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Genevieve Dable-Tupas *Editors*

Functional Foods and Nutraceuticals

Bioactive Components, Formulations and Innovations

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Preface

This book, *Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations*, presents scientific information on the importance of functional foods to human health and the probable mechanisms of nutraceuticals in the prevention, treatment, and management of diseases. This book comes at a time when there is an urgent need to address the increasing cases of metabolic diseases and high rates of deaths resulting from the lack of proper knowledge or deviation from good eating habit. This book is designed to serve a useful purpose as substitute for the unverifiable sources of information on the internet and in the very old literatures, which are doused with all sorts of promotional propaganda for profit-making while leading people astray. The general public should understand what and why they should eat certain foods or use certain dietary supplements. This book serves this purpose while balancing the evidences of their health-promoting benefits and the associated risks.

The global demand for functional foods and nutraceuticals has continuously evolved through the years with a projected global nutraceutical market increase from $241 billion market in 2019 to $373 billion in 2025 at a compound annual growth rate of 7.5%. The first chapter of this book on functional foods and health benefits presents the evolution of our understanding about the terms and concepts of functional foods and nutraceuticals. Evidence-based health benefits of functional foods and/or nutraceuticals are also presented which are further discussed in the succeeding chapters. The following chapters are discussions of the different groups of functional foods and/or nutraceuticals, their sources, bioactive components, health benefits, and risks. Also included are emerging areas of the industry. Topics on regulations and safety were also taken into consideration.

The chapter contributors of this book are respected authors and professionals from key institutions around the world. The primary target audience for this book are users of functional foods, food supplements, and nutraceuticals, food technology students, nutritionists, researchers on natural products and phytochemistry, chemistry students, and public and private health practitioners. Additionally, the general public will find this book useful. The chapters are well thought of and crafted using simple English so that even the general public will be able to understand.

Our sincere appreciation goes to the chapter contributors for their great contributions, patience, and cooperation during the editorial process. Their hard work and dedication have significantly contributed to the completion of this book. Our special thanks go to our families for their support and patience during the whole process. To the management of Springer, we will remain thankful for your guidance and support. To the readers, we hope you will find this book helpful and informative. May our efforts in coming up with this book shed light in your never-ending quest for knowledge as we welcome your views.

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2. Screening of Natural Antidiabetic Agents (2019, with C. Egbuna, S. Palai, I.E. Ebhohimen, A.G. Mtewa, J.C. Ifemeje, and T.L. Kryeziu) in S. Kumar and C. Egbuna (Editors) *Phytochemistry: An In-Silico and In-Vitro Update* (Springer, Singapore)
3. In-Vitro Assays for Antimicrobial Assessment (2019, with J. Kurhekar and M.C.B. Otero) in S. Kumar and C. Egbuna (Editors) *Phytochemistry: An In-Silico and In-Vitro Update* (Springer, Singapore)
4. Phytochelatins and Heavy Metal Tolerance in Plants (2018, with M.C.B. Otero) in C. Egbuna (Editor) *Phytochemistry: Volume 3: Marine Sources, Industrial Applications, and Recent Advances* (USA: Apple Academic Press)

She has authored and mentored several researches both in clinical and preclinical areas mostly on natural products research. Her research interests include natural products research, clinical epidemiology, genomics, and their translation for the benefit of the community.

Chapter 1
Functional Foods and Health Benefits

Genevieve Dable-Tupas, Maria Catherine B. Otero, and Leslie Bernolo

1.1 Introduction

Population demographics, socio-economic changes, increase in life expectancy, and increase in cost of health care have attracted researchers to conduct studies on how these changes can be managed efficiently (Eufic 2006). Functional foods and nutraceuticals have been identified as one of the leading food categories where research and development efforts are concentrated (Kindle 2001). Hence, major companies have become more curious and eventually ventured in the development of foods for health and wellness market. During the 1990s, food industries, including manufacturers of functional foods had 10 to 20% annual increase in sales. In the US, functional foods market has reached $18.5 billion. According to Hasler (2002), the market for functional foods will continue to be strong for the next several years, most especially because consumers have developed interest in self-care, aging demographics and increasing healthcare costs. In 2018, the functional food ingredients market was valued at $68.60 billion and is forecasted to reach $94.21 billion by 2023 at a compounded annual growth rate (CAGR) of 6.6% (Markets and Markets 2019).

The concept of functional foods originated in Japan in the mid-1980s when the Japanese government commenced providing financial assistance to research programs focused on the capability of certain foods to influence physiological

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functions (Litwin et al. 2018). However, up to this day, there is still no single definition of functional foods.

International groups in dietetics and nutrition, such as the International Life Sciences Institute, International Food Information Council, the European Commission, the American Dietetic Association, and the Academy of Nutrition and Dietetics, agree that *"functional foods provide health benefits beyond basic nutrition."* But there is no consensus on whether medical foods, foods for special dietary use, and food supplements are qualified as functional foods. Table 1.1 shows the evolution of the meaning of functional foods.

In this chapter, we operationally define functional foods as *"whole foods along with fortified, enriched, or enhanced foods that have a potentially beneficial effect*

Table 1.1 Evolution of functional foods definition

Organization	Definition of functional foods	Reference
National Academy of Sciences' Food and Nutrition Board	Any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains	Thomas and Earl (1994)
International Life Sciences Institute	Foods that, by virtue of the presence of physiologically-active components, provide a health benefit beyond basic nutrition	Hasler (2002)
Encyclopedia of Food Sciences and Nutrition	Food made from natural ingredients, consumable as part of the daily diet, and has certain function when taken which serves to regulate body processes such as enhancement of biological defense mechanisms, prevention of specific diseases, recovery from specific illness, control of physical and mental disorders and slowing of aging process	Leon (2003)
10th International functional food conference	Natural or processed foods that contain known or unknown biologically-active compounds which provide a clinically proven documented health benefit for the prevention, management, or treatment of chronic diseases	Martirosyan and Singh (2015)
17th International conference organized by the US Department of Agriculture (USDA) and Agricultural Research Service (ARS)	Natural or processed foods that contain known or unknown biologically-active compounds which provide a clinically proven documented health benefit for the prevention, management, or treatment of chronic diseases ***in effective non-toxic amounts***	Martirosyan and Singh (2015)
Academy of Nutrition and Dietetics	Whole foods along with fortified, enriched or enhanced foods with potential beneficial effect on health when eaten as part of a varied diet on a regular basis at effective levels based on significant standards of evidence	Wolfram (2017)
British Nutrition Foundation	Encompassing a very broad range of products from foods developed for a particular functional ingredient to staple foods fortified with a nutrient that are seldom present	British Nutrition Foundation (2018)

on health when consumed as part of a varied diet on a regular basis at effective levels" (Crowe and Francis 2013). We further characterize functional foods as those:

1. found in conventional food forms with inherent sensory characteristics;
2. containing physiologically functional components that are not consumed in medicinal/therapeutic levels;
3. that confer scientifically-proven physiologic benefit/s when consumed as part of the regular diet, but not in the pill or isolated forms;
4. scientifically-proven safe for long-term consumption of the intended population;
5. containing functional components (whether nutrient or phytochemical) that are naturally present, or are added to the food (Sion et al. 2018),
6. may be used to prevent and treat certain diseases.

Another confusing term often used interchangeably with functional food is nutraceuticals. Nutraceuticals is a term derived from 'nutrition' and 'pharmaceuticals' (Kalra 2003). It was coined in 1989 by the founder of Foundation for Innovation in Medicine, Dr. Stephen De Felice, and is defined as 'any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease' (Cencic and Chingwaru 2010). Just like functional foods, nutraceuticals is defined differently in many countries. According to Trottier et al. (2010), when functional food aids in the prevention and/or treatment of diseases and/or disorders other than anaemia, it is called a nutraceutical. In Canada and US, nutraceuticals is defined as 'a product produced from foods but sold in pills, powders, and other medicinal forms not generally associated with food (Bull et al. 2000; Nasri et al. 2014). Hence, the simplest difference between functional foods and nutraceuticals is that a functional food is essentially a food, but a nutraceutical is an isolated or concentrated form used as medicine to improve health, impede aging, prevent chronic diseases, and increase life expectancy (Nasri et al. 2014).

Despite the immense interest in health research, the design of functional foods and nutraceuticals requires high development costs and produces critical challenges, which are sometimes difficult to handle for food companies (Villaño et al. 2016). Thus, introducing functional food to the market entails a series of steps involving research, communication with government agencies, and effective public marketing (IFT 2015). Currently, research efforts have been intensified more to increase understanding of the mechanisms in functional foods. Nutrigenomics and biotechnology are two of the most popular emerging discipline and technology that are known to affect the future of functional foods. According to Pavlidis et al. (2015), nutrigenomics is an emerging science which look into a specific area of nutrition using molecular tools to assess and understand different responses gathered through a certain diet applied between individual and groups. Biotechnology, on the other hand, is a technology that uses biological systems or living organisms to develop new products (NUST 2019).

1.2 Classification of Functional Foods

According to the International Life Sciences Institute, food has three important roles in the body. First is the provision of nutrients so the body can carry out its day to day activities, and for growth and development. Second, food gives satisfaction and improves well-being, especially food that tastes, looks, and smells good. The third, and the most recently added function of food relates to the regulation of physiologic processes in the body by components other than the main nutrients from the food (Sion et al. 2018). The appreciation of this role of food for health fueled global research on functional food science.

There are 3 general classes of functional foods (Table 1.2), based on how these are prepared: conventional foods, modified foods, and food ingredients. Conventional food are whole, unmodified food, such as vegetables and fruits, fish, dairy, and grains, that naturally contain bioactive components that confer health benefits (Crowe and Francis 2013). Conventional food represents the top functional food items recognized by consumers (Food Insight 2009).

Modified foods are normal food items that have been enhanced, enriched, or fortified with functional food components. Some examples of modified foods are fruit juices with calcium, folate-enriched breads, beverages with plant extracts, and iodized salt. Functional food components range from macronutrients, essential micronutrient needed in higher levels, or non-nutrient components like phytochemicals, which are derived from plants, microorganisms, marine organisms, and other inorganic raw materials (Hasler and Brown 2009; Crowe and Francis 2013). The health benefits of common functional food components/ingredients will be discussed in the next section of this chapter.

Food fortification is a general term that describes the supplementation of food components, whether nutrient or non-nutrient, to improve the properties of functional food (FAO/WHO 1994; Dary and Hurrell 2006). Another technical term used in food modification is enrichment. Enriched foods are equivalent to fortified foods, where the "fortificants" are food components normally found in the raw food, but were lost during processing (FAO/WHO 1994; Hoffpauer and Wright 1994). An emergent innovation in food fortification is Biofortification, where the nutrient composition of the food source (animal or plant) is increased

Table 1.2 Classification of functional foods

Classes	Description	Examples
Conventional food	Whole, unmodified food that naturally contain bioactive components that confer health benefits	Vegetables and fruits, fish, dairy, and grains
Modified foods	Food items that have been enhanced, enriched, or fortified with functional food components	Fruit juices with calcium, folate-enriched breads, beverages with plant extracts, and iodized salt
Synthetic food ingredients	Functional components that are synthesized in the laboratory	Inulin-type fructans

via selective breeding or feeding, treatment, or genetic engineering (Calvo and Whiting 2013; Cashman 2015).

The third class of functional foods is synthetic food ingredients, such as inulin-type fructans, that serve as prebiotics (Crowe and Francis 2013). Inulin and oligofructose are well-studied prebiotics known to selectively stimulate beneficial gut microorganisms. Inulin-type fructans are dietary fiber complexes produced using *Aspergillus niger* β-fructosidase (Roberfroid 2007). It is estimated that this area of functional foods will have the highest growth in the next 15 years because of its various applications in almost all food groups (Functional Food Ingredients Market 2018).

International standards and guidelines applied for evaluating functional food are stipulated in the Codex Alimentarius Guidelines for Use of Nutrition and Health Claims (CAC/GL 23-1997). Under this set of guidelines, food manufactures of functional food may label their products with approved health claims when the defined criteria are met. These claims are:

1. Nutrient function claim, which describes the physiologic role of the nutrient in normal functioning, growth, and development;
2. Other function claim, which describes the specific benefits of non-nutrient components on physiologic functions of the body;
3. Reduction of disease risk claim, which relates on the consumption of food or food ingredients to decreased risks for a specific disease or condition.

1.3 Health Benefits

Since time immemorial, it has been common knowledge that eating fruits and vegetables is good for prevention of diseases and promotion of health. However, scientific studies were lacking. With the advent of global researches on functional foods, many of the health claims in the past are now supported by scientific evidence. Indeed, evidence has shown that functional foods can really prevent and/or serve as adjunct in the management of a number of diseases. Randomized controlled trials and systematic reviews presented several health benefits on the use of functional foods. These benefits have been attributed to specific phytochemical components contained in these food sources (Table 1.3). Shown in the table below are some important phytochemicals found in functional foods that are shown to provide health benefits.

There are more phytochemicals contained in functional foods that are not included in the table above which will be discussed in the succeeding chapters of this book. These evidence-based health benefits of functional foods are expected to expand in the future owing to its increasing usage and marketability which demand for more research to be conducted.

Table 1.3 Important phytochemicals in functional foods and their health benefits

Phytochemicals	Functional Food Sources	Health Benefits
Anthocyanins	Red, purple, & blue veggies: Red cabbage, straw/black/blueberries, radishes, plums, & eggplant; elderberry; black rice; bananas	Reduction of cholesterol (Wallace et al. 2016); Prevention of Cardiovascular diseases (Mink et al. 2007; Qin et al. 2009; Cassidy et al. 2011; Hassellund et al. 2012; Jennings et al. 2012; McCullough et al. 2012; Hassellund et al., 2013); Antiobesity or Weight loss (CAB Reviews 2010; Bertoia et al. 2016; Azzini et al. 2017); Cancer prevention (Wang and Stoner 2008; Lin et al. 2017); Enhances cognitive function (Whyte and Williams 2012; Wallace and Giusti 2013)
Beta-carotene	Sweet potato; carrots; spinach; butternut squash; cantaloupe; lettuce; bell pepper; apricot; broccoli; pea; other yellow and orange colored vegetables and fruits	Precursor of Vit. A (Grune et al. 2010; Gul et al. 2015); Inhibits the peroxidation of lipids induced by free radicals (Antioxidant) (Grune et al. 2010; Gul et al. 2015); Prevention of sunburns and photodamage (Krutmann 2008; Stahl and Sies 2012), porphyria (Sassa 2006; Mir 2018) and psoriasis (Rollman and Vahlquist 1985)
Catechins	Green tea, apples (peel on), apricots, cherries, peaches, blackberries, black grapes, strawberries, blueberries and raspberries	Antioxidant (Cabrera et al. 2006); Lowers blood pressure and low density lipoprotein (Kim et al. 2011; Khalesi et al. 2014)
Flavonoids	Citrus fruits, berries, purple grapes, onions, black and green tea, cocoa, legumes, soy, & whole grains	Antioxidant (Brunetti et al. 2013; Banjarnahor and Artnti 2015), Decreases inflammation, may protect against heart disease, stroke & cancer (Serafini et al. 2010; Perez-Cano and Castell 2016)
Lutein	Green leafy vegetables: kale, collards, spinach, romaine & broccoli; yellow corn	Protection from eye disease by absorbing damaging blue light that enters the eye (Johnson 2002); May reduce risk of age-related macular degeneration (Ugusman et al. 2014)
Lycopene	Tomatoes, watermelon & pink grapefruit, pink guava, papaya, seabuckthorn and rosehip	Decreases risk for prostate cancer (Ilic et al. 2011; Ilic and Misso 2012; Wang et al. 2015; Chen et al. 2015; Rowles et al. 2017) and Cardiovascular diseases (Cheng et al. 2017); Cholesterol lowering action (Ried and Fakler 2011); Protection against Metabolic Syndrome (Senkus et al. 2018); Strong antioxidant effect (Chen et al. 2013)
Quercetin	Yellow fruits & veggies: Apples/pears, citrus fruits, onions, some berries, black/green tea	Blood pressure reduction (Serban et al. 2016); Antioxidants, decreases inflammation, & may help protect from heart disease, stroke & cancer (Shah et al. 2016)

(continued)

Table 1.3 (continued)

Phytochemicals	Functional Food Sources	Health Benefits
Resveratrol	Red/purple grapes, red wine, purple grape juice, cocoa/dark chocolate & peanuts	Promotes cardiovascular health; decreases blood pressure and body mass index (Fogacci et al. 2018)
Rutin (vitamin P or Rotuside)	Buckwheat; Asparagus; elderflower tea; unpeeled apples; figs	Anti-inflammatory (Guardia et al. 2001; Gunawardena and Munch 2014; Horcajada et al. 2014; Ganeshpurkara and Salujaa 2017); Neuroprotection against brain ischemia (Pu et al. 2007; Khan et al. 2009; Javed et al. 2012); Improves endothelial function by enhancing nitric oxide production (Ugusman et al. 2014)
Zeaxanthin	Green leafy vegetables: kale, collards, spinach, romaine & broccoli; yellow corn	Protection from eye disease by absorbing damaging blue light that enters the eye (Johnson 2002); May reduce risk of age-related macular degeneration (Ugusman et al. 2014)

1.4 Summary

Presented in this introductory chapter are the common phytochemicals contained in functional foods with evidence-based health benefits. In view of a variety of definitions found in literatures regarding functional foods, we operationally defined functional foods as *"whole foods along with fortified, enriched, or enhanced foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels"* (Crowe and Francis, 2013). Functional foods differ from another related term nutraceuticals in that functional foods are essentially foods while nutraceuticals are derived from foods but are used as medicines. A tabulated classification of functional foods was also presented to improve understanding of the term. Overall, numerous claims on the health benefits of functional foods have been documented with evidence-based researches. Henceforward, evidence-based health benefits are more likely to surface in the coming years owing to the current popularity of functional foods. Hopefully, this will encourage people to go back to eating natural, unprocessed healthier food sources to promote health and prevent diseases.

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Chapter 2
Nutraceuticals: History, Classification and Market Demand

Onyeka Kingsley Nwosu and Kingsley Ikechukwu Ubaoji

2.1 Introduction

Over the past few years, an increasing number of dietary supplements have become available in supermarkets and health food shops and even also available for purchase in pharmaceutical shops.

The term "nutraceutical" is used to define these nutritionally or medicinally functional foods. Nutraceuticals, which have also been called medical foods, designer foods, functional foods, phytochemicals and nutritional supplements, comprise such everyday products as "bio" yoghurts and fortified breakfast cereals, as well as vitamins, herbal remedies and even genetically/living modified foods and supplements. Many different terms and meanings are used in different countries, which can result in confusion. Nutraceuticals is a comprehensive umbrella term that is used to define any product resulting from food sources with extra health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to promote general well-being, prevent malignant processes and control symptoms. Generally, nutraceutical is said to be a "food, or parts of a food, that provide health benefits, including the prevention and treatment of disease (Cencic and Chingwaru 2010).

In recent times, nutraceuticals have attracted substantial interest owing to their prospective nutritional, safety and therapeutic effects. They could have a role in a plethora of biological processes, including gene expression, antioxidant defenses, cell proliferation, and maintenance the integrity of the mitochondria.

Nutraceuticals therefore, may be used to improve health, avoid chronic diseases, delay the aging process (and in turn increase life expectancy), or just maintain

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functions and integrity of the body. They are considered to be healthy sources for prevention of life threatening diseases such as diabetes, renal and gastrointestinal disorders, as well as diverse infections.

A wide range of nutraceuticals have been shown to enforce vital roles in immune status and vulnerability to certain disease states. They also reveal diseases adjusting indications related to oxidative stress including allergy, Alzheimer's disease, cardiovascular diseases, cancer, eye conditions, Parkinson's diseases and obesity (Pandey et al. 2010).

2.2 Nutraceuticals

Nutraceuticals refers to foods having a medicinal effect on health of human beings. The word 'nutraceuticals' comprises of 'Nutrients' and 'Pharmaceuticals'. According to AAFCO 1996, 'Nutrient' means a feed constituent in a form and at a level that will aid, sustain a life of human being or animal while 'Nutraceutical' means any non-toxic food constituent that has scientifically proven health benefits together with prevention and treatment of diseases. Products isolated or purified from food are sold in medicinal forms not usually associated with food. A nutraceutical has a functional benefit that provides protection against chronic diseases.

