur-containing compounds, which might inhibit synthesis of nitrites by bacteria in the stomach (Milner 2001); isoflavones, which could have antioestrogenic effects (Adlercreutz and Mazur 1997); and phytosterols, which could affect signal transduction pathways and apoptosis (Awad and Fink 2000).

36.4.1 Carotenoids

Commonly consumed green, yellow red and yellow orange vegetables and fruits contain more than 40 carotenoids (Khachik et al. 1997) including beta-carotene, lutein, lycopene and xanthines. Various carotenoids may serve as cancer preventive agents (Krinsky 1998). Among the promising carotenoids is lycopene found in tomato and tomato-based products. Inverse relationship exists between intake of tomatoes or plasma lycopene levels and risk of cancer at various sites, particularly the prostate, lung and stomach (Giovannucci 1999). The health benefits of lycopene have been attributed to its antioxidant properties, although other mechanisms of lycopene action are possible, including the modulation of intercellular communication, hormonal and immune system changes and enhancement of gap junctional communication (Obermuller-Jevic et al. 2003). In breast cancer cells, lycopene can interfere with insulin-like growth factor I-stimulated tumour cell proliferation (Karas et al. 2000). Lycopene inhibits the proliferation of cancer cells in test tube research (Levy et al. 1995). Some carotenoids are converted to vitamin A, which is required for growth and the normal development and differentiation of tissues. It has also been suggested that carotenoids act as antioxidants in tissues, deactivating free radicals (Potter 1997).

36.4.2 Allium Compounds

Allium compounds are found in the allium vegetables, which include onions, garlic, scallions and chives. It is these compounds that account for the distinctive flavour and aroma of allium vegetables, as well as for the many reported medicinal effects. Allium compounds contain sulphur. In garlic, the amino acid allin is enzymatically converted to allicin when garlic cloves are crushed. Allicin is unstable and converts rapidly to sulphide compounds, such as diallyl sulphide and allyl methyl trisulphide. An average clove of garlic contains several milligrams of sulphide. The sulphur compounds inhibit cell proliferation of cancer cells, modulate cell cycle activity and interfere with hormone action in cancer cells (Hirsch et al. 2000; Munday and Munday 2001; Nakagawa et al. 2001; Pinto and Rivlin 2001).

Garlic also contains allixin, a phenolic compound. Allium compounds may have anti-carcinogenic mechanisms involving the induction of enzymatic detoxification systems. Allium vegetables have also been hypothesized to protect against cancer by inhibiting the bacterial conversion of nitrate to nitrite in the stomach.

36.4.3 Phytoestrogens

Phytoestrogens, including isoflavones and lignans, are found in foods of plant origin. These are diphenolic compounds with a variety of structures. Sources of phytoestrogens, isoflavonoids and lignans include legumes, whole grains, fruits and berries (Clarke et al. 1996). Phytoestrogens have numerous biological effects. They are antiviral, anti-proliferative and growth inhibiting. Phytoestrogens exhibit both weak estrogenic and anti-oestrogenic effects (Wiseman 1996) and can compete with steroid hormones for various enzymes and receptors. They also stimulate production of sex-hormone-binding globulin in the liver. In these ways, they may alter steroid hormone metabolism and, by inhibiting growth and proliferation of hormone-dependent cancer cells, may alter cancer risk (Potter 1997).

36.4.4 Isoflavonoids

Isoflavonoids, common in soy foods, inhibit angiogenesis, cell cycle progression and aromatase enzyme and stimulates sex-hormone-binding globulin synthesis and hence antioxidant properties (Murkies et al. 1998). In some tissues, soy acts as a mild pro-oestrogen (bone and brain), while in others it acts as an antioestrogen (breast and uterus). The evidence supporting an understanding of the cancer-preventive potential of soy protein comes from a combination of evidence in cell culture, animals and humans (Heber 2004). In addition, the studies of population eating soy protein indicated that they had a lower incidence of breast cancer and other common cancers compared to populations such as the US population where soy foods were rarely eaten (Yamamoto et al. 2003). Soy protein, isoflavones have been shown to influence protein synthesis, intracellular enzymes, growth factor action, malignant cell proliferation, differentiation and angiogenesis, providing strong evidence that these substances may have a protective role in cancer (Kim et al. 2002).

36.4.5 Phenolic Compounds

Phenolic compounds are found in freshly harvested vegetables and fruits and relatively large amounts in teas and wines. These include caffeic, ferulic and ellagic acids. Ellagic acid is found in high concentrations in fruits and nuts (Birt and Bresnick 1991; Hollman and Venema 1993). Specifically strawberries, raspberries, blackberries, walnuts and pecans possess a number of anti-carcinogenic properties (Barch et al. 1996). Phenolic compounds are involved in the induction of the detoxification systems. Some phenolic compounds have been found to inhibit N-nitrosation reactions by trapping nitrate to form C-nitrosophenolic compounds (Potter 1997).