These nutraceuticals include food supplements, herbal products, probiotics and prebiotics, and medical foods meant for prevention and treatment of diseases. Key nutraceuticals possess multiple therapeutic effect hence attract more consumer interest. Increased awareness towards preventive therapies and cumulative disposable income, promising pricing environment growth in pharma retail chain and rise in healthcare spending are mainly responsible for the driving force increasing market demands for nutraceuticals.

2.2.1 History

The concept of Nutraceuticals went back as far as 3000 years ago. Hippocrates (460–377 B.C) stated 'let food be thy medicine and medicine be thy food '. In the early 1900s the United States of America food manufacturers started adding small quantity of iodine to salt to prevent goiter. The term nutraceuticals was coined in 1989 by Stephen DeFelice who was the Chairman and Founder of the Foundation for Innovation in Medicine, Cranford, New Jersey (Bieselkl 2001). According to DeFelice, nutraceutical can be said to be "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." However, the term nutraceutical as commonly used in marketing has no regulatory definition (Zeisel 1999).

In England, Japan and other countries, nutraceuticals are already becoming part of dietary landscape. Diet was first considered by Germany, France and the United Kingdom as a more important factor than exercise or hereditary factors in achieving good health. Canada defined them as 'product of foods but sold in pills, powders, (potions) and other medicinal forms not normally associated with food'. In India, nutraceuticals are seen as the food components made from herbal or botanical raw materials, which are used for preventing or treating different types of chronic and acute maladies (Thakur et al. 2010). Nowadays, nutraceuticals are one of the most rapidly growing segments of the industry with an expected compound annual growth rate (CAGR) of 7.5% (Healthcare Packaging 2019). The global nutraceutical market is estimated to increase from $241 billion market in 2019 to $373 billion in 2025 (Healthcare Packaging 2019). The definite use of nutraceuticals has been to achieve desirable therapeutic outcomes with reduced side effects. Herbal Nutraceuticals are powerful instruments in sustaining health and act contrary to nutritionally induced acute and chronic diseases by promoting optimal health, longevity and quality of life.

2.2.2 Scope

The philosophy behind nutraceuticals is focused on prevention. Most times it can be used in the context of Dietary supplements and/or functional food.

(a) **Dietary Supplements:** Dietary supplements are products envisioned to complement the diet that accepts or contains one or more of the following dietary ingredients: a mineral, a vitamin, an amino acid, a herb or other botanical, constituent, metabolite, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, extract, or combinations of these ingredients (Zeisel 1999). Dietary supplements are not intended to treat or remedy disease whereas nutraceuticals emphasize more on the expected results of these products, such as prevention or treatment of diseases.
(b) **Functional Food:** As defined by the United States of America Institute of Medicine's Food and Nutrition Board, functional food is "any food or food ingredient that may offer a health benefit beyond the traditional nutrients it contains". The functional food concept is – "Food products to be taken as part of the usual diet in order to have helpful effects that go beyond basic nutritional function". Functional foods contain physiologically active components obtained either from plants or animal sources (Ernst 2001).

2.2.3 Classification

Nutraceuticals or functional foods can be classified on the basis of their sources: natural or traditional and unnatural or non-traditional.

(a) On the basis of natural source, it can be classified as the products obtained from plants, animals, minerals, or microbial sources. This classification can be referred to as Traditional Nutraceuticals.
(b) Nutraceuticals as prepared via biotechnology: this classification can be referred to as Non-Traditional Nutraceuticals.

2.2.3.1 Traditional Nutraceuticals

They are natural products with no changes to the food. They contain numerous natural components that convey benefits beyond basic nutrition, like omega-3 fatty acids in salmon, saponins in soy or lycopene in tomatoes. The traditional nutraceuticals can be divided on the basis of:

(a) Chemical Constituents.
    (i) Nutrients.
    (ii) Herbals.
    (iii) Phytochemicals.
(b) Nutraceutical Enzymes.
    (i) Chemical Constituents.
(c) Probiotic Microorganisms.

Nutrients The nutrients include amino acids, fatty acids, minerals and vitamins with recognized nutritional functions. Most foods contain vitamins that aid in curing diseases like stroke, cataracts, osteoporosis and heart diseases. Minerals found in plants, animals and dairy products are useful in osteoporosis, anemia and in building strong bones, teeth, muscles, and improve nerve impulses and heart rhythm. Foods that contain fatty acids like omega-3 PUFAs are potent regulators of the inflammatory processes, maintenance of brain function and reduction in cholesterol deposition (Chauhan et al. 2013).

Herbals Herbal nutraceuticals help to improve health and avert chronic diseases. Most of these are analgesic, anti-inflammatory, astringent, antipyretic and antiarthritic. Some of the herbals contain flavonoids like apiol, psoralen that are diuretic, carminative and antipyretic. Peppermint (Menthapiperita) contains menthol as an active component that help cure cold and flu (Ehrlich 2009). Some of the plants contain tannin which is claimed to aid in the management of depression, cold, stress, cough, hypertension and asthma while proanthocyanadin found in some herbals are useful in the treatment or prevention of cancer, ulcers and urinary tract infections (Chauhan et al. 2013).

Phytochemicals Phytochemicals are plant nutrients with particular biological activities that promote human health (Zhao 2007). They are also referred to as Phytonutrients. They work by serving as substrate for biochemical reactions, cofactors or inhibitors of enzymatic reactions, absorbents that bind to and eradicate unwanted constituent in the intestine and improve the absorption and/or stability of indispensable nutrients among others (Zhao 2007).

Nutraceutical Enzymes These are enzymes that are derived from plant, animal and microbial sources. Enzymes are an essential part of life, without which our bodies would cease to function optimally. Medical conditions such as blood sugar disorders, digestive problems and obesity have their symptoms eliminated by enzyme supplements in the diet.

Probiotic Microorganisms Probiotics mean ‘for life’. They are defined as live microorganisms, which when consumed in tolerable amounts, confer a health effect on the host (Michail et al. 2006). These microorganisms are responsive bacteria that promote healthy digestion and absorption of some nutrients. They most importantly act to mob out pathogens, like yeasts and other bacteria and viruses that may cause disease and develop a communally advantageous symbiosis with the human gastrointestinal tract (Holzapfel et al. 2001). They possess an antimicrobial effect through altering the microflora, averting adhesion of pathogens to the intestinal epithelium, competing for nutrients necessary for pathogen survival, producing an antitoxin effect and retrogressing some of the consequences of infection on the intestinal epithelium, such as secretory changes and neutrophil migration. For instance, probiotics can cure lactose intolerance by enhancing the production of a specific enzyme (ß-galactosidase) that can hydrolyze the offending lactose into its component sugars (Oak and Jha 2019).

2.2.3.2 Non-Traditional Nutraceuticals

These are the artificial foods developed via biotechnology. The bioactive components in food samples are engineered to produce products for human-wellness. They can be grouped into fortified nutraceuticals and recombinant nutraceuticals.

Fortified Nutraceuticals These are nutraceuticals from agrarian breeding or added nutrients and/or ingredients. Examples include cereals with added vitamins or minerals, milk fortified with cholecalciferol used in vitamin D deficiency, flour with added folic acid, prebiotic and probiotic fortified milk with Bifidobacteriumlactis HN019 used in diarrhea, respiratory infections and severe illnesses, in children (Sazawal et al. 2010), and orange juice fortified with calcium.

Recombinant Nutraceuticals Recombinant nutraceuticals include the making of probiotics and the extraction of bioactive components by enzyme/fermentation technologies as well as genetic engineering technology. Also, energy-providing foods, such as bread, alcohol, fermented starch, yoghurt, cheese, vinegar, and others are produced using modern biotechnology. Examples include cows with lactoferrin deficiency is engineered with recombinant human lactoferrin (rhLf) to be able to solve the lactoferrin deficiency (Hyvonen et al. 2006).

2.3 Herbals as Nutraceuticals

Herbs play a significant role in the maintenance of the quality of human life through the abundant source of bio-constituents. The herbal bioactive constituents are an essential category of nutraceuticals which have plenty of health promoting medicinal properties in addition to minerals, vitamins and other active compounds. The herbs harbor a widespread variety of active phytochemicals like flavonoids, terpenoids, saponin, and polyphenols. These herbal bioactives are most times commonly used by people who seek conventional health care as a food supplement. In this regard, it is seen as using herbs as nutraceutical (Chauhan et al. 2013). Example of herbs used as nutraceuticals are presented in Table 2.1.

Table 2.1 The common herbals used as nutraceuticals

	Biological Name	Common Name	Part of the Plant	Bioactive compounds	Benefits to Health
1	*Zingiber officinale (Zingiberaceae.)*	Ginger	Rhizomes	Zingiberene and gingerols	Hyperglycemia, chronic bronchitis, stimulant and throat ache
2	*Panax ginseng (Araliaceae)*	Ginseng	Root	Ginsenosides and Panaxosides	Stimulating immune and nervous system
3	*Allium sativum (Liliaceae)*	Garlic	Bulbs	Alliin and Allicin	Antibacterial, anti-inflammatory, antifungal, antigout, antithrombotic, hypotensive, antihyperlipidemic
4	*Aloe barbadensis Mill. (Liliaceae)*	*Aloe vera*	Gel	Aloins and aloesin	Dilates capillaries, anti-inflammatory, emollient, wound healing properties
5	*Curcuma Longa (Zingiberacae)*	Turmeric	Rhizome	Curcumin	Anticancer, anti-inflammatory, antiseptic, antiarthritic.
6	*Allium cepa Linn. (Liliaceae)*	Onion	Bulb	Allicin and alliin	Hypoglycemic activity, antibiotic and antiatherosclerosis
7	*Ginkgo biloba (Ginkgoacea)*	Maiden Hair Tree	Leaves	Ginkgolide and bilobalide	Antioxidant, memory enhancer, increased peripheral blood flow, treatment of post-thrombotic syndrome

(continued)

Table 2.1 (continued)

	Biological Name	Common Name	Part of the Plant	Bioactive compounds	Benefits to Health
8	*Cassia angustifolia (Leguminosae)*	Senna	Leaves	Sennosides	Purgative
9	*Echinacea purpurea (Asteraceae)*	Echinacea	Leaves	Alkylamide and echinacoside	Antiviral, anti-inflammatory and immunomodulator
10	*Glycyrrhizaglabra (leguminosae)*	Liquorice	Root	Glycyrrhizin and liquirtin	Anti-inflammatory and anti-allergic.
11	*Hydrastiscanadensis L. (Ranunculaceae)*	Goldenseal	Root	Hydrastine berberine and canadine	Antimicrobial, astringent, antihemorrhagic, treatment of mucosal inflammation, dyspepsia, gastritis
12	*Marrubiumvulgare L. (Lamiaceae)*	Horehound	Whole plant	11-oxomarrubiin and vulgarcoside A	Expectorant, antitussive, choleretic
13	*Salix alba L. (Salicaceae)*	White Willow	Bark	Salicin	Antiinflammatory, analgesic, antipyretic, astringent, treatment of rheumatic and arthritic.
14	*Hypericumperforatum (Hypericaceae)*	St. John's wort	Aerial part	Hypericin and hyperforin	Antidepressant, against HIV and hepatitis-c virus
15	*Valerianaofficinalis Linn. (Valerianaceae)*	Valeriana	Root	Valerenic acid and valerate	Menstrual pain, intestinal cramps, bronchial spasm, tranquillizer, migraine
16	*Aeglemarmelos Corr. (Rutaceae)*	Bael	Unripe fruits	Marmelosin	Treatment of diarrhea and dysentery, digestive, appetizer.

Source: Modified from Chauhan et al. (2013). https://creativecommons.org/licenses/by/4.0/

2.3.1 Herbal Nutraceutical Safety and Regulation

The processes of manufacturing and the quality of plant material used for herbal nutraceuticals are under the regulations of food laws, which are deficient of the specificity required for botanical drugs. This indeed may have serious consequences. Toxin contamination like fungal and bacterial toxins, adulterations and numerous other types of impurity of herbal nutraceuticals conceivably remain undetected simply because there is an almost total absence of specific quality control. Lack of quality control not only increases the potential threats to the consumer, it may also result in a total lack of the drive to conduct suitable research that determines the possible benefits of nutraceuticals or ensures their safety (Ernst 2001).

Food and medicine regulation is intended to protect consumer's health, increase economic viability, and harmonize well-being and the engendering fair trade on foods within and between nations. If indeed an assertion was made that implied medicinal benefit concerning a herbal nutraceutical product, the product would be required to conform with the regulatory requirements for medicinal products, in respect of safety, efficacy, and quality testing and marketing authorization procedures (Pandey et al. 2010). For herbal nutraceutical industries, two challenges are apparent; regulatory uncertainty and integrity of labeling claims.

For years, the Food and Drug Administration (FDA) regulated dietary supplements as foods to ensure that they were safe and wholesome and also ensure that the labeling was truthful and not deceptive. The FDA may establish good manufacturing practices for nutraceuticals as long as these regulations are molded after the less strict regulations for foods as divergent to those for drugs.

The Indian government in 2006 passed Food Safety and Standard Act to incorporate and streamline the many regulations covering foods, dietary supplements and nutraceuticals. The act recommended the creation of the Food Safety and Standards Authority (FSSA). The FSSA was expected to unify the existing eight laws i.e. harmonization of the laws to align with the international regulations and science-based standards, maintain clarity and uniformity on novel food areas and also help curb corruptions.

Importance of Herbal Nutraceutical Regulation:

(I) Allows better legal security and more predictable environment.
(II) Supports innovation (food, herbal and drink products).
(III) Prevents imbalanced competition from manufacturers using untruthful or misleading claims.
(IV) If confident claims cannot be made, the regulation does not gratify anyone to make negative claims about the product.

2.3.2 Global Market/Demands of Herbal Nutraceutical

The nutraceutical industry is divided into two main segments which include dietary supplements and herbal/natural products. In the Global market, nutraceutical has become a multi-billion dollar industry with about USD 117 billion (INR 5148 billion) as estimated cost of investment (Rishi 2006). According to a report, India is growing at 21% per annum in her total market for nutraceuticals. As of 2014, it appreciated at INR 44bn (€621 m), but could be worth more than INR 95bn in the following 4 years (http://www.horiba.com/scientific/products/particle/characterization/applications/nutraceuticals). In Canada, the nutraceutical industry has an estimated potential to grow up to 50 billion US dollars. According to the nutrition business journal in 2017, the United States of America (USA) is the largest market in the world followed by Japan which has a steady average growth rate of 9.6% per annum. The nutrition business journal generally classified the contributions of the different countries in the nutraceutical market – USA 37%, Europe 33%, Japan 18% and rest of the world as 12%. These two rapidly increasing sectors of the industry were at 19.5% per year for dietary supplements and 11.6% per year natural/herbal products (Chauhan et al. 2013). Table 2.2 shows some commercially available nutraceuticals.

2.4 The Future Prospects of Nutraceutical Industry

The world is becoming more sophisticated and interesting. Foods are becoming more attractive, appealing and fortified to meet the increasing demand of healthy nutrition. With the invention and recent advances in Living Modified Organisms (LMOs) and Genetically Modified Foods (GMFs), food for all agenda in the future will be attained or rather malnutrition would soon be history but new challenges may ensue. With the popping-in of tablets as nutrients to provide the body with complete Recommended Dietary Allowance (RDA) including fiber to ensure intestinal or bowel emptying, the prospects of nutraceuticals are limitless. The target of food and nutrition societies for a world rid of hunger in the future is possible with the potentials of nutraceuticals. At the core of the value-added market performance is the increasing responsiveness on the part of consumers as to how nutraceuticals

Table 2.2 Some commercially marketed nutraceuticals

Product	Group	Content	Company
Calcirol D-3®	Calcium supplement	Calcium and vitamins	Cadilla healthcare limited, India
Coral calcium	Calcium supplement	Calcium and trace minerals	Nature's answer, Hauppauge, NY, USA
WelLife®	Amino acid supplement	Granulated-L-glutamine	Daesang America Inc., Hackensach, NJ, USA
Proteinex®	Protein supplement	Predigested proteins, vitamins,	Pfizer ltd., Mumbai, India
Daytime restore & nighttime repose	Restful sleep	Ginseng, Ginkgo biloba,	Xigo health
CogniSure	Amino acid supplement	Proline-rich polypeptide complex	Metagenics Inc
Omega woman	Immune supplement	Antioxidants, vitamins and phytochemicals	Wassen, Surrey, U.K.
PNerplusTM	Neuropathic pain supplement	Vitamin and other natural supplement	NeuroHelp, San Antonio, Texas, USA
GRD	Nutritional supplement	Proteins, vitamins, minerals and carbohydrates	ZydusCadila Ltd. Ahmedabad, India
BiovincaTM	Neurotonic	Vinpocetine	Cyvex nutrition, Irvine, CA, USA

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can contribute to good health. With the established growing consumer demand for nutraceuticals, not only will consumers in nearby future use supplement products to support overall dietary intake, they are more likely to regard supplementation as an effective way to improve health. The undertone is, would the leading world economic nations not consider the economic politics and drown this vision or would it be given the attention it so deserves?

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Chapter 3
Dietary Supplements: Types, Health Benefits, Industry and Regulation

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3.1 Introduction

Nowadays, diet is thought to be much richer than it used to be. The people's ignorance of the basic principles of nutrition has led a large part of the population to a non-balanced diet that is high in both calories and fat and low in proteins, vitamins and minerals (Kourkouta et al. 2016). This long-term situation has led to the emergence of various degenerative diseases. In an effort to address this concern, nutritional supplements was proposed to proffer solution to this problem (Oikonomou

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C. Egbuna, G. Dable-Tupas (eds.), *Functional Foods and Nutraceuticals*,
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2009). *Dietary supplements are products that contain one or more concentrated nutrients with the aim of supplementing an individual's daily diet, when his or her diet is not balanced or if nutrients are lacking*. Dietary supplements do not belong to the category of common food neither medicines nor special dietary products and not intended for specific categories of people (www.nutrinews.gr).

Supplements offer the missing ingredients to the body in order for the latter to be kept in good physical and mental condition. In effect, the human system wouldn't be exhausted and at the same time injuries and fatigue are avoided (Kourkouta et al. 2016). The production as well as the consumption of dietary supplements have considerably increased in the last decade. Majority of these supplements are supplied in the form of tablets or powder. Although, the increased intake is supposed to offer health benefits, too much consumption may result in higher amounts of vitamins and minerals which the body may not be able to tolerate. As a result, consumers are exposed to health risks due to excessive consumption of dietary supplements. The problem becomes more serious if people take these supplements by themselves, without prescription or medical supervision (Beitz et al. 2004).

3.2 Dietary Supplements

Dietary supplements are products that are ingested in addition to the regular diet to provide additional health-promoting nutrients. According to the Dietary Supplement Health and Education Act (DSHEA), a dietary supplement is a product that is intended to supplement the diet; contains dietary ingredients including vitamins, minerals, amino acids, herbs, and botanicals; is intended to be ingested as a pill, capsule, tablet, or liquid; and is labeled as being a dietary supplement (ODS 2011; Ronis et al. 2018). Dietary supplements are widely used. They are generally taken to improve and maintain overall health. For women in particular, supplements are intended to support bone integrity and prevent osteoporosis. The most commonly

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used supplements are multivitamins, mineral supplements, calcium supplements, and omega-3 fatty acids or fish oil (Bailey et al. 2013). About a quarter of the supplements are used based on the advice of health-care providers. Thus, most decisions to use supplements are made by the consumers themselves. Despite their popularity, the health benefits of dietary supplements are questionable. Lack of vitamins will certainly cause deficiency diseases such as scurvy, beriberi, pellagra, and rickets. However, the vitamin content of a normal well-balanced diet is sufficient to avoid these diseases. Studies aimed at determining effects of supplements often give conflicting results. There seems to be no current scientific consensus whether vitamins or any other dietary supplements prevent disease or have health benefits in well-nourished individuals (Moyer 2014).

3.3 Historical Overview

From the beginning of human civilization, the people's diet mostly comprises of plant foods and seafoods that could be easily gathered. Hunter-gatherers later contributed meat products by big game. This was the diet of most humans until about 10,000 BC, at which time the development of agriculture and animal husbandry provided more meat and grains for the whole family. Then was little knowledge about vitamins, minerals, proteins, carbohydrates and fats and their role in human nutrition. Various people in the continents of the Earth developed nutritious cuisines with mostly local products that sustained their health. By trial and error, they chose a variety of foods and cooking methods that led to physical strength, health, and fertility. The common wisdom of native cultures knew which foods and herbs had special properties for energy, nutrition and extra health benefits for children, pregnant women and elders. Everyday diets were "supplemented" to make up for deficiencies as far back as native cultures. Native Americans, for example, knew that drinking tea made from pine bark and needles which contains high concentration of ascorbic acid could cure scurvy. This condition was later found by science to be a vitamin C deficiency disease. In 1749, Dr. James Lind discovered that citrus fruits which have high vitamin C content prevented scurvy (Shils and Shike 2006; Fraga 2009). The discovery of the role of vitamins was a major scientific achievement in understanding the association existing among nutrition, health and disease. By the 1920s and 1930s, significant strides occurred in the world of vitamin research and mass marketing of synthesized vitamin C flourished in 1935 under the brand name Redoxon. In nearly 75 years since the marketing of vitamin C pill started, large steps have been made in vitamin and other dietary supplement products (Kourkouta et al. 2016). The mainstream scientific community gradually became intrigued by the potential health benefits of dietary supplements and numerous research projects (epidemiologic, clinical, in vivo and in vitro) were initiated in the 1980s. This interest was fueled in part by studies demonstrating that nutrient antioxidants, (vitamins C, A, E, β-carotene and Selenium) have a role in protecting cells from oxidative free radical damage. Furthermore, epidemiological studies suggested that a diet rich in fruits and vegetables and abundant in antioxidants, nutrients, and other substances, can reduce the risk of coronary heart disease and certain cancers (Fortmann et al. 2013; Leenders et al. 2014).

3.4 Balanced Diet and Dietary Supplements

Dietary supplement can be defined as any vitamin, mineral, chemical substance, herbal product, botanicals, amino acids, or other ingestible preparation that is added to the diet to benefit human health. Dietary supplements are used worldwide and represent a broad category of ingestible products that are distinguishable from conventional foods and drugs (Watson et al. 2010; Berginc and Kreft 2014). It is common knowledge that nutrition plays a very important role in health. However, a fundamental question that everybody asks these days that most people are very aware of is: "Will conventional and balanced diet without supplements be able to cover all the needs of the human body for a healthy lifestyle until old age?" Nutritionists and health professionals argued for years that people can get the most important food requirements for their body needs each day from a conventional, balanced and regular daily diet. Today's dietary guidelines from health and nutrition agencies all over the world cover more than 40 nutrients that are subdivided into 6 categories: carbohydrates, fats, proteins, vitamins, minerals and water. Daily nutrient recommendations are collectively known as dietary reference intakes (DRIs). A healthy diet is one that favors "real" fresh whole foods that have been sustaining people throughout the millennia. Whole foods supply the needed vitamins, minerals, protein, carbohydrates, fats, and fiber that are essential to good health. A balanced diet is a mix of food from the different food groups (vegetables, legumes, fruits, grains, protein foods, meat, and dairy). Variety involves eating different foods from all the food groups that helps to ensure that you receive all the nutrients necessary for a healthy diet. The components of the Mediterranean diet have been evaluated as substantially beneficial to human health (Katz and Meller 2014; Widmer et al. 2015). The World Health Organization (WHO) makes the following recommendations for a balanced and healthy diet: (a) eat roughly the same amount of calories that your body is using, (b) a healthy weight is a balance between energy consumed and energy that is 'burnt off', (c) limit intake of fats, and prefer unsaturated, than saturated fats and trans fats, (d) increase consumption of plant foods, particularly fruits, vegetables, legumes, whole grains and nuts, (e) limit the intake of sugar, salt/sodium consumption from all sources and ensure that salt is iodized, (f) eat a diet with essential micronutrients such as vitamins and certain minerals (WHO 2003). In contrast, many commercially prepared foods or fast foods as they are called, often lack nutrients and contain inordinate amounts of sugar, salt, saturated and trans-fats, all of which are associated with the development of diseases.

3.5 Global Market of Supplements

Globally, the nutrition and supplements market stood at over US $90 billion. Research analysis of various markets with large nutrition and dietary supplements industries show interesting sales numbers and growth projections (Nasdag 2015; CMR 2015). The global mineral supplement market is increasing due to a growing geriatric population, demand from pregnant women, and rising urban population. With the increasing population of baby boomers, spending on mineral supplements is anticipated to increase. The mineral supplement market consists of macro-minerals, calcium, phosphorous, zinc, alkaline pH booster, sodium, potassium, chlorine, sulfur, magnesium, and trace minerals: iron, boron, manganese, chromium, copper, iodine, cobalt, fluoride, selenium, colloidal silver. The global mineral supplements market was valued at $9.9 million in 2014 and was expected to grow at a rate of 7.5% during the period 2015–2020 (http://www.researchandmarkets.com/research/xjrcm6/global_mineral).

Vitamin and poly-vitamin supplements are very popular and their use as supplements, supported by commercial advertising is growing every year. The global vitamin supplements market, was estimated to be valued at US$37 billion in 2014, and is expected to grow at 6.5% annually from 2014 to 2020. This is mainly attributed to growing demand among consumer about preventative healthcare. Furthermore, increasing cost of healthcare pushes people to turn towards vitamin supplements in the hope that this could prevent disease. Vitamin supplements included vitamins C, E, B, A, β-carotene, K, niacin, folic acid, etc) (https://www.psmarketresearch.com/market-analysis/ vitamin-supplements-market). According to recent United State (US) Food Drug Administration (FDA) testimony, dietary supplements, including vitamins, were consumed by 158 million Americans in the year 2000, which is more than 50% of the US population. Surveys showed that alternative medical therapies and herbal supplements were used by over 80 million people in the USA (Kennedy 2005; Wilson et al. 2006).

3.6 Supplement Formulations

Food supplements are in various packages, sizes and types, depending on how they are taken. There are tablets, capsules, powders, oral ampoules, effervescent tablets, chocolates and mastics which is available in syrup form or otherwise. More specifically, food supplements can be taken in any of the following forms: (a) oral pills or powders for relatively quick absorption; (b) sublingual drops or oral disintegrated tablets, for ease of intake and to limit the damage of their active substance; (c) nose spray or drops to further improve their absorption; (d) injectables through intravenous and intramuscular injections for quick absorption and action; (e) bone-anchored for slow and gradual absorption and prolonged action (American Diabetes Association ADA 2001). Some supplements are not well absorbed, others are almost completely destroyed by the stomach fluids and a few others irritate the mucosa of the stomach and intestine. Powders and liquid preparations are useful for those who have difficulty of swallowing the capsules or tablets. Supplements in the form of sprays can cause intense local irritation or possible lung aspiration. Injecting supplements is always done under the guidance of a physician. On the other hand, bone-anchored supplements can only be taken in hospitals due to surgical technique, monitoring and laboratory control required to prevent local inflammations and complications (Teixeira 2013). If there is intense change in color of urine when taking a supplement, this means that a large portion of the supplement is excreted because either it cannot be absorbed, or the dose is in excess. This must lead us to reflect on the effectiveness and possible harm that supplements can cause (Mulholland and Benford, 2007).

3.7 Classification of Supplements

According to the National Agency of Medicines, dietary supplements are divided into two categories depending on their intended use (EUFIC 2009):

(1) Food supplements as food product which supplements the usual diet.
(2) Foodstuff for particular nutritional uses such as beverages, which due to their special composition, are intended for certain population groups e.g. for healthy infants or children between the ages of two and five or for special categories of persons with disordered metabolism, or for categories of persons who are in a special physiological condition.

Supplements can also be distinguished depending on their origin (natural or synthetic). They are classified comparably to their texture or form in which they are available as follows: (a) Vitamin and mineral supplements, whether they are combined in the form of multivitamins or multi-minerals or not, (b) Protein supplements in the form of liquid or tablet in combination or not with carbohydrates, fats, vitamins and minerals, (c) Amino acids of every form and composition, (d) Supplements for gaining weight, (e) Meal surrogates in the form of powder, wafers or biscuits, (f) Carbohydrate supplements with or without electrolytes and vitamins, (g) Supplements which have natural anabolic effect, and which are not included in the "banned substances list", (h) "Activator" supplements of growth hormone and other hormones, (i) Supplements of basic fatty acids, (j) Foodstuffs or food ingredients such as yeast, garlic, kelp, royal jelly, (k) Herbs. There are thousands of supplements in the market. Many of them are very good, others moderate and some provide very little benefit. The main reason for their low efficiency is their manufacturing method and the source of their basic substances (Rovira et al. 2013). Some classes of supplements, their examples and contents are presented in Table 3.1.

Table 3.1 Classification of supplements, examples and contents

Class	Example	Contents
Activator	Amino acids	Contains growth hormone and other hormones
Carbohydrate	Dextrose	May contain vitamins and electrolytes
Food and Food stuff	Fish oils, mineral and vitamins	Contain garlic, kelp, royal jelly, yeast
Herbs	Ginseng, Fiber	Contains amino acids, other plant source
Minerals	Selenium, multimineral tablets	Contains only minerals
Multivitamins and multiminerals	Vitamin D, calcium supplement	Contains both mineral and vitamins
Oil supplements	Cod liver oil, primrose oil	Contains oil base, with vitamins, minerals
Vitamins	Vitamin C, vitamin B	Contains only vitamins

3.7.1 Vitamins and Minerals as Supplements

Today, multivitamin, multimineral, vitamin, and mineral supplements are the most widely used dietary supplements (Bailey et al. 2013). Although adequate intake of these micronutrients is required to maintain optimal health, the possibility of toxicity increases with increasing dose (Mulholland and Benford 2007). Because dietary micronutrient deficiency is increasingly rare in developed countries, most supplement consumers actually have excess vitamin and mineral intake. Despite the widespread belief that vitamin and mineral supplements are beneficial to health, recent reviews of vitamin and mineral supplement trials in community-dwelling adults with no nutritional deficiencies have concluded that there is no clear evidence of beneficial health effects. These include primary or secondary prevention of chronic diseases including cardiovascular disease, cancer, and cognitive decline, as well as effects on overall mortality (Guallar et al. 2013; Fortmann et al. 2013). Indeed, there is evidence for possible harm from consumption of individual vitamins and mineral in excess. Toxicity following consumption of water-soluble vitamins is rare. However, photosensitivity and neurotoxicity have been reported at doses higher than 500 mg/day of pyridoxine (vitamin B6) (Ziegler and Filer 1996), and cases of pyridoxine-associated chronic sensory polyneuropathy have been reported in elderly patients consuming multivitamin supplements (De Kruijk and Notermans 2005). Reports of toxicity associated with overconsumption of supplemental antioxidant fat-soluble vitamins are more prevalent. Vitamin E is a family of eight related tocopherols and tocotrienols, of which α-tocopherol is the form generally used in supplements. Doses of 800–1200 mg/day can result in bleeding associated with antiplatelet action, and doses above 1200 mg/day can result in diarrhea, weakness, blurred vision, and gonadal dysfunction (Ziegler and Filer 1996). Moreover, vitamin E supplementation following radiation therapy in a randomized trial of head and neck cancer patients was associated with increased cancer recurrence in the first 3.5 years of follow-up (Bairati et al. 2005), and meta-analysis has suggested an increase in all-cause mortality after high-dose vitamin E supplementation (Miller et al. 2005). Toxicity has also been associated with consumption of supplemental vitamin A and its provitamin carotenoid precursors. Excess vitamin A supplementation has been suggested to be associated with adverse effects on bone health, including low bone mineral density and increased fracture risk (Melhus et al. 1998). In addition, women consuming large amounts of vitamin A supplements during pregnancy have been reported to have increased incidence of congenital abnormalities (Rothman et al. 1995). There is also a case report of intrahepatic cholestasis in a patient with chronic hypervitaminosis A after 12 years of supplement consumption, which resolved after supplements were ceased (Ramanathan et al. 2010). Toxicity can arise from excess consumption of minerals as well as vitamins. In particular, there is an increased risk of hyperchromatosis, an iron storage disease associated with liver injury after excess consumption of iron or multimineral supplements (Barton et al. 2006).

3.7.2 Fish Oil and Omega-3 Fatty Acids as Supplements

Omega-3 fatty acids are essential fatty acids that cannot be synthesized de novo in humans and therefore must be provided through the diet (Spector and Kim 2015). A link between fish oil and ischemic heart disease was suggested by a widely publicized study from 1971 involving Eskimos (Greenlanders) from the west coast of Greenland (Bang et al. 1971). Greenlanders eating a traditional meat and fish diet rich in polyunsaturated omega-3 fatty acids had significantly lower levels of plasma total lipids, plasma cholesterol, plasma triglycerides, and pre β-lipoprotein (equaling very low-density lipoprotein) than both Danes and Greenlanders living in Denmark. The authors hypothesized that this diet contributed to the low incidence of ischemic heart disease and diabetes among Greenlanders. Since then, polyunsaturated omega-3 fatty acids taken in the form of fish oils, krill oil, or mixtures of docosahexaenoic and eicosapentaenoic acids, also known as DHA and EPA, purified from fish oils have become widely used dietary supplements. These fatty acids have metabolites with anti-inflammatory properties and have electrical stabilizing effects on ion channels in cardiac myocytes (Sierra et al. 2004; Leaf et al. 2008). They have been linked to anti-cancer and cardio-protective effects (Gogos et al. 2000; Harris and Isley 2001). However, the therapeutic benefits on cardiovascular diseases are still controversial owing to disparate findings from different clinical trials (Glück and Alter 2016). It appears that fish oil and omega-3 fatty acids are well tolerated, even at doses of 1000–2000 mg/day, and there is little evidence of toxicity. However, simultaneous consumption of fish liver oils that also contain vitamin A and multivitamin supplements could result in hypervitaminosis A. Furthermore, fish oils and omega-3 fatty acid supplements may exacerbate anticoagulation and promote bleeding in patients taking anticoagulant medications such as warfarin (Gross et al. 2017).

3.7.3 Protein Powders and Infant Formula as Supplements

Protein powders consisting of the dairy proteins casein and whey and of vegetable proteins in soy protein isolate (SPI) are popular supplements among athletes and body builders. These proteins are also the basis of infant formulas. The dairy proteins appear to have little toxicity except in individuals with allergies to cow's milk protein, although excessive consumption may result in ketosis. In contrast, there is an ongoing debate with regard to the potential safety of SPI. This debate is related primarily to the presence of weakly estrogenic compounds—the isoflavones: genistein and daidzein, which are among the 100 phytochemicals that remain bound to the protein isolate (Fang et al. 2004). These compounds can reach potentially estrogenic levels after SPI consumption in soy formula–fed infants and in children, men, and postmenopausal women taking soy protein supplements. Concerns have focused on potential estrogenic effects in early development resulting in reproductive toxicity, infertility, demasculinization, and increased promotion of estrogen-responsive cancers such as breast and endometrial cancer (Messina,2016). Animal studies of SPI and soy formula toxicity have however been contradictory. Akingbemi et al. (2007) reported that perinatal exposure to diets made with soy resulted in suppressed steroidogenesis, decreased testosterone secretion, and increased Leydig cell proliferation in rats. Similarly, marmoset monkeys fed soy infant formula had suppressed serum testosterone concentrations (Tan et al. 2006). Increased testis size and increased Leydig cell numbers per testis were also observed in these monkeys at adulthood, consistent with compensated Leydig cell failure. In adult female ovariectomized mice, feeding SPI increased growth of human breast cancer cell xenografts, consistent with an estrogenic effect (Allred et al. 2001). In contrast to the small number of animal studies with SPI suggesting estrogenicity, lifetime feeding studies in rats fed with SPI revealed that the sole protein source in soy formulas had no effects on sex organ weights, serum sex steroids concentrations, or fertility (Badger et al. 2009).

3.7.4 Botanical Supplements

Traditional herbal medicine can be said to be the precursor both for drugs used in modern medicine that are based on plant compounds (such as aspirin and morphine) and for contemporary botanical dietary supplements. Herbal and botanical products have sustained popularity given the fact that these natural (i.e., derived from plant root, leaves, or bark) substances were among the oldest therapeutics. Estimates published by the CDC as part of the National Health and Nutrition Examination Survey

2003–2006 reported that 20% of adults use a supplement containing at least one botanical ingredient (Bailey et al. 2010). A common motivation for taking these substances is to "improve overall health". Accordingly, the FDA regulates the majority of botanicals as dietary supplements and not as drugs developed for the treatment or prevention of specific maladies (FDA 2016). Botanical use is correlated with non-smoking and higher self-reported health (Bailey et al. 2013). Alarmingly, patients frequently do not report herbal supplement use to primary care physicians (Wu et al. 2011), a concern because many botanical supplements may interact with prescribed medications. On their own, bioactive constituents of botanicals can have acute adverse effects that require hospitalization. This review describes acute adverse effects and herb-drug interactions of the most common botanical and herbal supplements. As a result of their plant-based derivation, botanical supplements consist of a mixture of organic compounds. Only a fraction of these compounds is biologically active, with a small subset of the active compounds having therapeutic and/or toxic mechanisms of action. Table 3.2 presents a list of commonly used and researched botanical supplements, their primary active constituents and typical use. Concurrent exposure to other compounds (e.g., pharmaceuticals, smoking) and the heterogeneity of herbal supplements often obfuscates the determination of toxic mechanisms in clinical cases, even when doses of the supplement are reported. As such, reports of adverse effects directly attributable to botanicals are generally rare (Di Lorenzo et al. 2015). In most such cases, effects are mild (e.g., nausea, fatigue, and headache). However, more serious clinical cases have appeared, most often relating to adverse effects falling under the general category of drug-induced liver injury and its associated mechanisms, namely mitochondrial dysfunction, oxidative stress, and alteration of bile acid homeostasis.

3.8 Advantages and Limitations

The advantages that nutritional supplements generally offer includes high content of nutrients in small volumes; special nutrient compositions; lack of undesirable accompanying substances such as fats, cholesterol and purines; and complete coverage of specialized sporting needs. Nevertheless, these formulations should be treated as a supplement of basic healthy diet and not as a replacement. Users of dietary supplements often increase the dosage or frequency. As a result, doses become less and less effective. Thus, the human organism is forced to work harder to eliminate the extra amounts of supplements taken in (Thomas 2001). All these factors lead to the appearance of side effects due to toxicity of dietary supplements, which depends on the factors including (a) The dosage, because exceeding the recommended dosage may cause side effects; (b) The duration of intake which is related to the fact that the human organism is overloaded, owing to specific substances that the supplement contains, till the substance is eliminated; (c) The special chemical properties of some substances and their interactions with other foods and substances; (d) The person's weight who takes those supplements; (e) Age, because

Table 3.2 Commonly used botanical supplements, their primary active constituents and typical uses

Scientific name	Common name	Uses	Active components	References
Genus *Echinacea*	Echinacea	Imuno-stimulant	Chicoric acid, alkylamides	(Hermann and von Richter 2012)
Allium sativum	Garlic	Antioxidant; antihypertension	Allicin, adenosine	(Hermann and von Richter 2012)
Ginkgo biloba	Ginkgo biloba	Memory improvement; lowering blood pressure	Terpenoids (ginkgolides)	(Mayo Clinic 2013a)
Panax ginseng	Ginseng	Overall health; antistress	Ginsenosides	(Hermann and von Richter 2012)
Camellia sinensis	Green tea extract	Antiproliferative; antioxidant	Catechins (ECGC, ECG)	(Chen et al. 2016)
Serenoa repens	Saw palmetto	Treatment of benign prostatic hypertrophy	Various phytosterols	(Mayo Clinic 2013b)
Hypericum perforatum	St. John's wort	Antidepressant	Hyperforin, hypericin	(Mayo Clinic 2013c)
Silybum marianum	Milk thistle	DILI; high cholesterol	Silymarin	(Mayo Clinic 2013d)
Piper methysticum	Kava kava	Reducing anxiety	Kavalactones	(Teschke 2010)
Cimicifuga racemosa, Actaea racemose	Black cohosh	Alleviating postmenopausal symptoms	Triterpene glycosides	(Mayo Clinic 2013e)
Valeriana officinalis	Valerian	Reducing anxiety	Valepotriates (terpene alcohols)	(Gharib et al. 2015)
Pausinystalia yohimbe	Yohimbe	Stimulant; erectile dysfunction treatment	Yohimbine	(Hermann and von Richter 2012)
Hydrastis canadensis	Goldenseal	Treatment of cold/ respiratory infection; alleviate menstrual complications	Hydrastine, berberine	(Hermann and von Richter 2012)

lots of supplements are not recommended for underaged persons or the elderly; and (f) The individual capacity, because each person reacts differently in the face of various substances (Oikonomou 2009). No supplement is innocent. For instance, some overdose of fat-soluble vitamins causes hypervitaminosis. Protein overdose damages kidneys and the liver. A lot of carbohydrate intake in the form of powder can cause fat increase. A large dose of fatty acids may lead to some inability of the organism to form some muscle proteins. Last but not least, performance-enhancing drugs may cause endocrine disorders (Troesch et al. 2012).