36.4.6 Flavonoids

Flavonoids represent the most important single group of phenolics in foods (Newmark and American Institute of Cancer Research 1996). Flavonoids are found in fruits, vegetables, coffee, tea, cola and alcoholic beverages. Quercetin, kaempferol and myricetin are flavonols widely distributed in vegetables and fruits. In plants, flavonoids function as potent antioxidants and metal chelators and as repellents to keep certain viruses, fungi and animals from feeding on the plant (Birt and Bresnick 1991). Consumption of flavonoids in general or quercetin-containing foods in particular (Le Marchand et al. 2000) has been associated with protection against cancer in some (Knekt et al. 1997) but not all (Hertog et al. 1995). Potential mechanisms for the anticancer effects of flavonoids include interaction with cytochrome-P-450 mixed function oxidase system and inhibition of tyrosine kinase activity and phosphoinositidase phosphorylation (Guthrie and Carroll 1998; Rodgers and Grant 1998). Flavonoids have been shown to possess anti-proliferative activity against ovarian, breast and stomach cancer cell lines (Ferry et al. 1996).

36.4.7 Green Tea Polyphenols

Tea is one of the most popular beverages in the world, and the consumption of tea has been associated with a decreased risk of developing cancer of the ovary (Zhang et al. 2002), oral cavity (Hsu et al. 2002), colon (Su and Arab 2002), stomach (Setiawan et al. 2001) and prostate (Jian et al. 2004). Experimental studies support that tea polyphenols have anti-mutagenic and anti-carcinogenic activities (Kuroda and Hara 1999). This beneficial health effect has been attributed to the catechins in tea: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Their biological benefits are due to their strong antioxidant and anti-angiogenic activity as well as their potential to inhibit cell proliferation and modulate carcinogen metabolism (Demeule et al. 2002; Kazi et al. 2002). The primary green tea phenols have growth-inhibitory effects, which selectively operate on cancerous cells and not on normal cells. Epidemiological studies support the potential preventive effect of green tea against cancer at various sites including the lung, oesophagus and skin (Yang et al. 1996). Zatonski et al. (1992) reported an inverse association between risk of gallbladder cancer and the amount of tea drunk throughout life.

36.4.8 Glucosinolates

Glucosinolates are found in cruciferous vegetables. During cooking and chewing, the plant enzyme, myrosinase, metabolizes glucosinolates to produce isothiocyanates and indoles. Epidemiological studies have consistently found strong inverse relationship between high consumption of cruciferous vegetables and cancer incidence (Steinmetz and Potter 1996). Cruciferous vegetables contain three groups of glucosinolates-derived compounds likely to be cancer protective: indole 3-carbinol (I3C) and related indole compounds; phenylethyl isothiocyanates (PEITC) and related isothiocyanates such as benzyl isothiocyanates (BITC) and dithiol thionine; and other thiol-containing compounds. The isothiocyanates and indoles have shown promising results against cancer at a number of sites in both animals and human (Hecht 1999; Huang et al. 1998; Kohlmeier et al. 1995).

36.4.9 Protease Inhibitors

Protease inhibitors are widely distributed in plants; cereals and pulses are particularly rich sources. The soybean-derived Bowman-Birk inhibitor (BBI) is major protease inhibitor (Kennedy 1998). Protease inhibitors competitively inhibit protease by the formation of complexes that block the enzyme's catalytic site. Proteases may be an important part of the invasive capacity of some cancer cells (Potter 1997).

36.4.10 Phytic Acid

Phytic acid (inositol hexaphosphate) is particularly found in cereals, nuts, seeds and pulses. Phytic acid forms insoluble salts with specific cations, thus altering both intestinal absorption of specific minerals and redox potential. While it has been shown to be anti-carcinogenic in experimental studies, the mechanisms are unclear but may involve control of cell proliferation (Potter 1997).

36.4.11 Monoterpenes

Monoterpenes are found in the essential oils of citrus fruits and other edible plants (Crowell and American Institute for Cancer Research 1996). The most common monocyclic monoterpenes, D-limonene and its metabolite perillyl alcohol, have been shown to be effective as cancer-protective agents against mammary, skin, liver, lung, stomach, pancreas and prostate (Crowell and American Institute for Cancer Research 1996). It is also used as a flavouring agent in non-alcoholic beverages, ice cream, sweets, baked goods, gelatins, puddings and chewing gum. Terpenoids such as D-limonene are believed to protect against cancer by inducing the family of enzymes called glutathione transferases (Potter 1997).

36.4.12 Curcuminoids

Curcuminoids, the major yellow pigment in turmeric and curry, are a common spice; colouring agent; and herbal drug. It has multiple effects on carcinogenic process including antioxidant activity, reduction in polyamine synthesis and inhibition of tumour initiation and promotion. Curcumine inhibits colon carcinogenesis and has a cytostatic effect on human breast tumour cells (Lin et al. 1994; Man-Ying and Fong 1994).

36.4.13 Plant Sterols

Plant sterols, including beta-sitosterol, campesterol and stigmasterol, are found in vegetables. Vegetarians, who experience lower rates of cancer of many sites, have been shown to have higher levels of the plant sterol, beta-sitosterol, in their faeces, as might be expected. One study in rats showed that inclusion of 0.2% beta-sitosterol in the diet decreased the occurrence of chemically induced tumours in the colon (Fiala et al. 1985; Messina and Barnes 1991; Raicht et al. 1980).

In conclusion diet is one of the most important factors related to cancer in two ways: some foods increase the risk of cancer, i.e. alcohol, aflatoxin, salted preserved foods, preserved meat and red meat, dairy products and other high-fat foods, high caloric intake and very hot drinks and foods. On the other hand, plant-based foods, i.e. fruits, vegetables, soya and whole grain cereals contain carotenoids, vitamins (B2, B6, folate, B12, C, D and E), calcium, zinc and selenium and non-nutrient plant constituents, i.e. allium compounds, flavonoids, etc. are helpful to prevent cancer.

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