3.9 Future Perspectives

Attitudes toward safety, efficacy, and values about what is important in food and life will be important in determining future needs involving supplement science in the countries we have discussed and perhaps elsewhere in the world. Safety is critical and requires better chains of custody and product characterization that exists at present for these products, particularly those involving global markets. Efficacy/health promotion claims for the product as being true and not misleading is also critical. Demonstrating efficacy requires clinical studies with well-defined products and rigorous experimental designs, and the studies must be replicable (NCCIH 2017). Finally, there are issues of personal choice and values, sometimes involving the efficacy of supplements as complementary and alternative therapies that are part of larger philosophical or religious world views and systems (Sebastian et al. 2017; González-Sarrías et al. 2017). Supplements intended to enhance sports performance (Kuhman et al. 2015) botanicals used for disease treatment (Costello et al. 2016) and those ingredients thought to slow aging (Delmas 2015) all require identification of valid biomarkers of efficacy as well as of exposure. The associations between supplement ingredients and health outcomes in chronic degenerative diseases must be clarified (Yetley et al. 2016; Pérez-Cano and Castell 2016). Collaborations among scientists in a number of countries are needed to drive supplement science forward. Irrespective of the type of health product, high quality science is fundamental to the success of any regulatory framework. Assessments of the safety, quality and efficacy of nutrients and other bioactive compounds are needed to provide the scientific information that regulators need (Taylor and Yetley 2008).

3.10 Conclusion

Scientists and health professionals agree that dietary supplements can be under certain conditions beneficial to human health but should not replace complete and balanced daily meals of food substances. The market for dietary supplements taken to improve the health or well-being of the customer is enormous. However, these products are not necessarily safe for everybody. Like regular drugs, supplements with active ingredients that provide a physiological or pharmacological effect are likely to also cause adverse effects in susceptible individuals. More attention to adverse effects and potential interactions is needed to avoid serious medical outcomes. Users and physicians alike should consult updated literature before beginning or advising a regimen involving these substances. Medical providers should be aware that a large fraction of the general population takes dietary supplements. They should therefore request information from patients about their supplement intake to provide optimal medical care. Self-prescription of dietary supplements should be avoided and patients, older people, pregnant women, young persons and people living with disabilities should be informed and advised by their doctors or pharmacists on dietary supplementation.

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Chapter 4
Bioactive Carbohydrates, Biological Activities, and Sources

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4.1 Introduction

A bioactive substance can be defined as a component that affect, causes a reaction, or triggers a response in the living tissue. These effects may be positive or negative depending on the dose or bioavailability of such substance (Guaadaoui et al. 2014). This is to say that bioactive compounds may occur as natural constituents or fortificants in food with the added health benefits that are more than the basic nutritional value of such food product (Lordan et al. 2011). Studies on bioactive compounds have attracted enormous interest in various fields, especially the field of nutrition and medical sciences.

Carbohydrates play an indispensable role in human physiology and pathogenesis of diseases because they are rich sources of bioactive compounds which can be used as functional foods or pharmaceuticals. Specific carbohydrates with a defined target are often referred to as bioactive substances. This includes carbohydrates such as glycoproteins, gangliosides and all carbohydrates with beneficial health effects, which include digestible carbohydrates with a low glycaemic index, or indigestible carbohydrates that are either resistant to fermentation or easily fermented.

Bioactive carbohydrates which are now used extensively several biotechnological and pharmaceutical applications can be derived from plants, animals and, microorganisms. The fact that most people do not take adequate fiber in their diet has made food fortifications with bioactive carbohydrates an important area of research in nutrition. Polysaccharides which are derived from plants are relatively nontoxic, and any side effects are minimal compared with those of synthetic compounds

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(Liu et al. 2015). The purpose of this chapter is to review the functional properties of bioactive carbohydrates and their sources.

4.1.1 Structure and Sources of Bioactive Carbohydrates

Carbohydrates are naturally found in almost all living organisms especially lower and higher plants (e.g. dietary fibers, herbs, wood plants, algae, fungi and lichen,), some microorganisms, and certain tissues of animals e.g. heparin, chondroitin sulfate, and hyaluronic acid (Singh et al. 2012; Liu et al. 2015). The structures of polysaccharides vary from linear structures to highly branched structures. These include:

(i) Homo-polysaccharides: These have the same type of monosaccharide being repeated in the chain.
(ii) Hetero-polysaccharides: These usually contain two or more different types of monosaccharides.

The repeating monosaccharide units in these polysaccharides can be bonded in a linear chain or a complex arrangement.

Although, carbohydrates play a central role in human physiology and certain disease progression, they are yet to be fully explored as bioactive compounds for preparations of nutraceutical or medicinal drugs (Hayes and Tiwari 2015). Their biological activities include prebiotics and immunomodulatory, anti-thrombotic, anti-viral, antimicrobial, antioxidant, antitumor and hypoglycemic properties among others (Hayes and Tiwari 2015; Xie et al. 2016b). Bioactive carbohydrates from plants and their potential health benefits were summarized in Tables 4.1 and 4.2 while the bioactive carbohydrates from animals are presented in Table 4.3.

4.2 Bioactive Carbohydrates from Lower Plants

4.2.1 Marine Algae

Marine organisms are a valuable source of bioactive metabolites. Marine macroalgae (seaweeds) metabolites have been gaining increasing research interest since they are a valuable source of new drug targets and due to their production of toxins in lethal algal blooms (O'Sullivan et al. 2010). Seaweeds are the types of marine macroalgae that are available commercially. Seaweeds of the marine macrophytic algae comprise of Chlorophycease (green algae), Phaeophycease (brown algae) and Rhodophycease (red algae). These organisms produce primary and secondary metabolites that have potentials for various biological activities. They are therefore widely used as food ingredients (especially the red and brown algae) and as nutraceuticals for human and animal health. Mata et al. (2010) reported that the brown algae have a higher content of bioactive compounds and stronger antioxidant activity than the red or green algae.

Table 4.1 Examples of bioactive carbohydrates from lower plants and potential health benefits

Bioactive carbohydrate	Sources	Potential health benefits
Phlorotannins	Brown and red marine algae	Antioxidant, antiviral, antidiabetic, anticancer and radioprotective activities.
		Gupta and Abu-Ghannam (2011)
Alginates	Brown seaweeds e.g. Laminaria and Ascophyllum species.	Wound healing, therapeutic agents, protein delivery, and cell transplantation
		Antibacterial, anti-tumor and prebiotics activities.
		Ueno and Oda (2014)
Fucoidan	Brown seaweeds e.g. *Ecklonia cava*	Antitumour, antioxidant, anticoagulant, antithrombotic, immunoregulatory, antiviral and antiinflammatory effects (Wang et al. 2019a, b)
Laminarin	Brown seaweeds (e.g. Laminaria and Saccharina species)	Anti-inflammatory, antitumour, anti-apoptotic, anticoagulant and antioxidant activities (Kadam et al. 2015)
Carrageenans	Red seaweeds e.g. *Porphyra haitanensis*	Antioxidant, anticoagulant (Pangestuti and Kim 2014)
Ulvans	Green seaweeds (Ulva)	Antioxidant, antiviral, antitumour, immunomodulating activities (Zong et al. 2012)
Sulfated rhamnan	Green seaweed *(Monostroma Latissimum)*	Antioxidant, antiviral, antitumour, immunomodulating activities (Raposo et al. 2013; Shang et al. 2018)
Sulfated arabinogalactans	Green seaweed (Codium)	Antioxidant, antiviral, antitumour, immunomodulating activities (Raposo et al. 2013; Udani et al. 2009)
Sulfated galacotans	Green seaweed (Caulerpa)	Antioxidant, antiviral, antitumour, immunomodulating activities (Raposo et al. 2013; Shang et al. 2018)
Sulfated mannans	Green seaweeds	Antioxidant, antiviral, antitumour, immunomodulating activities (Raposo et al. 2013; Shang et al. 2018)
β- Glucans lichenan	Lichens, *Cetraria islandica/ Umbilicaria proboscidea/ Thamnolia vermicularis var. subuliformis/Peltigera canina*	Immunomodulating Activity (Vester Boler and Fahey 2011)
Schizophyllan	Fungi *(Schizophyllum commune)*	Immunostimulatory (Wang et al. 2017)
Scleroglucan	Fungi *(Athelia rolfsii)*	Immunostimulatory (Synytsya and Novák 2013)
Lentinan	Mushroom (*Lentinula edodes*)	Antitumor, immunomodulatory (Zhang et al. 2011)

Table 4.2 Examples of bioactive carbohydrates from higher plants and their potential health benefits

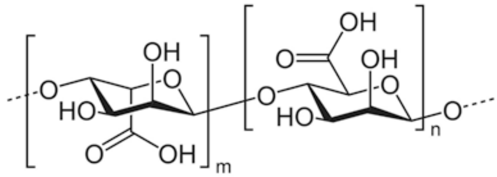
Bioactive carbohydrate	Sources	Potential health benefits
Cellulose	Fruit, grains, nuts, vegetables,	Increased stool bulk and bowel movement regulation (Wang et al. 2013)
Hemicelluloses	Vegetative and storage tissues of annual/ perennial plants, fruit, legumes, and nuts	Antioxidant activity, antithrombotic activity, immunomodulating activity, cholesterol lowering, free radical elimination and bowel movements regulation (Wang et al. 2018)
Pectins	Plant primary cell wall, soft tissues of fruit and vegetable	Immunomodulating activity, cholesterol-lowering effect, delayed gastric emptying and small intestine transit time (Kamhi et al. 2013)
β-Glucans	Oats, barley and grains	Cholesterol lowering effect, lipid regulation, blood glucose control, reduction of high blood pressure, immunostimulatory (Wang and Ellis 2014; Xu et al. 2013)
Resistant starch	Cooked and cooled potatoes, rice, green bananas, legumes	Hypoglycemic activities, hypocholesterolemic effects, prevention of colonic cancer, prebiotic roles, inhibition of fat accumulation, enhanced absorption of minerals (Toivonen et al. 2014)
Gums like Galactan, xylan, xyloglucan, glucuronic mannan, galacturonic rhamnosan type	Locust bean gum, gum arabic, and guar gum	Delayed postprandial glycemia, lipemia, and lipoprotein composition, increased satiety, delayed gastric emptying, Hypocholesterolemic effect, hypotriglyceridemic effect (Uebelhack et al. 2014)
Inulin	Chicory root, onion, garlic, wheat.	Prebiotic roles, Hypolipidemic effects, stimulation of mineral absorption (i.e.calcium, magnesium) (Uebelhack et al. 2014)
Konjac glucomannan	Konjac plant	Hypocholesterolemic effect, weight reduction (Tester and Al-Ghazzewi 2013)
Ginseng polysaccharides	Panax ginseng root	Antirotavirus activity (Xie et al. 2016b)
Acanthopanax senticosu polysaccharides	Acanthopanax senticosus leaves	Antioxidant activity and immunobiological activity (Maslowski and Mackay 2011)

The carbohydrates content of the seaweeds are very high (Gupta and Abu-Ghannam 2011; Rajasulochana et al. 2012). Sulfated polysaccharides, such as phlorotannins and diterpenes are the biologically active compounds from marine algae that are most widely studied for their potent anti-viral, anti-tumor, and anti-cancer properties. Several studies have also been carried out on the prebiotic health capabilities of these polysaccharides from seaweeds (Gupta and Abu-Ghannam 2011). A variety of polysaccharides, such as alginates, fucoidans, and laminarans have also been found in brown seaweeds (Table 4.1). While fucoidans and laminarans are soluble in water, the high-molecular-mass alginates are alkali-soluble polysaccharides.

Table 4.3 Examples of bioactive carbohydrates from higher animals and potential health benefits

Bioactive carbohydrate	Sources	Potential health benefits
Chitin and Chitosan	Crustaceans, insect cuticles, cell walls of fungi, shells of mollusks	Bacteriostatic and fungi static influences, antiviral, drug encapsulation, fat absorber, and wound dressing materials (Paul et al. 2015; Younes and Rinaudo 2015)
Heparin/Heparan sulfate	Golgi of animal cells	Anticoagulating, signaling and development, antimicrobial, anti-inflammatory and anticancer activities (Nikitovic et al. 2014)
Hyaluronic acid	Connective, epithelial, and neural tissues of animals	Chondroprotective effects, immunomodulatory (Lam et al. 2014)
Chondroitin sulfate/dermatan sulfate	Animal granules of mast cell, animal tissues from porcine intestine, bovine trachea, and shark cartilage	Anti-inflammatory, modulating cellular growth and signaling, maintaining the extracellular matrix Integrity (Zhang et al. 2014a; Zhao et al. 2015b)

Fig. 4.1 Alginate



Alginic acid or alginate (Fig. 4.1) is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic and α-L-guluronic acid residues arranged in a non-regular, block -wise order along the chain (Ferreira et al. 2012). Alginates usually have highly different physicochemical heterogeneity which can affect their quality and lead to different applications. Besides, the ability of alginate produced by brown seaweed to chelate metal ions (particularly those of sodium and calcium) and to form highly viscous solutions, have made them be of great use in the food and drug industries (Gupta and Abu-Ghannam 2011). The alginate gel that is formed by the induction of divalent cations have potentials for wound healing, therapeutic agents, protein delivery, and cell transplantation (De Leon-Peralta et al. 2016; Jain and Bar-Shalom 2014; Pipeleers and Keymeulen 2016).

Fucoidans (Fig. 4.2) are branched polysaccharide sulfate ester with L-fucose 4-sulfate building blocks as the major component. Their backbone contains α-linked – l-fucose residues with various substitutions. These polysaccharides are mainly found in brown seaweeds, even though their structures differ among various brown seaweed species (Ferreira et al. 2012; Gupta and Abu-Ghannam 2011).

Laminaran (or laminarin) appears to be the food reserve of all brown algae. It is the major sugar found in the Laminaria species and its structure and composition differ from one algae species to another (Gupta and Abu-Ghannam 2011). The structure of Laminarin contains β(1 → 3)-glucan with β(1 → 6)-branches and its

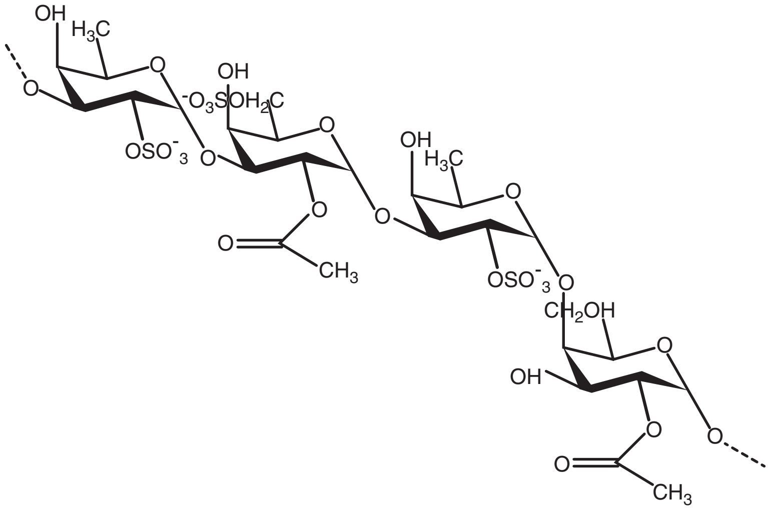


Fig. 4.2 Fucoidan

biological functions are determined by environmental factors, such as water, salinity, temperature, nutritive salt, depth of immersion, waves and sea current. Laminarins have been established to possess antibacterial, anti-tumor and prebiotics activities.

Furthermore, sulphated exopolysaccharides are produced by several species of microalgae. They have found applications in food, nutraceuticals, and medicine due to their various biological activities (Beluhan and Ranogajec 2011). The exocellular polysaccharides of all marine microalgae are heteropolymers which consist majorly of xylose, galactose, and glucose at varying degrees, except those of *Gyrodinium impudicum*, which are homopolymer of galactose. Other sugars such as fucose, rhamnose, and fructose also exist in marine microalgae (Raposo et al. 2013).

4.2.2 Mushrooms

Edible mushrooms are great sources of dietary fiber which are consumed by many populations because of their sensory characteristics, unique aroma and great taste (Beluhan and Ranogajec 2011; Wang et al. 2013). They represent an excellent source of bioactive carbohydrates (Villares et al. 2012). Polysaccharides that are isolated from mushrooms have been established as biological response modifiers (BRM) (El Khoury et al. 2011). They are present in the fungal cell wall with different forms of glycosidic linkages, such as (1 → 3) and (1 → 6) – β-D-glucans. The glucan moieties showed both α and β linkages with the β being more abundant and having more biological activities (Villares et al. 2012).

The biological activity of mushroom polysaccharides differs among species and is dependent on the length as well as the branching of the chain, chain rigidity, and helical conformation (Ahmad et al. 2012). These activities have been attributed to the (1 → 3)-β-glucan and (1 → 3), (1 → 6)-β-glucan, with the most positive effect on immune stimulation obtained with (1 → 3)-β-glucan (Villares et al. 2012). Mushroom polysaccharides that have demonstrated therapeutic effect against human cancers include lentinan from shiitake, active hexose correlated compound (AHCC) also from shiitake, schizophyllan from splitgill, maitake D-fraction from *Grifola frondosa* and polysaccharide-K or polysaccharide P (PSK/PSP) from *Trametes versicolor* (Friedman 2016). PSP and PSK have been reported to be non-toxic with prolonged use during the treatment of cancers. They suppress DNA/RNA synthesis in the cancer cells and also enhance the immune function (Wang et al. 2019a, b). The glucan portion of PSK consists of a β-1-4 main chain and β-1-3 side chains, with β-1-6 side chains that bind to a polypeptide moiety through O- or N-glycosidic bonds. PSK has a molecular weight of approximately 100,000 Dalton. This molecular weight of PSK has a great influence on their biological activities as β-glucan with high molecular weight have stronger biological activities compared to those with low molecular weight (Liu et al. 2015).

PSP is also a proteoglycan with a molecular weight of approximately100,000 Dalton which contains a polysaccharide and polypeptide portion (Wang et al. 2015). It is used widely as an immune modifier and it is famous for its immunoregulatory, anticancer, anti-inflammatory and antiviral activities (Chen and Huang 2018). The polysaccharide component in PSP consists of monosaccharides with α-1,4 and β-1,3 glucosidic linkages. PSP and PSK are chemically similar such that it may be difficult to distinguish them. However, PSK contains fucose in its structure while PSP has rhamnose and arabinose in its structure.

4.3 Bioactive Carbohydrates from Higher Plants

Polysaccharides obtained from plant sources constitute a large family of biopolymers which forms part of herbal ingredients that are widely used in many Asian countries (Guo and Cui 2014). Several bioactive polysaccharides extracted and separated from such plants have attracted a lot of research interest that examines their biological activities (Xie et al. 2016b).

4.3.1 Beta-Glucans

Beta-glucans (β-Glucans) are naturally occurring polysaccharides comprising a group of β-D-glucose polysaccharides naturally occurring in cell walls of cereals, bacteria, and fungi. The structure of β- glucan is similar to that of cellulose except that the β-(1 → 3)- linkages introduce a twist to the chain. This twist in the chain gives stability to β-glucan and reduces its ability to form aggregates, hence, the solubility of β-glucan is greatly affected by such a trend (Ahmad et al. 2012; Sikora et al. 2013). Other names for β-Glucans include β- Glycans, β-1, 3-glucan, and β-1, 3/1, 6-glycan (Synytsya and Novák 2013).

The effectiveness of β- glucan against various diseases and disorders have been established in various studies. Some of these scientific reported the tendency of β-glucan to reduce the onset of colorectal cancer (Xu et al. 2013), increase stool bulk and provide assistance against constipation (Ahmad et al. 2012), reduce glycemic index, flatten postprandial blood glucose levels and insulin rises, prevent insulin resistance, reduce serum cholesterol levels, produce short-chain fatty acids (Wang et al. 2013), prevent coronary heart disease, prevent hepatic damage (Karaduman et al. 2010), and promote the growth of beneficial gut microflora (Ahmad et al. 2012; El Khoury et al. 2011).

Additionally, it is important to note that β-Glucans obtained from different sources do have variations in their structure. The physicochemical properties of β-glucans, therefore, have a direct relationship with the characteristics of their primary structure. This includes- molecular weight, linkage type, degree of branching, and conformation (Synytsya and Novák 2013). The most common forms of β-glucans are those comprising D-glucose units with β-1 → 3 links. β-glucan found in yeast and mushrooms contains 1 → 3-glucan linkages and occasionally 1 → 6 linkages. Meanwhile, the β-glucans from grains (i.e., oats and barley) contains 1 → 3 and 1 → 4 linkages (Synytsya and Novák 2013). Mushrooms form α (1 → 3) or β (1 → 3) or (1 → 6) linkages but it can also form heteroglycans containing other sugars like arabinose, mannose, fucose, galactose, xylose, etc. They can also bind to protein residues as it is found in PSP (polysaccharide–protein) complexes. The yeast-derived β-1 → 3/1 → 6 glucan supposedly has greater biological activity than the 1 → 3/1 → 4 counterparts (Ahmad et al. 2012). Different β-glucan linkages are shown in Figs. 4.3 and 4.4.

These structural differences have a significant effect on the activity of β-glucans. *In vitro* studies have suggested that large molecular weight β-glucans (e.g. zymosan) can have a direct effect on the activation of leukocytes and have a bearing on their antioxidant, antimicrobial, phagocytic, and cytotoxic activities. Other factors that may impact on the immunomodulatory activities of β- glucans include frequency, location, and length of the side-chains. Conversely, intermedi-

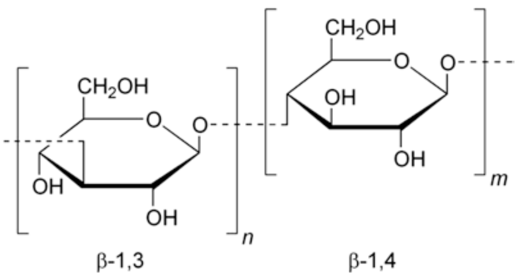


Fig. 4.3 β-1 → 3/1 → 4 Glucan

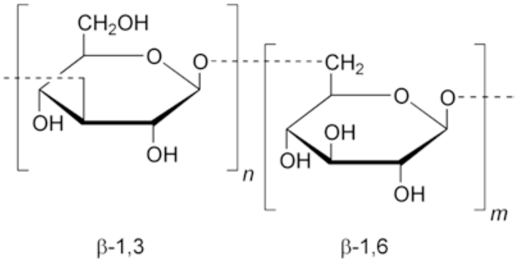


Fig. 4.4 β-1 → 3/1 → 6 Glucan

ate or low molecular weight β-glucans (e.g. glucan phosphate) possess biological activity *in vivo*, but their cellular effects are less clear. Very short β-glucans i.e. <5000–10,000 molecular weight (e.g. laminarin) are generally considered inactive (El Khoury et al. 2011).

4.3.2 Cellulose and Hemicelluloses

Cellulose is the most abundant organic chemical on earth because it is the main component of plant skeleton (Credou and Berthelot 2014). Apart from plants that are the dominant cellulose suppliers, algae, bacteria, and fungi also produce cellulose. Thus, millions of tons are biosynthesized annually, thereby leading cellulose to be considered an almost inexhaustible polymeric raw material (Klemm et al. 2011). The conventional sources of cellulose are wood pulp and cotton linters (Kamhi et al. 2013). Cellulose obtained from seed hairs of the cotton plant occurs in almost pure form. In contrast, cell wall of woody plants provides a composite material mainly made of cellulose, hemicelluloses, and lignin. It may also contain pectin, extractives such as waxes, or even proteins (Kamhi et al. 2013). The sources and potential health benefits of bioactive carbohydrates from higher plants were presented in Table 4.2.

Cellulose consists of several hundreds of β – (1 → 4) linked D-glucose units in a linear chain. A good number of these polysaccharide chains are arranged in parallel arrays to form cellulose microfibrils. Each of the polysaccharide chains in the microfibrils is bound together by hydrogen bonds (Fig. 4.5) which makes the microfibrils to be exceedingly tough and inflexible. Furthermore, the microfibrils are bundled together to form macrofibrils (Synytsya and Novák 2013). This tensile strength of the cellulose makes it a very useful organic molecule because it does not bind with water nor change form in the digestive tract (Onofrei and Filimon 2016).

Cellulose in the form of its dietary fiber plays a very important role in human nutrition as they are crucial for healthy digestion and health of the human gut. Dietary cellulose is thought not to be digested in the stomach and small intestine since about 85% can be recovered in ileostomy contents from subjects fed diets containing usually eaten foods. However, in the large intestine, it is fermented by gut microflora leading to the production of short-chain fatty acids, methane, hydrogen and carbon dioxide (Chen et al. 2011).

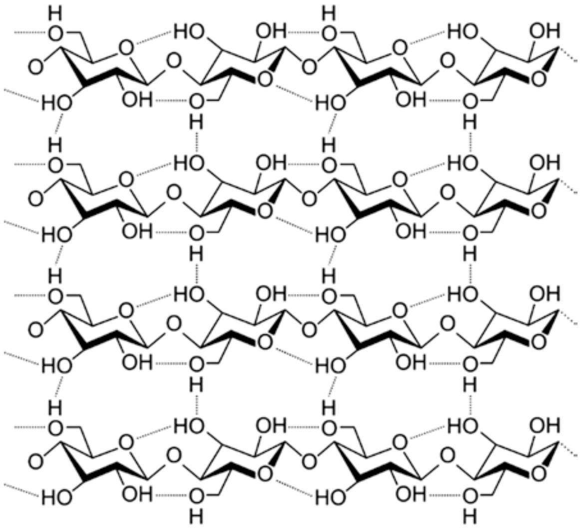


Fig. 4.5 Cellulose

Hemicelluloses are water-soluble polysaccharides of low degree of polymerization (100–200). While cellulose is made of a linear chain of glucose homopolymer, hemicelluloses are branched heteropolymers made of many different sugars such as glucose, mannose, galactose, xylose, and arabinose. Sugar ratio changes from plant to plant (Kamhi et al. 2013).

4.3.3 Glucomanan

These are fibrous polysaccharides with molecular formula $C_{24}H_{42}O_{21}$ that has a high molecular weight (average: 1,000,000 Daltons) and can absorb up to 50 times its weight in water, making it one of the most sticky dietary fibers (Tester and Al-Ghazzewi 2013). Glucomanan is a fermentable dietary fiber that is usually extracted from the tuber of the elephant yam (*Amorphophallus konjac* or *Amorphophallus rivieri*). It consists of a polysaccharide chain of β-D-glucose and β-D-mannose with acetyl groups attached in a molar ratio of 5:8. This ratio varies among different species with β-1–4 linkages (Tester and Al-Ghazzewi 2013). The basic polymeric repeating unit has the pattern: GGMMGMMMMMGGM, with branching through β-(1 → 3)- and β-(1 → 6)-glucosyl linkages (Fig. 4.6). Acetate groups occur on C-6 in every 9–19 units of the main chain. The fact that the human salivary and pancreatic amylase cannot split β-1, 4 linkages, glucomannan passes relatively unchanged into the colon, where it is highly fermented by resident colonic bacteria (Tester and Al-Ghazzewi 2013).

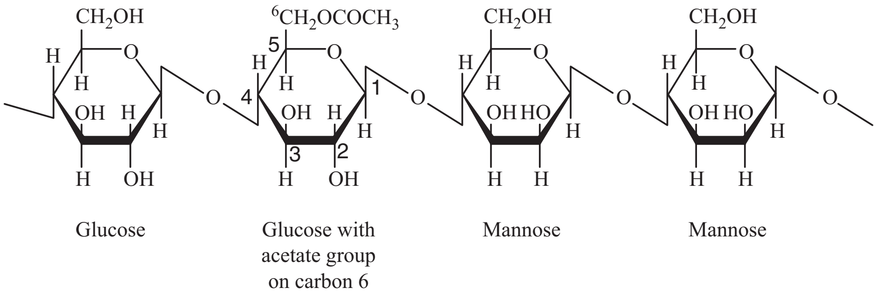


Fig. 4.6 Glucomanan

4.3.4 Pectins

Pectins are natural plant carbohydrates which are complicated in terms of their chemical composition as well as their physico-chemical structure. It is a heteropolysaccharide which is often present in the primary cell walls of terrestrial plants. It is a polymer with a core of alternating α-1,4-linked D-galacturonic acid and α-1,2- L-rhamnose units, including a variety of neutral sugars such as arabinose, galactose, and a few units of other sugars. The amount, structure and chemical composition of pectin may be different among various plants. They may also differ within a plant over time, just as they may differ in various parts of a plant (Kamhi et al. 2013). Pectins are soluble in alkaline water. They provide flexibility to plants and play a role in plant growth. They equally play important roles in the cell wall structure, cell–cell adhesion, cell development, morphogenesis, defense, signaling, cell expansion, wall porosity, binding of ions, growth factors and enzymes, pollen tube growth, seed hydration, leaf abscission, and fruit development (Kamhi et al. 2013).

Many bioactive pectic polysaccharides have been isolated from plants. They contain some defined structural units: homogalacturonans (HG), rhamnogalacturonan I (RGI), and substituted galacturonans such as rhamnogalacturonan II (RGII) (Ho et al. 2016). The familiar components of pectin with anti-cancer potentials are HG and RGI. The most predominant region of pectin is HG (Fig. 4.7), composed principally of a homopolymer of (1 → 4)-linked α-D-galacturonic acid (GalA) partially methylated at C-6 (Ho et al. 2016).

The degree of methylation (DE) refers to the ratio between methylated and non-methylated GalA. Pectin with high DE has 50% or more methyl ester groups on the HG backbone and are known as HM pectin while pectin with low DE (LM pectin) have fewer than 50% methyl ester groups on the HG backbone. The methyl-ester content is particularly important in pectin research as it strongly determines the physical properties of pectin. The GalA residues at O-2 and O-3 may also be partially esterified with acetic acid in certain plant species such as sugar beet (Jiang et al. 2016). Similarly, the ratio between acetylated and non- acetylated GalA is referred to as the degree of acetylation (DAc).

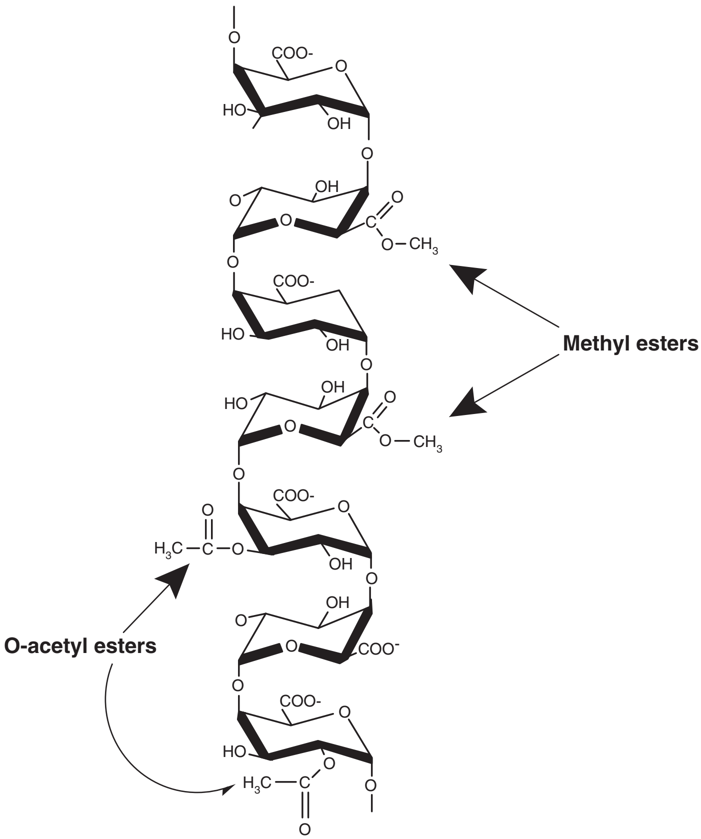


Fig. 4.7 Homogalacturonans (HG)

The second well-characterized component of pectin is the 'hairy' regions also known as rhamnogalacturonan I (RGI) regions. RGI consists of a backbone composed of a repeating disaccharide of GalA and rhamnose (Rha) residues [−4)-α-D-GalA – (1,2)-α-L-Rha-(1-]$_n$ (Ho et al. 2016). They are highly branched structures with neutral sugar side chains of varying degrees of polymerization attached to O-4 or O-3 position on the α-L- rhamnose residues (Toivonen et al. 2014) (Fig. 4.8).

The side chains consist mainly of α-L-arabinose and/or β-D-galactose residues. The major types of side-chain present are:

(i) Arabinan (Ara) which is composed of (1 → 5)-α-L-Ara units and often ramified with short (1 → 3)-α-L-Ara or single α-L-Ara nonreducing units at O-2, O-3 or O-5 positions
(ii) Galactan (Gal) which has linear, type I (1 → 4)-β-D-Gal or branched, type II (1 → 3,6)- β-D-Gal, depending on the plant source.

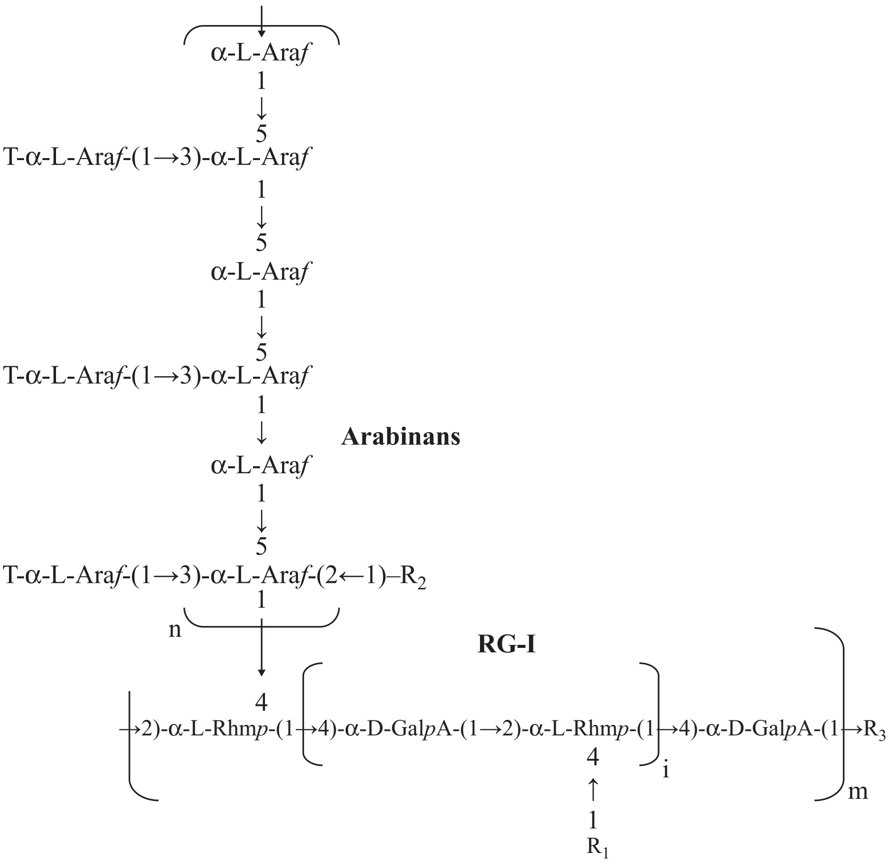


Fig. 4.8 Rhamnogalacturonan I (RGI)

(iii) Arabinogalactan I (AGI) consisting of a basal chain of (1 → 4)-β-D-Gal substituted at O-3 with short (1 → 2)/(1 → 3)-α-L-Ara or single α-L-Ara non-reducing units.

(iv) type II arabinogalactan (AGII) which has a backbone of (1 → 3)-β-D-Gal heavily substituted at position 6 by mono- and oligosaccharide Ara and Gal side chains (Udani et al. 2009).

Studies have highlighted the potential importance of these neutral sugar chain-containing regions (Jiang et al. 2016). Pectins are also highly variable depending on source and extraction. Minor amounts of other sugars such as fucose, glucose, mannose, xylose, glucuronic acid, and methyl esterified glucuronic acid are sometimes present in the side chains. In some cases, phenolics are also present in the side chains (Dhingra et al. 2012).

4.4 Bioactive Carbohydrates from Animal Products

4.4.1 Heparan Sulfate/Heparin

Heparin and heparan sulfates are closely related linear anionic polysaccharides. They belong to the group called glycosaminoglycans (GAGs), which exhibit several important biological activities. These polysaccharides which exhibit poly-dispersity, are synthesized in the Golgi apparatus of animal cells. Heparin that possesses highly sulfated, linear structure are considered as an important member of glycosaminoglycans. It is composed of repeated units of sulfonated hexuronic acid (1 → 4) D-glucosamine. Heparan sulfate is an extracellular glycosaminoglycan that is widely distributed in membranes. However, heparin is found primarily intracellularly in the granules of mast cells.

Heparin has received a lot of scientific attention due to its anticoagulant activity just as interest has increasingly grown to value the various roles that heparan sulfate plays in normal and pathophysiology (Nikitovic et al. 2014). Activities of Heparin and heparan sulfates include anticoagulation (Kamhi et al. 2013), signaling and development (Zhang et al. 2014a), and infectious disease, inflammation, and cancer (Garcia et al. 2014). Uronic acid residue in heparin which consists of α-l-iduronic acid (IdoA) or β-d-glucuronic acid (GlcA) can be sulfated at the second oxygen position. The residue of glucosamine can present unmodified (GlcN), N-sulfonated (GlcNS), or N-acetylated (GlcNAc), with various O-sulfations at the third and sixth oxygen positions (Fig. 4.9) (Nikitovic et al. 2014).

4.4.2 Hyaluronic Acid

The hyaluronic acid also called hyaluronan, is an anionic, non-sulfated glycosaminoglycan with high molecular weight. For instance, the human synovial HA averages about seven million Da per molecule, or about twenty thousand disaccharide monomers (Cyphert et al. 2015). Hyaluronic acid is composed of alternating units of D-glucuronic acid, and N-acetyl- D- glucosamine (Fig. 4.10). It exists naturally in the body performing essential biological functions. It is the component of the extracellular matrix (ECM) which explains why it is widely distributed in connective, epithelial, and neural tissues (Elmorsy et al. 2014).

Many studies have documented the chondroprotective effects of hyaluronic acid (HA) *in vivo* and its influence on the articular cartilage. It was also documented that exogenous HA could promote the synthesis of proteoglycan, modulate the functions of immune cells, and reduce the activity of proinflammatory cytokines (Elmorsy et al. 2014). Hyaluronic acid with special structure is also reasoned to be a prominent signalling molecule that can interact with cell surface receptors and thereby modulate cell adhesion, migration, and proliferation (Lam et al. 2014).

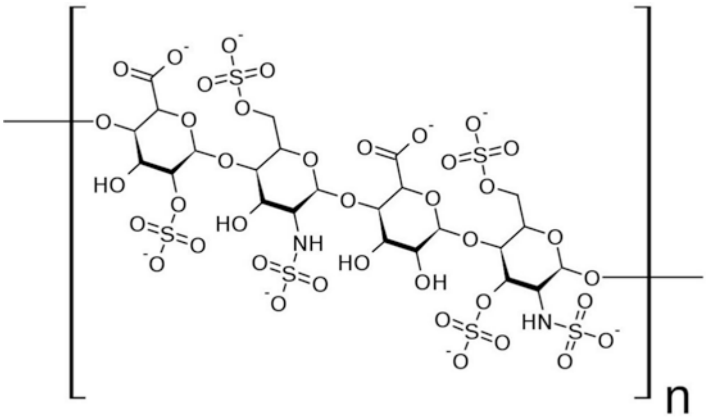


Fig. 4.9 Heparin sulfate

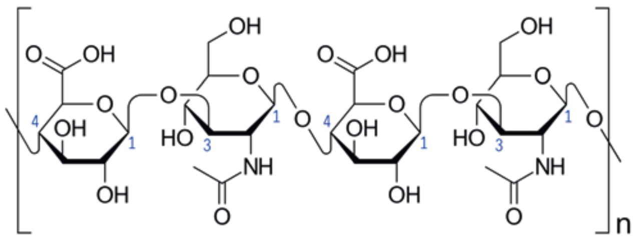


Fig. 4.10 Hyaluronic acid

4.4.3 Chitin and Chitosan

Chitin and chitosan are linear polysaccharides. The source of chitosan is chitin, a natural biopolymer (Fig. 4.11a) most abundant in exoskeletons of crustaceans, insect cuticles, cell walls of fungi, shells of mollusks, etc. (Paul et al. 2015). Chitin consists of 2-acetamido- 2-deoxy- β -D-glucose monomers (N-acetyl glucosamine units) linked through β (1 → 4) linkages (Ramya et al. 2012).

Hydrolysis of chitin is generally used for the industrial production of the glucosamine monomer and chito- oligosaccharides (Despras et al. 2014). Multidimensional utilization of chitin derivatives is as a result of certain functional properties such as their bacteriostatic and fungi static power, polyelectrolyte and cationic nature, presence of reactive groups and high adsorption capability. All these functions make them very versatile biomolecules (Hayes et al. 2008; Hayes 2012). Chitosan is a polymer of deacetyl α-(1, 4) glucosamine units that can typically be obtained by deacetylation of chitin (Fig. 4.11b) with sodium hydroxide (Yuan et al. 2015) after demineralization and deproteinization of the crustacean shells or exoskeletons. The

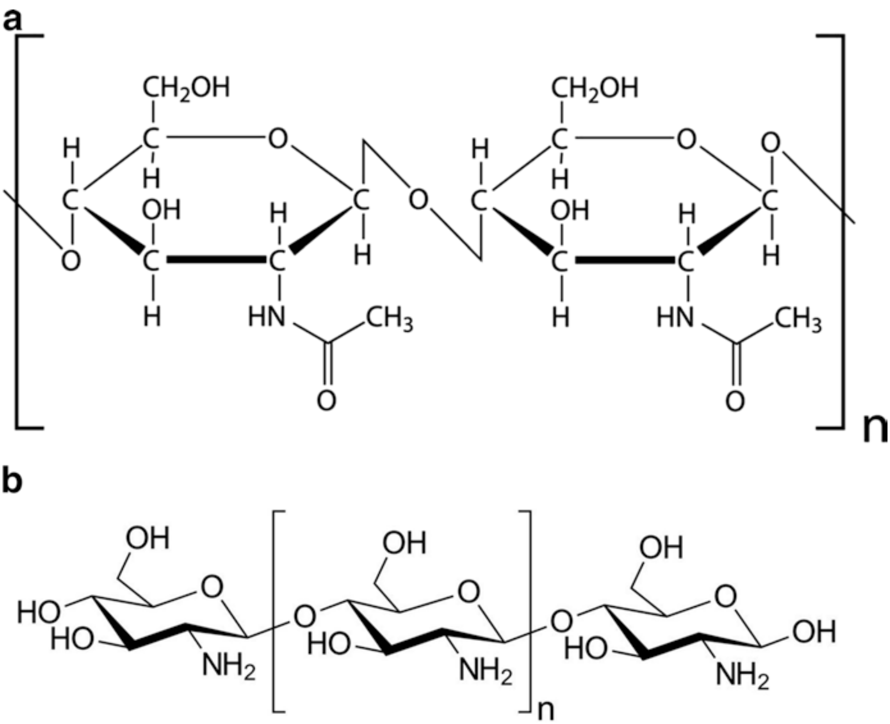


Fig. 4.11 (**a**) Chitin. (**b**) Chitosan

degree of deacetylation (DDA) of chitin ranges from 60 to 100% (Yuan et al. 2011). Chitosan as a heteropolysaccharide also includes linear β-1,4-linked units which often exists in the form of granules, sheets, or powders (Younes and Rinaudo 2015).

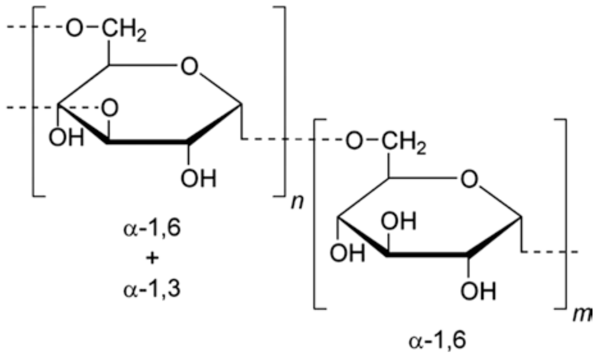
Chitin is the pre-constructed organic framework on which calcium carbonate growth takes place. Chitin, so similar to cellulose in structure, also reacts in many ways similar to cellulose, aside from being biodegradable and non-toxic. It is currently being used for medical applications such as drug encapsulation, fat absorber, and wound dressing materials. The chitin/chitosan—calcium phosphate system has the potential use as a virus filter given the adsorptive properties of hydroxyapatite (Singh et al. 2012) which may allow the attachment of drugs for future treatment of a serious viral disease.

4.5 Bioactive Carbohydrates from Microorganisms

4.5.1 Dextran

Dextran is a family of glucans produced by polymerization of the α-D-glucose portion of sucrose in a reaction catalyzed by the enzyme dextransucrase. It is a high molecular-weight polysaccharide which is composed of α-1,6 linking glucose as the

Fig. 4.12 Dextran



backbone and α-1,4 linking glucose as the side chain (Fig. 4.12). Dextran can be produced commercially when *L. mesenteroides,* a bacterial species, is cultured on sucrose (Table 4.4). Several other microorganisms also produce dextrans, but according to Chen and Huang (2018), dextran which is extracted from different microbial strain possesses varying structures. Dextran can be used for the separation and purification of biomacromolecules. Due to its biocompatibility, it also can be applied as the plasma expander for biomedical application (Friedman 2016).

4.5.2 Xanthan

Xanthan gum is a high molecular weight branched polysaccharide that consists of glucose, mannose and glucuronic acid (Fig. 4.13). It is produced naturally by the bacterium *Xanthomonas campestris* and manufactured commercially by aerobic fermentation of glucose or sucrose solutions with pure cultures of *Xanthomonas campestris* (Gupta and Diwan 2017). The primary structure of xanthan gum is a linear (1 → 4) linked β-D-glucose backbone (similar to that of cellulose) with a trisaccharide side chain on every other glucose at C-3 (Shang et al. 2018).

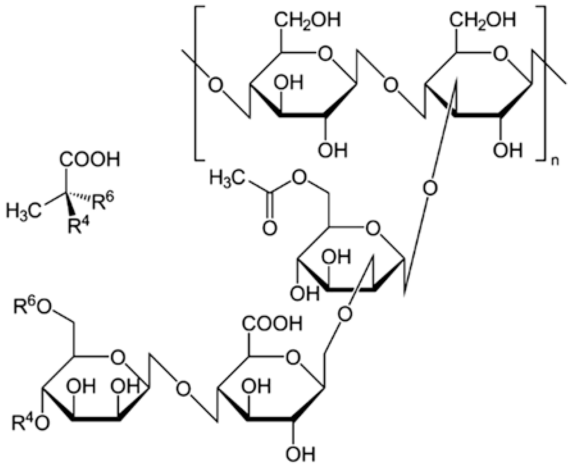
4.6 Relationship Between the Structure and Activities of Bioactive Carbohydrates

Carbohydrates (especially polysaccharides) have diverse chemical structure, composition, molecular weight, potential, and linking sequence, all of which result in different functionality and biological activity (Xie et al. 2016b). Xie et al. (2014, 2016b) indicated that polysaccharides have a different molecular weight (Mw) and monosaccharide composition. They also exhibit differences in their configuration and position of glycosidic linkages. Their structure range from those that are linear

Table 4.4 Examples of bioactive carbohydrates from microbes and their potential health benefits

Bioactive carbohydrate	Sources	Potential health benefits
Dextran	*L. mesenteroides*	Anticoagulation, Plasma/volume expander in biomedical applications (Khalikova et al. 2005)
Xanthan gum	*Xanthomonas campestris*	Antioxidant, antibacterial and biofilm inhibitory activities (Munir et al. 2017)

Fig. 4.13 Xanthan Gum



to those which are highly branched (Zhao et al. 2010). Therefore, it is important to understand how the structure of these polysaccharides influences their biological activities.

The three-dimensional arrangement of a polysaccharide is greatly influenced by the type of monomer, linkage type and position, and the number and position of branches occurring within the polymer chain. This factor, in addition to the molecular size, determines the behavior of a polysaccharide (Xie et al. 2016b). Some physical properties, such as solubility, viscosity, and gelation, may also influence the bioactivity since they can affect bioavailability (Sletmoen and Stokke 2008). Some studies have suggested that polysaccharides which have significantly different average molecular weights but have fractions with similar monosaccharide compositions can possess the same biological activity (Luo et al. 2010). Therefore, the elucidation of molecular structures of bioactive polysaccharides is very important for predicting their bioactivities.

The development of bioanalytical technology has tremendously helped to understand the structure of polysaccharides and apply their functions. Through the continuous investigation on the structure of plant polysaccharides, many new groups of physiologically active compounds has been discovered. Similarly, nutraceutical applications of bioactive carbohydrates are also related to their structural resistance to digestion in the upper gastrointestinal tract reaching the colon and becoming vital nutrients for the proliferation of probiotic microflora, or serving as intermediates for microbial conversion into other bioactive compounds (Hornung et al. 2018).

Several groups of polysaccharides have been studied over the last few decades to explore their bioactivities, especially on human health. Techniques such as centrifugation, ultrafiltration, ultrasound-assisted extraction, and pulsed electric energy has been very useful in the extraction and purification of bioactive polysaccharides (Zhu et al. 2016, 2017). The characterization of polysaccharide often comprise of preliminary, physical, and structural properties by glycosaminoglycans (GAG), uronic acid, and sulfate content, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gel permeation chromatography (GPC), Fourier-transform infrared spectroscopy (FTIR), UV-Vis spectroscopy, thermal gravimetric analysis (TGA), and X-ray powder diffraction (XRD). With the advancement of these extraction and identification techniques, a lot of new polysaccharides are continuously discovered from various resources.

4.7 Biological Roles of Bioactive Carbohydrates

Oligosaccharides and polysaccharides are very important biomolecules with multiple functions in all living organisms. The great variations in the structure of different polysaccharides are responsible for the flexibility of the precise regulatory mechanisms of several cell-cell interactions in higher organisms. Among macromolecules, polysaccharides have the best potential for carrying biological information because they have a high potential for structural variability (Wang et al. 2013). It is well known that the biological functions of polysaccharides are intimately related to their structural features (Xie et al. 2016a).

Many bioactive polysaccharides have been extracted from medicinal plants and this has led to many research studies in the field of pharmacology and biochemistry as a result of their potential biological activities. More importantly, polysaccharides derived from plants are comparatively non-toxic and most of them have insignificant side effects (Xie et al. 2014).

4.7.1 Prebiotics Carbohydrates

Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activities of probiotics (i.e. the beneficial bacteria) in the colon (Elsabee and Abdou 2013). Polysaccharides in the human diet are very good sources of prebiotics because they usually promote the growth of probiotics and intestinal biodiversity (Shang et al. 2018). Many polysaccharides found in foods cannot be completely digested by the human digestive system. These indigestible polysaccharides which include cellulose, hemicellulose, β-glucan, pectin, mucilage, gums and lignin are collectively referred to as dietary fiber. Most of these polysaccharides are usually resistant to digestion in the human alimentary system. A very good example is the resistant starch which is a starch fraction that can only be fermented by the large intestinal microbiota. The reason for this is that the human genome does not encode adequate gastrointestinal enzymes that can metabolize polysaccharides. Hence, the degradation of polysaccharides can only be achieved through a series of enzymes derived from intestinal microbiota (Karaduman et al. 2010).

The intestinal microbiota is a dynamic organ that plays a key role in maintaining health. It is a complex aggregation of microscopic organisms in the gut which may involve more than 100 trillion microorganisms (Gilbert et al. 2016). The diversity and density of these microorganisms are highest in the colon (Hornung et al. 2018). These microbes participate in vital physiological functions for the host. They also establish complex interactions with each other. The interactions could range from a mutual relationship to competitive relationships that may directly or indirectly influence the well-being of the host (Partida-Rodríguez et al. 2017). One evidence for this is that the germ-free animals are more vulnerable to germs than the colonized animals (Shang et al. 2018).

Dietary polysaccharides are known to impact gut microbial ecology and studies have indicated that gut microbiota can impact host nutrition, immune modulation, resistance to pathogens, intestinal epithelial development and activity, and energy metabolism (Jacobs et al. 2009). Bacteria in the human gut produce hundreds of polysaccharide degrading enzymes, which account for roughly 2.62% of the total enzymes encoded by the intestinal microbiome (Karaduman et al. 2010). Hence, these polysaccharides serve as unique carbon sources for the intestinal bacteria during fermentation. Arabinogalactans, galactomannans, glucomannans, laminarin (a glucan), and other mixed polysaccharide products are examples of bioactive polysaccharides that can be metabolized by human colonic bacteria (Kalia et al. 2011). Jiang et al. (2016) documented that apple pectin can increase Firmicutes phylum, decrease Bacteroidetes phylum and ameliorate the fat accumulation and body weight in diet-induced obese rats. According to Wang et al. (2011) for any carbohydrate to be defined as prebiotic carbohydrates, it must meet the following criteria:

(i) The carbohydrate must be resistant to

- gastric acidity
- hydrolysis by mammalian enzymes
- gastrointestinal absorption
- fermentation by intestinal microflora.

(ii) The carbohydrate in question must have selective stimulation on the growth and activity of intestinal probiotic bacteria

The beneficial effect of many polysaccharides is mainly dependent on their fermentability since human alimentary system cannot digest most of the polysaccharides completely. Other benefits include physiochemical properties such as water-holding capacity and bile acid-binding ability. The natural polysaccharides therefore, benefit human health by slowing gastric emptying, improving the bowel function, providing substrates for microbial fermentation, modulating the structure of gut microbe, and protecting the immune system (Minamida et al. 2014).

Certain gut bacteria can break down polysaccharides into short-chain fatty acids (SCFAs). Among other functions, SCFAs furnish energy to the colon and modulate immune responses. Besides, SCFAs protect against colitis/colorectal cancers keep the epithelial barrier function, and improve epithelial proliferation (Hamer et al. 2008). For example, butyric acid improves colonic health by supplying energy to the epithelial cells. Depending on its concentration, butyric acid can boost proliferation and differentiation of human cells and elicit apoptosis of tumor cells (Maslowski and Mackay 2011).

One condition that is frequently reported to be associated with deranged microbiota is metabolic syndrome. Many polysaccharides have been reported to successfully ameliorate metabolic syndrome (Cani et al. 2008). A study by Liu et al. (2017) documented that apple-derived pectin can reduce weight gain and excessive accumulation of fat in diet-induced obese mice. Soluble dietary fiber also suppressed weight gain and accumulation of fat by increasing energy expenditure and modulating gut microbiota (Wang et al. 2018).

4.7.2 Antioxidant Activity

Polysaccharides play an important role as free radical scavengers and antioxidants for the prevention of oxidative damage in living organisms. Oxidative stress also evolves from the regulation of most cellular processes, including proliferation, differentiation, stress responses, and cell death. It is one of the direct factors which is responsible for various diseases and aging (Wang et al. 2012). This makes the antioxidant effect of polysaccharides to be one of their main promising bioactivities. Numerous polysaccharides with antioxidant properties have been discovered from animals, plants, and microorganisms (Kofuji et al. 2012). Probiotic bacteria can synthesize extracellular polysaccharides (EPSs). EPSs possess physiological and therapeutic activities of commercial importance. These class of biomolecules also play the role of removing reactive oxygen species (ROS) that are formed in the intestine by various metabolic reactions (Wang et al. 2013).

The antioxidant properties of polysaccharides have a direct relationship with their structures. The main physicochemical properties, which determine their antioxidant effects include:

- water solubility
- molecular weight higher than 90 ku
- backbone chain of β- (1 → 3)-D-glucose
- β- (1 → 6)-side chain
- degree of branching
- functional groups (polyhydroxyl, carboxymethyl, acetyl, formyl, etc),
- triple-helical structure (Kofuji et al. 2012; Yuan et al. 2015).

Seaweeds are rich in antioxidants as they possess diverse functional polysaccharides. Similarly, β-glucan extracted from barley exerts significant antioxidant activity, in addition to other various biological activities. The magnitude of antioxidant activity exhibited by β-glucan is a function of different physiologic properties (e.g., structure and molecular size) of the β-glucan. This is also dependent on the source of the β-glucan and extraction method that is used (Kofuji et al. 2012).

The antioxidant activities of *Lycium barbarum* Polysaccharides (LBP) is an example of antioxidant polysaccharide that has been widely studied. *Lycium barbarum* is being used traditionally in China as a food supplement and medicinal herb (Gao et al. 2017). LBPs displayed significant radical scavenging effect on ABTS, DPPH as well as superoxide radicals (Zhang et al. 2014b). Furthermore, LBPs exhibited significant reducing power, ferrous ion chelating potency, and superoxide scavenging ability (He et al. 2012; Jin et al. 2013). All these stated antioxidant activities of LBPs is as a result of the plant's ability to improve the activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

A water-soluble polysaccharide was extracted from *Hyriopsis cumingii* (HCp) by Hu et al. (2010). The polysaccharide has a structural component of $\alpha-(1 \rightarrow 4)-$ D-glucan branched with a single α- D-glucose at C-6 of every five residues on average and an average molecular weight of about 2.65X 10^5. Pharmacological studies revealed that this polysaccharide significantly increases the total SOD activity of brain tissue and lowers malondialdehyde content effectively (Hu et al. 2010). In another study, Ngo et al. (2008) identified the antioxidant effects of chitosan Oligosaccharides (NA-COS) which is produced by acidic hydrolysis of crab chitin. Their study indicated that NA-COS have significant potentials for scavenging free-radical in a cellular system. They were shown to inhibit myeloperoxidase activity as well as reduce free-radical oxidation of DNA and membrane proteins. They were also shown to stimulate an increase in intracellular gluthathione levels.

4.7.3 Immunomodulatory and Immunostimulatory Activities

One of the desirable features of the innate immune system is to quickly recognize and respond to an invading pathogen so as to control infection. There are different types of natural immunotherapeutic operators that incorporate monoclonal antibodies, malignant growth immunizations, interferons, interleukins, state invigorating variables, colony-stimulating factors, gene therapy, and non-specific immune-modulating agents (Pandya et al. 2018). A wide scope of polysaccharides is likewise equipped for connecting with the immune system to upregulate or downregulate explicit portion of the host reaction and can, therefore, be classified as immune modulators (Wang et al. 2013).

The immunomodulatory properties of naturally derived polysaccharides which include heteroglycans and proteoglycans have been recognized and documented. Such polysaccharides are of certain molecular weight and structure. Their immunomodulating activities include activation of macrophages, monocytes, dendritic cells, natural killer (NK) cells, lymphocyte- activated killer cells, tumor-infiltrating lymphocytes, and other lymphocytes. Another important activity of these polysaccharides which have been documented is the prompt release of various cytokines including interleukins, interferons, tumor necrosis factor and colony-stimulating factors. Such polysaccharides are thus considered multicytokine inducers and this is probably because of initiation of gene expression of diverse immunomodulatory cytokines and cytokine receptors (Novak and Vetvicka 2008).

β-glucans have been documented to be potent immunomodulators with effects on both innate and adaptive immunity (Tian et al. 2013). Dectin-1, which is a type II trans- membrane protein receptor that binds β-1,3 and β-1,6 glucans, can initiate and modulate the innate immune response (Schorey and Lawrence 2008). Dectin-1 is expressed on cells responsible for the innate immune response and has been found in macrophages, neutrophils, and dendritic cells (Schorey and Lawrence 2008). Dectin-1 accepts β-glucans found in the bacterial and fungal cell wall. Since β-glucans are absent in human cells Dextrin-1 can conveniently trigger effective immune responses including phagocytosis and proinflammatory factor production which leads to the elimination of infectious agents (Schorey and Lawrence 2008).

The immunomodulatory activities of polysaccharides are related to their structures. Differences in molecular weight, tertiary structure/conformation, and composition all affect bioactivities of polysaccharide. Generally, polysaccharides with a configuration of β-1-3, 1-4, or 1-6 branch chains are necessary for activity while complex branch-chained polysaccharides with anionic structures and higher molecular weights have greater immunostimulating activities (Kim and Kim 2017). These differences in bioactivity may be due to differences in receptor affinity or receptor-ligand interaction on the cell surface (Li et al. 2016, 2017). The immunostimulatory activities of many polysaccharides have been documented in some studies in healthy human adults. Studies in healthy animals also indicated many immune stimulating effects of various glucan products (Ahmad et al. 2012; Kamhi et al. 2013; Tian et al. 2013).

The major manner through which polysaccharides from plants activate macrophages is through their interaction with specific receptors on cells. These receptors are known as pattern recognition receptors. The macrophages are involved in immune regulating processes. Macrophages play a significant role in all phases of host defense that are both innate and adaptive immune responses to infection (Zhao et al. 2015a, b). Macrophages play an essential role in all kinds of complex microbicidal functions, including the surveillance, chemotaxis, phagocytosis, and eventual degradation of the target organisms (Niu et al. 2017). Carbohydrates can interact with macrophages and regulate these cells through diverse mechanisms. The possible mechanism is that macrophages bind and thus interact with the polysaccharides through toll-like receptor 4 (TLR4), CD14, dectin-1 and mannose receptor, among others (Hollmig et al. 2009). Once the receptors are activated, there will be downstream signal and generation of pro-inflammatory factors.

Since polysaccharides can regulate the function of macrophages, they can also increase the host immune defense. Studies on the functions of macrophages have demonstrated that glycans can enhance their functions and this includes the activation of phagocytic ability (Niu et al. 2017), increased cytotoxic activity against tumour cells, reactive oxygen species (ROS) and nitric oxide (NO) production. It also includes secretion of cytokines and chemokines, such as tumour necrosis factor (TNF-α), interleukin-1β (IL-1β), IL-6, IL-12 and many more (Rabinovich and Toscano 2009). For example, a study by Udani et al. (2009) documented that a polysaccharide from the cones of *Juniperus scopulorum* showed significant immunomodulatory effect to the murine macrophages. The study certified that the polysaccharide caused an increased expression of macrophage iNOS and NO. Also, there was enhanced secretion of cytokines such asIL-1, IL-6, IL-12, IL-10 and TNF-α.

The immunostimulating properties of mushrooms have been documented in many Eastern countries (Friedman 2016). The bioactive polysaccharides in mushrooms are β-glucans. These substances also increase the host immune defense by enhancing the function of macrophages and natural killer cells (Friedman 2016). The mechanism for induction of cellular responses by β-glucans may be due to their specific interaction with several cell surface receptors like complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1. β-Glucans are also known to demonstrate anticarcinogenic activities. They can prevent oncogenesis through their protection against potent genotoxic carcinogens (Novak and Vetvicka 2008).

Anti-angiogenesis is a possible pathway by which β-glucans can decrease tumor proliferation and prevent tumor metastasis. β-Glucan can, therefore, a supportive therapy to cancer chemotherapy and radiotherapy as demonstrated by their positive role in the restoration of hematopiesis followed by bone marrow injury (Moreno-Mendieta et al. 2017). Immunotherapy using monoclonal antibodies is a novel strategy for cancer treatment. These antibodies trigger the activity of complement system and alter tumor cells with iC3b fragment. It is difficult for tumor cells as well as other host cells to trigger complement receptor 3-dependent cellular cytotoxicity and initiate tumor-killing activity because they do not have β-glucan as a surface component. This mechanism will, therefore, be elicited only in the presence of β-glucans (Carvalho et al. 2016).

4.7.4 Antitumor Activities

Malignant neoplasm is a dreaded disease that is very harmful to human health. Most antitumor chemicals kill cancer cells but at the same time, they seriously damage normal cells (Chen et al. 2014). This has led to numerous studies seeking alternative cancer therapy. A good number of polysaccharides have strong antitumor activities with broad application prospects. The ability of bioactive polysaccharides that have structures containing a β-1,3 1,4 or 1,6 has been extensively demonstrated to enhance the immune system and therefore indirectly reducing tumorigenesis as well as tumor growth in animals (Tomé et al. 2015). Similarly, prolonged survival due to treatment with nutraceuticals and drugs derived from polysaccharides have been documented in numerous controlled clinical trials (Ina et al. 2016). Anti-inflammatory and anti-allergy effects of some polysaccharide products have also been demonstrated in controlled human and animal studies. Plant polysaccharides with antitumor effect can function in tumorigenesis of various cell lines mainly through the suppression of tumor growth, apoptosis induction, enhancement of immune function and coordination of chemotherapy drugs (Tian et al. 2013).

Microbial polysaccharides (which are mainly derived from bacteria and fungi) with significant antitumor activity have also been found. They can be used for suppressing the growth of tumor cells, induction of apoptosis, immunopotentiation, synergistic chemotherapy drugs and other ways that can produce an antitumor effect.

Polysaccharides mainly exert antitumor effects through two major mechanisms (Pandya et al. 2018):

(i) The direct effect of polysaccharides on tumor cells.
(ii) Enhancing the immune function of the organism (indirect anti-tumour activities)

4.7.4.1 Direct Effect of Polysaccharides on Tumor Cells

This can be effected through six different mechanisms:

(a) *Direct inhibition to the growth of a tumor cell.* Polysaccharides can have a direct antitumor effect on tumor cells that present cytotoxic effects (Chen and Huang 2018). For example, a polysaccharide obtained from the root of *Sanguisorba officinalis* L (sowp) could suppress the growth of H22 ascites tumor cells, prolong the survival time of ascites tumor mice, and also reduce toxicity to normal cells (Friedman 2016; Zong et al. 2012).
(b) *Induction of tumor cell apoptosis.* Apoptosis is a spontaneous, active cell death process that is prevalent in multicellular organisms and the inherent mechanism of the relative stability of the cells to maintain the number of cells. Obstacles or abnormalities to this mechanism may lead to a tumor or other lesions. Induction of tumor cell apoptosis is therefore a novel method of cancer treatment. The mechanism of tumor cell apoptosis induced by polysaccharide is by inhibiting the telomerase activity and inducing the apoptosis of tumor cells through a change in the mitochondrial membrane potential, blocking of the cell cycle, affecting the manifestation of apoptosis-related genes, regulation of the expression and activity of Caspase protease (Chen and Huang 2018; Zong et al. 2012).
(c) *Inhibition of tumor angiogenesis*. The control of tumor-associated angiogenesis is a promising mechanism for limiting cancer progression since angiogenesis is vital for tumor growth and metastasis (Weis and Cheresh 2011). Solid tumor growth and metastasis have a close relationship with the formation of neovascularization. New blood vessels provide adequate nutritional supply to the

tumor thereby allowing for rapid growth. *Sargassum fusiforme* polysaccharides (SFPS) are documented to have an effect on inhibiting the proliferation of tumor vascular endothelial cells, and its mechanism has to do with down-regulating the manifestation of VEGF-A and VEGFR2 in tumor vascular cells (Chen et al. 2017).

(d) *Affecting the signaling pathway of tumor cells*. A variety of extracellular and intracellular signals play an important role in tumor cell apoptosis. Such signals include tyrosine protein kinase (TPK), cyclic adenosine monophosphate (cAMP), phosphatidylinositol (PI), nitric oxide (NO), nitric oxide synthase (NOS), and many others. Epidermal growth factor receptor (EGFR) is similar to receptor tyrosine kinase (RTK), which is responsible for regulating cell proliferation. The classical pathway of EGFR is Erk (extracellular signal-regulated enzyme) pathway, which inhibits MT-1 human malignant breast cancer cells growth, reduces Erk expression and inhibits proliferation of tumor cells through the Erk signaling pathway (Zhao et al. 2010).

(e) *Changes to the biochemical properties of tumor cell membranes.* Cell membrane growth characteristics have a lot of influence on the adhesion of the tumor membrane proteins and tumor cells. The biochemical properties of any cell membrane are essential to maintain the normal physiological activity and function of cells. Changes in the content of phospholipid membrane sialic acid conversion can kill the tumor cells. Therefore, a decrease in the sialic acid content will affect the tumor metastasis, antigen exposure associated with the tumor, immune response cell activation, etc. Phospholipids are converted to phosphatidylinositol phosphate by their enzymatic enzymes, which are involved in the activation of oncogenes and induction of tumors (Chen et al. 2014; Zhao et al. 2010).

(f) *Affecting oncogene*. Oncogenes are activated through established chromosomal alterations such as gene fusion, focal amplification, and translocation (Hnisz et al. 2016). The study of oncogenes and tumor suppressor genes has provided strong evidence and targets for elucidating the mechanism of cancer and the development of gene therapy and antineoplastic drugs. Genes involved in antitumor effects include the Bcl-2 family, p53 family, C-myc, and so on (Chen and Huang 2018; Levine and Puzio-Kuter 2010). The anti-apoptotic genes in Bcl-2 family are Bcl-2, Bcl-xl, Bcl-w, Mcl-1, etc. Apoptotic genes are Bax, Bak, Bad, Bid, Bim, and so on. p53 is a tumor suppressor gene, c-myc overexpression will promote tumor cell proliferation. Bcl-2/Bax ratio is also effective for promoting apoptosis or inhibiting cell apoptosis (Levine and Puzio-Kuter 2010).

4.7.4.2 Enhancing the Immune Function of Organism

The role which the immune system plays in tumor progression cannot be overemphasized. This is the main reason why tumor immunotherapy is considered as one of the important treatment strategies for cancer (Carvalho et al. 2016). While some polysaccharides may have no direct ability to inhibit tumor cells, they may provide antitumor effect by enhancing the immune function of the body. The immunomodulatory effect of polysaccharide is therefore considered as an important mechanism for their antitumor effect (Carvalho et al. 2016). Polysaccharides also activate the reticuloendothelial system (RES), remove aged cells, foreign bodies and pathogens. Furthermore, they promote the formation of IL-1, IL-2, TNFα, INF-r, NO, etc. which regulate antibodies in the body and complement their formation. All these improve the body's antitumor immunity (Carvalho et al. 2016). The indirect antitumor activities of polysaccharides can be done by:

(a) *Activation of macrophages*. Macrophages are important immune cells with multiple functions, they can actively swallow and clear the granular foreign antigens or directly kill pathogenic microorganisms, also can secrete a variety of bioactive substances, which almost are involved in all immune responses of the body. Polysaccharides can stimulate macrophages and enhance the phagocytosis of macrophage. They also promote the production and release of mononuclear factors, activate lymphocytes, in order to activate the immune response of tumor cells (Levine and Puzio-Kuter 2010).
(b) *Activation of Lymphocytes*. T and B lymphocytes play critical roles in cellular immunity as well as humoral immunity. B cells produce antibodies to mediate humoral immunity, whereas T cells induce cell-mediated immunity. In effect, B lymphocytes produce antibodies while T lymphocytes help kill tumor cells and help to control immune responses. Lentinan which is a β-glucan polysaccharide extracted from the fruit body of shiitake (*Lentinus edodes*) is a typical T cell activator (Yu et al. 2009; Zhang et al. 2011).
(c) *Activation of NK cells*. NK cells are naturally occurring non-specific immune killer cells. They move freely in the blood to lyse cancer cells and other cells virus-infected cells (Tian et al. 2013).
(d) *Promotion of antibody formation and complement activation.* Complement is a series of proteins with prokaryotic activity in the blood that can fight against pathogenic microbes through synergistic actions with antibodies or phagocytic cells (Meri 2016).
(e) *Promoting the secretion of cytokines.* Cytokine (CK) plays a vital role in the mechanism of antitumor effect. NO, IL-2, TNF-α, TNF-β, INF-γ, and other cytokines can activate the immune response of tumor cells thereby reflecting the antitumor activity (He et al. 2017). Interleukin-2 (IL-2) is synthesized by T cells, particularly, $CD4^+$ T cells when stimulated by antigen or mitogen. Other cells such as mononuclear macrophages, B cells, NK cells, etc. can also produce IL-2. IL-2 is a naturally occurring cytokine with immune-modulating and antineoplastic effects. It regulates lymphocyte activation, activates macrophages, and participates in specific immunity and non-specific immunity (Pandya et al. 2018; Tian et al. 2013).
(f) *Promoting the synthesis of dendritic cells.* Dendritic cells (DCs) are antigen-presenting cells, and also immune -regulatory cells. DC can initiate an initial lymphocyte-mediated immune response. Polysaccharides can trigger innate

immune responses as well as the adaptive immune system by the induction of DC recruitment and maturation. Specifically, β-glucans were reported to enhance the antigen-presenting function of DCs, thereby inducing tumor-specific cytotoxic T cells (Li et al. 2012).

(g) *Enhancing erythrocyte immunity.* Red blood cells transport the antigen foreign body to both liver and spleen to be removed by the immune adherence function of C3b receptor (CR1) on the cell membrane. And the C3b receptor of erythrocyte membrane has the characteristics of cluster distribution and polyvalency, which is more favorable for the combination and elimination of antigenic substances. Therefore, the maintenance of erythrocyte normal immune adhesion can effectively prevent tumor proliferation. Pumpkin polysaccharides could inhibit H22 cell proliferation and enhance immune adsorption capacity of red blood cell (Li et al. 2012). Jujube polysaccharides could enhance the immune function of erythrocytes in mice and had obvious antagonistic effects on the inhibition of red blood cell immune function caused by cytophosphamide (CY) (Chen and Huang 2018).

4.7.5 Anti-diabetic Activity

Consumption of fiber-like polysaccharides has been frequently indicated to protect against the incidence of diabetes. High intake of dietary fiber can improve glucose metabolism and predict higher glucose control (Brauchla et al. 2012). Consumption of viscous fiber can particularly slow down glucose absorption and this will prevent quick peaking of blood glucose (Wang et al. 2013). Hence, a high carbohydrate diet accompanied by a diet rich in fiber can give better control of blood glucose. Also, reduction in plasma cholesterol levels will be achieved in diabetic patients without raising plasma insulin or triglyceride concentrations (Uebelhack et al. 2014). Polysaccharides can influence the pathogenesis of diabetes through the modification of microbiota homeostasis and gut barrier.

Polysaccharides from plants which have been documented to have hypoglycaemic effect include cellulose, protein-bound polysaccharides, glucomannan, mannose guar gum, pectin/pectin fibres, caryophylline, and mucilaginous fibre (Brauchla et al. 2012; Wang et al. 2013). These polysaccharides can initiate insulin secretion, carbohydrate digestion, and absorption (Liu et al. 2015). Carbohydrate digestion and absorption are also regulated by L-arabino-D-xylan, cinnzeylanin, cinnzeylanol and D-glucan, which are extracted from *Cinnamomum zeylanicum* blume (Solomon and Blannin 2007). Fructo- oligosaccharide extracted from plants and microorganisms are reported to significantly decrease advanced glycation end products (AGEs), glycosuria, and plasma triglycerides, as well as very low density lipoproteins (Bharti et al. 2013).

Polysaccharides feature prominently as anti-diabetes therapy in numerous Chinese herbs (Chan et al. 2012). β- Glucans, and many other viscous plant polysaccharides have been demonstrated to have physiological activities that have been documented to reduce postprandial glucose levels in the serum (Brauchla et al.

2012). This effect has been pinned to the ability of this polymer to form an unstirred water layer which can resist the convective effects of intestinal contractions which in turn decreases sugar absorption by the small intestine (Friedman 2016). Many studies have also demonstrated that plant polysaccharides can regenerate the integrity of pancreatic tissues which leads to an increased insulin output by the functional β-cell and in effect lowers the blood glucose levels. These polysaccharides have also been established to ameliorate the sensitivity of peripheral cells to circulating insulin (Hu et al. 2013).

Diabetes-associated dyslipidemia which is caused either by insulin resistance or adipocytokines is a major risk factor for cardiovascular diseases. In diabetes, the adipose cells are insulin-resistant so the insulin-mediated uptake of free fatty acids in skeletal muscles is impaired. There will, therefore, be increased circulation of free fatty acids flowing to the liver, resulting in increased synthesis of triglyceride and the assembly of very low-density lipoprotein (VLDL). The characteristic of dyslipidemia in diabetic patients, therefore, include hypertriglyceridemia. Hyperglycemia and low insulin may also lead to increased VLDL production (Friedman 2016). Adiponectin is reduced in diabetes and this increases the uptake of free fatty acid by the muscle and reduces the plasma-free fatty acid level. This mechanism is independent of insulin-resistance. Furthermore, high-density lipoprotein (HDL) may also decrease. Indigestible polysaccharides can also reduce fat absorption partly by binding to fat molecules in the digested food product and also increasing their excretion (Uebelhack et al. 2014). β-Glucans extracted from barley, which mainly contains β-(1,3-1,4)-d-glucan, have been demonstrated to reduce blood lipid levels, including cholesterol and triglyceride levels (Talati et al. 2009).

4.7.6 Antimicrobial Activity

β-Glucan has been demonstrated to increase resistance to infectious challenge (Friedman 2016) by eliciting broad anti-infective effects. The protective effect of β-glucan has been established for microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Pneumocystis carinii*, *Listeria monocytogenes*, *Leishmania donovani,* and Influenza virus (El Khoury et al. 2011; Synytsya and Novák 2013). Other studies hypothesized that systemic β-glucan treatment leads to increased migration of neutrophils into a site of inflammation and also improve antimicrobial function (Gilbert et al. 2016). β-glucan administration enhances microbial killing by monocytes and neutrophils.

4.7.7 Wound Healing Activity

Wound healing takes place in three overlapping stages in most organs or tissues. These stages are:

(i) Inflammation
(ii) Proliferation and
(iii) Remodeling (Goh et al. 2016).

In all stages, especially the second and third, multiple families of growth factors play diverse vital and integrated roles. There has been a lot of attempts made for engineering polysaccharide scaffolds to bind and improve growth factors, which demonstrate a better effect on the repair of a wound than free growth factors (Goh et al. 2016; Wu et al. 2016). Macrophage activity is known to perform a significant function in wound healing from surgery or trauma. Therapy with β-glucan has provided improvements such as fewer infections, reduced mortality, and stronger tensile strength of scar tissue in both animal and human studies (Xie et al. 2016a).

4.8 Conclusion

Polysaccharides are natural biomaterials which are inexpensive and are readily available. Lots of polysaccharides have been developed into functional foods. Many of the bioactive polysaccharides are already existing in food and pharmaceutical products as functional and bioactive ingredients. These myriads of bioactive polysaccharides with different structures from different sources and physicochemical properties shall continue to be a large source of material for medical applications and the production of healthy and sustainable food. More importantly, information on the mechanism of antitumor activities by polysaccharides is a promising area of research towards the development of novel therapies such as adjuvant immunotherapy for the treatment of malignancies which can be used in combination with chemotherapy, radiotherapy or surgery.

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Chapter 5
Bioactive Peptides and Their Natural Sources

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5.1 Introduction

Bioactive peptides (BAPs) are particular amino acid sequences with constructive influences on bodily functions and conditions. They are also studied for their positive effects on human life (Kitts and Weiler 2003). Currently, there are more than 3791 different sorts of BAPs that have been reported by the database 'Biopep'. BAPs are generally of organic origin. They are formed by linking amino acids with peptide bonds or amide bonds. They are different from proteins as proteins consist of polypeptides with very high molecular weight (MW). BAPs and proteins have crucial roles in the metabolism of living organisms. They possess hormone and drug-like actions that can be categorized on the basis of their biological activities such as anti-thrombotic, antimicrobial, opioid, antihypertensive, mineral binding, immunomodulatory and antioxidative.

The activity of peptides after being released from the parent protein is determined by the composition and sequence of amino acids. Several natural processes within the body are governed by the specific amino acid sequence of the proteins (Fields et al. 2009). Proteins can be categorized as exogenous if they are obtained through some external source and endogenous if they are taken from amino acids produced within the body via different processes (Carrasco-Castilla et al. 2012). Proteins from various natural sources are recognized for their nutritional and functional properties. The nutritional abilities of proteins are linked with the amino acid residue of the proteins in addition to the physiological consumption of specific amino acids after they are being digested and absorbed whereas, the functional properties of the proteins are associated with the physiochemical properties of the source material (Friedman 1996; Vercruysse et al. 2005). Different proteins obtained

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from animals and plants are the potential sources of a wide range of BAPs, as the BAPs are encoded in the structure of these proteins (Bhat et al. 2015a, b).

A number of BAPs have several common structural features such as length of peptide residues between 2 and 20 amino acids (Moller et al. 2008), and in many of the BAPs hydrophobic amino acids are present along with the lysine, arginine, or proline groups. BAPs have also displayed resistance against the activity of digestive peptidases (Kitts and Weiler 2003).

BAPs are now taken as a new class of biologically dynamic regulators, which can ensure prevention against oxidation and microscopic degradation of foods. They can be utilized potentially for the treatment of several medical disorders, hence can improve the quality of life (Lemes et al. 2016). In the past few years, nutraceuticals (Chauhan et al. 2013) and functional foods (Haque et al. 2008) have garnered significant amount of attention, specifically for their influence on human health and their application in the prevention of ailments. Therefore, BAPs have also secured a promising spot among the modern biologically important substances (Przybylski et al. 2016).

As BAPs can be released from proteins of almost all organisms, therefore, the number of BAPs accessible from proteins is boundless. This provides an alternative to individuals who are opposed to products from certain groups of organisms. For instance, different sources like that of plant proteins and myco-proteins are available for vegetarians who do not want to consume the BAPs from animal origins.

The BAPs have been successfully isolated and identified from several natural sources such as meat, fish, collagen, dairy, egg, plant, marine and fungal resources. This chapter aims to provide the detail account of BAP obtained from these sources.

5.2 Bioactive Peptides and Their Sources

Peptides and proteins are of supreme importance among all the macronutrients present in foods, as they provide the raw materials essential for protein biosynthesis and also serve as important source of energy (Walther and Sieber 2011; Dziuba and Dziuba 2014). They also play crucial roles in complicated series of organic reactions that take place during metabolism and absorption of foods depicting their remarkable nutritional importance and diverse biological activities (Hartmann et al. 2007; Moller et al. 2008).

BAPs are primarily encoded inside bioactive proteins (Meisel and Bockelmann 1999). They are discovered and isolated from several different sources such as animals, plants and other natural products. Among them the bovine milk (Mohanty et al. 2016), cheese (Pritchard et al. 2010), and other dairy products (Choi et al. 2012) are the greatest contributors of BAPs and bioactive proteins. Other than these BAPs can also be obtained from other animal sources such as bovine blood (Przybylski et al. 2016), gelatin (Lassoued et al. 2015) eggs, meat etc. BAPs can also be obtained from plants and vegetable proteins such as maize, soy (Singh et al. 2014), mushrooms, pumpkin, sorghum (Moller et al. 2008), wheat (Matsui et al. 1999) and rice

(Selamassakul et al. 2016). *In vivo,* the encoded BAPs can be isolated through gastro-intestinal (GI) digestion because of the enzymatic action of certain enzymes such as trypsin or other microbial enzymes. *In vitro*, BAPs can be released through food processing or ripening via microbial enzymes such as in *Lactobacillus helveticus* (Dziuba and Dziuba 2014). BAPs have been recognized and separated from animal and plant sources along with the hydrolysates of several other products (Table 5.1).

5.2.1 Bioactive Peptides from Meat Sources

BAPs isolated from different animal proteins have been noted to possess certain biological effects (Bhat et al. 2015a, b).

Meat by-products and muscle proteins are a promising source of angiotensin-converting enzyme (ACE) inhibiting peptides with both *in vitro* and *in vivo* biological activities for hypertension treatment. Katayama et al. (2007) conducted an investigation and isolated two new ACE inhibiting peptides from the muscles of pig via treatment with pepsin. The sequence of the peptides was found to be KRQKYD and EKERERQ. The IC_{50} value for ACE inhibition was found to be at 26.2 and 552.5 μ M for KRQKYD and EKERERQ respectively. KRQKYD was administrated orally to spontaneously hypertensive rats (SHR) at a dose of 10 mg/kg of the animal weight. Temporary hypotensive activity was observed after 3–6 h of administration (Katayama et al. 2007).

In another study, an antihypertensive peptide was identified by hydrolyzing porcine skeletal myosin with enzymatic activity of pepsin. Protein hydrolysate was first obtained which was further purified with different chromatographic techniques and afterwards the peptide sequence was identified to be KRVITY. The IC_{50} value of the peptide was determined to be 6.1 μ M. Additionally, the antihypertensive activity of KRVITY peptide and VKAGF, which was identified (Ukeda et al. 1992), were investigated *in vivo* in rats. Both of the peptides, particularly KRVITY, depicted remarkable reduction in blood pressure (BP) (Muguruma et al. 2009).

A novel BAP was isolated from hoki frame fish that functions as the retarding agent for the peroxidation of lipids and acts as a free radical scavenger. It depicted strong anti-oxidizing action greater than α-tocopherol (Kim et al. 2007). Three dipeptides i.e. Arg-Try, Lys-Tyr, and Tyr-Tyr isolated from royal jelly fish consisting of Tyr unit at C-side depicted potent hydrogen peroxide, and hydroxyl-radical scavenging action (Kim et al. 2001a; Wu et al. 2003; Guo et al. 2009). Highly antioxidant peptides were also obtained from the muscle of mackerel, *Scomber austriasicus* (Wu et al. 2003) It has been considered that protein hydrolysis of the muscle of yellow stripe trevally, *Selaroides leptolepis*, via flavoenzyme release functionally active bio-peptide (Kim et al. 2001a).

In simulated gut digestion study ACE inhibitory peptides were liberated from pork meat by means of pepsin and pancreatin. The protein hydrolysate was obtained first from the digest which was further purified with Reversed Phase High Performance Liquid Chromatography (RP-HPLC) and characterized by means of

Table 5.1 Some sources and activities of BAPs

Source	Parent protein	Amino acid sequence	Enzyme	Activity	References
Pig muscle		KRQKYD, EKERERQ	Pepsin	Angiotensin-converting enzyme (ACE) inhibition	Katayama et al. (2007)
	Skeletal myosin	KRVITY, VKAGF	Pepsin	Reduction in blood pressure (BP), Antihypertensive	Ukeda et al. (1992)
	Myofibrillar proteins, Actin	EELDNALN, DAQEKLE, VPSIDDQEELM, IEAEGE, DSGVT	Actinase E	Antioxidant	Saiga et al. (2003)
Royal jelly fish		Arg-Try, Lys-Tyr, Tyr-Tyr		Antioxidant	Kim et al. (2001a), Wu et al. (2003), and Guo et al. (2009)
Yellow stripe		Flavoenzyme		Functionally active	Kim et al. (2001a)
Chicken dark meat	β-actin	YASGR		Antioxidant	
Tuna muscle			Pepsin, papain	ACE inhibition, Antihypertensive	Qian et al. (2007)
Beef		FHG, DFHING, GFHI, GLSDGEWQ	Commercial enzyme	Antihypertensive, Antimicrobial, Cytotoxic effects	Jang et al. (2008)
Blood	Serum albumin		Trypsin	Dipeptidyl peptidase-4 inhibitor (DPP-IV) inhibition, ACE inhibition, Antioxidant	Arrutia et al. (2016a)
Milk	Lactoferrin	VPP, IPP	*Lactobacillus* proteases	Antihypertensive	Moller et al. (2008)
	α-lactalbumin	Tyr-Gly-Leu-Phe		Antihypertensive	
	β-lactoglobulin	Tyr-Leu-Leu-Phe		Antihypertensive	Sipola et al. (2002)
	Casein			Opioid, ACE inhibition, Antimicrobial	Boutrou et al. (2015)
Collagen		EIIICIII, EIIICIV	Collagenase	ACE inhibition	Kim et al. (2001b)

		GAHypGLHypGP	*Aspergillus oryzae* protease	ACE inhibition	Saiga et al. (2008)
Skin gelatin		His-Gly-Pro-Leu-Gly-Pro-Leu	Tryptic	Antioxidant	Mendis et al. (2005)
Hemoglobin	β globin	Tyr-Pro-Trp-Thr		Opioid	Nyberg et al. (1997)
	Globular chain	PVNFKFLSH		Antinociceptive, anti-hyperalgesic	Lippton et al. (2006)
Egg		KIVSDGNGM, VSDGNGM		Antioxidant	Miller et al. (1993)
		IRW		Antidiabetic	Yu et al. (2011)
Soya	Glycinin		Trypsin, pronase,	ACE inhibition	Kumar et al. (2012)
Fruit stone		HNLPLL, MLPSLPK	Alcalase	Antioxidant, ACE inhibition	Bamdad et al. (2011)
Rice	Prolime			Anti-leukemia	Yu et al. (2006)
Sponge				Anticarcinogenic	Pettit et al. (1993)
Fungi		FR235222		Antimicrobial, Antiplasmodial	Yang et al. (2018)

Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometer (MALDI-TOF/TOF MS). In this study 22 different peptides were evaluated *in vitro* for ACE inhibition. Among all of them peptides with sequences PTPVP and KAPVA showed the highest bioactivity with IC_{50} values of 256.41 and 46.56 μ M respectively (Escudero et al. 2010).

In one study, BAPs were isolated from chicken dark meat. The isolated peptides were evaluated against hypochlorite ions and peroxyl radicals for their antioxidant potential. The isolated peptides were purified with High-Performance Liquid Chromatography (HPLC). One of the peptides exhibited remarkable antioxidant activity when evaluated against the peroxyl radical. This amino acid sequence of the peptide was determined, and the first five amino acids were found to be YASGR which correspond to amino acid number 143–147 of chicken β-actin (Fukada et al. 2016).

ACE inhibitory peptide was separated from the dark muscle of tuna (*Thunnus obesus*) hydrolysate. The hydrolysate was prepared by treating the muscle with the enzyme alcalase, neutrase, pepsin, papain, alpha-chymotrypsin, and trypsin, respectively. Amongst all the hydrolysates, the hydrolysate of pepsin showed the maximum ACE inhibitory ability. The peptides obtained from fish also depicted antihypertensive action. The study concluded that the peptide obtained from the dark muscle of tuna can serve as useful element for pharmaceuticals and functional food against hypertension and related ailments (Qian et al. 2007).

Four different peptides with the sequence FHG, DFHING, GFHI and GLSDGEWQ were isolated from beef sarcoplasmic proteins via commercial enzymes. The peptides depicted remarkable antihypertensive activities. The IC_{50} values of the peptides against ACE inhibition was determined to be 52.9(FHG), 64.3(DFHING), 117(GFHI) and 50.5(GLSDGEWQ) μ g/ml. The peptides were also investigated for their antimicrobial potential. For this purpose, different bacterial strains such as *Escherichia coli*, *Listeria monocytogenes, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Bacillus cereus* were used. The peptides not only showed antimicrobial activity against at least one of the above microorganisms but also exhibited cytotoxic effects against carcinogenic cells (Jang et al. 2008).

Porcine liver was studied for the generation of BAPs. Two potential ACE inhibitors were isolated from protein hydrolysates of the porcine liver. The IC_{50} values of the peptides were found to be at 0.31 and 0.18 mg/ml. Oral administration of the peptides in SHR indicated significant reduction in the blood pressure of the animal. The study concluded that both of the peptides have ACE inhibiting and antioxidant abilities along with the property of lowering the blood pressure (Inoue et al. 2013).

Blood is a remarkable source of proteins and signifies an amazing source of BAP. Though disposal of blood is a huge problem for meat handlers and because of this the "serum albumin", a major blood protein has garnered little interest. In a study, conducted by Arrutia et al., serum albumin was hydrolyzed via enzymatic action of trypsin. A biologically active sequence of the peptide was obtained. This bioactive peptide when evaluated showed the following activities: ACE inhibition activity, Dipeptidyl peptidase-4 inhibitor (DPP-IV) inhibition and antioxidant activity (Arrutia et al. 2016a).

A study reported the antioxidant activity of peptides isolated from hydrolysates of porcine myofibrillar proteins, these peptides were generated by the action of actinase E or papain and was further purified by Ion Exchange Chromatography (IEC). The antioxidant activity of the peptide was determined with, DPPH radical scavenging activity, linoleic acid peroxidation system and metal-chelating activity assays. The investigation reported five antioxidant peptides with the following sequences EELDNALN, IEAEGE, DSGVT, VPSIDDQEELM and DAQEKLE. The peptide with the sequence DAQEKLE from actin protein, exhibited the maximum antioxidant activity (Saiga et al. 2003).

From the above discussion it is evident that meat from different animal sources such as mammals, birds, fishes provide a variety of BAPs. The BAPs isolated from the meat proteins and hydrolysates possess wide-ranging biological activities such as antihypertensive activity, anti-oxidant activity, antimicrobial activity and certain enzyme inhibition activity.

5.2.2 Bioactive Peptides from Dairy Sources

Bovine milk, dairy goods, and cheese are the dominant sources of BAPs and bioactive proteins among food sources (Mohanty et al. 2016). Apparently, this may be the reason of why milk is essential beyond nourishment in the early months of the life (Moller et al. 2008). Milk proteins have a wide range of biological abilities. For example, lactoferrin (Lf) exhibits antimicrobial activity where immunoglobulins have an immune-protective influence. A small number of hormones and certain growth factors are present in colostrum, which play significant roles in post-natal development (Park and Nam 2015).

Milk proteins are packed with BAPs, these peptides are separated during food processing or gastrointestinal digestion (Meisel and FitzGerald 2003). In an investigation, opioid peptide was separated from dairy products, these peptides have promising pharmacological properties analogous to morphine hence relevant in some central nervous system (CNS) problems (Haque et al. 2008).

HPLC-MS and tandem mass spectrometry (MS-MS), were used to separate a different BAP from milk of human mothers of pre- and full-term infants. The isolated peptides were of varying molecular weight such as opioid and phosphopeptides. The isolation of several peptides from human milk confirms that human milk is susceptible to greater casein proteolysis compared to bovine milk. The results of the study strengthen the significance of maternal milk for the infants (Ferranti et al. 2004).

Lactoferrin (Lf), is the milk protein of all mammals. It is glycoprotein in nature and is known for its iron-binding properties. Other than remarkable iron binding properties it also depicts antimicrobial and immunomodulating abilities. Peptides derived from Lf showed remarkable antihypertensive activities. Both Lf and peptides derived from Lf have been reported to affect the production of cytokines which are associated with the immune and inflammatory functions of the body (Moller et al. 2008).

Hypertensive subjects showed lowering in BP after consuming the fermented milk containing the BAPs. *Lactobacillus helveticus* fermented milk contains the BAP, Val-Pro-Pro and Ile-Pro-Pro. These peptides showed lowering in blood pressure of SHR. Two additional peptides Tyr-Pro and Lys-Val-Leu- Pro-Val-Pro-Gln were also isolated from fermented milk. These two peptides depicted ACE inhibitory activity in SHR (Jäkälä and Vapaatalo 2010).

Whey fraction of the milk is also enriched with BAPs. In one investigation, whey proteins concentrate was hydrolyzed with trypsin and the obtained peptides were separated by means of ultra and nanofiltration membranes. β-lactoglobulin peptides were obtained in very large amount from the concentrate as it was explicitly cleaved (Arrutia et al. 2016b). Antihypertensive peptides have also been isolated from the whey portion of milk protein. Whey proteins α-lactalbumin and β-lactoglobulin produce two tetrapeptides (by enzymatic proteolysis) α-lactorphin (Tyr-Gly-Leu-Phe) and β-lactorphin (Tyr-Leu-Leu-Phe) respectively. These peptides have shown BP reduction in SHR (Sipola et al. 2002).

Different BAPs have been isolated from the milk of cow. Cow milk proteins exhibit wide ranging biological properties such as anti-carcinogenic, opioid, mineral-binding, immunomodulatory, ACE inhibitory, cytotoxicity, anti-thrombotic and antibacterial activities. The natural properties of BAP vary depending upon the source of milk proteins from which they are isolated. Different BAPs have been isolated and characterized from milk protein hydrolysates of mare, buffalo, sheep, camel, goat and yak milk has been reported (El-Salam and El-Shibiny 2013).

In a research made by Boutrou et al., 69 different BAPs were released from casein milk protein. Significant interest was shown to β-casomorphins and caseinomacro-peptide derived peptides. These BAPs were categorized as opioid, ACE inhibitors, and antimicrobial peptides (Boutrou et al. 2015).

The milk of donkey is a very useful product for the food industry, owing to its nutraceutical, nutritional, and functional properties. Endogenous peptides isolated from donkey milk have been studied for their ACE inhibitory and antioxidant potentials. In a study, two novel endogenous antioxidant peptides, namely GQGAKDMWR and EWFTFLKEAGQGAKDMWR were isolated from donkey milk. In the same study two other peptides with sequences REWFTFLK and MPFLKSPIVPF having ACE inhibitory actions, were also identified (Chiozzi et al. 2016).

5.2.3 *Bioactive Peptides from Collagen Sources*

Connective tissue converts the force of contraction into movement, by joining the muscles to the bones. The connective tissues joining bones and muscles are known as tendons. Fibrillar collagen is the major component of tendons. There are several sorts of collagens present in the extracellular matrix. Collagen is the major structural component of connective tissues. All collagens contain collagen triple helices which is primarily the recurrence of proline enriched tripeptide Gly-X-Y (Gelse et al. 2003).

Gelatin is a soluble protein compound which is produced as a result of partial hydrolysis of collagen (Johnston-Banks 1990). In a study, ACE inhibitory peptides were isolated from the bovine skin gelatin hydrolysate. Five different proteases such as α chymotrypsin, Alcalase, Pronase E, Neutrase, and trypsin were employed in the study. The treatment of hydrolysate with enzyme was followed by ultrafiltration and was finally treated with collagenase. Subsequently two BAPs with excellent ACE inhibitory activity were isolated. The first peptide was named as EIIICIII and the second peptide was named as EIIICIV, with IC_{50} value of 4.7 μ M, and 2.55 μ M respectively (Kim et al. 2001b).

Skin gelatin of fish Hoki on hydrolysis via enzymes released peptides that showed potent biological action. Hydrolysis via tryptic enzyme released peptide, which exhibited powerful action on DPPH and superoxide anion. Tryptic hydrolysis released another peptide with specific sequence His-Gly-Pro-Leu-Gly-Pro-Leu with strong radical scavenging ability (Mendis et al. 2005).

Chicken collagen was hydrolyzed by an *Aspergillus oryzae* protease and ACE inhibitory hydrolysate were also obtained. The subsequent hydrolysate was further treated with four different proteases, amino G, protease A, protease FP, and protease N. Consequently, four different peptides exhibiting ACE inhibitory potential were isolated and the IC_{50} values of each were determined. The peptides were named as GAHypGLHypGP, GAHypGPAGPGGIHypGERG, GLHypGSRGERGERGLHypG and GIHypGERGPVGPSG with IC_{50} values of 29.4, 45.6, 60.8 and 43.4 μ M respectively. It was concluded in the study that low molecular weight chicken collagen hydrolysates had long-lasting hypotensive effects *in vivo* and can serve as possible antihypertensive therapeutic agents (Saiga et al. 2008).

Fish peptides have significant biological activities against bacteria, viruses, and fungi and used as immunomodulator, and antitumor agents. Recently, it is suggested, that almost all fish antimicrobial peptides possess antibacterial or bacteriostatic activities against several Gram-negative and -positive bacteria. A novel 20-residue antimicrobial peptide, pelteobagrin, was isolated from the skin mucus of yellow catfish (Rajanbabu and Chen 2011).

In a study BAPs are obtained from the hydrolysis of squid (Dosidicus gigas) by- collagen, by means of enzyme Protease type XIV. The protein hydrolysate was further purified by ultrafiltration. After treatment with ultrafiltration membranes the BAPs from squid exhibited enhanced antioxidant and antimutagenic activities but the antiproliferative activity did not improve after ultrafiltration (UF) (Suárez-Jiménez et al. 2019). In another study, collagen remains from squid skins were treated with Esperase and hydrolysate was obtained. This hydrolysate was further fractionalized into three peptide fragments. The peptide sequence with the lowest molecular weight exhibited maximum ACE inhibition ability (Alemán et al. 2013).

5.2.4 Bioactive Peptides from Hemoglobin Sources

In 1971, the first biologically active peptide was obtained from hemoglobin (Hb) (Moeller et al. 1997). Hemoglobin is familiar as an origin of series of peptides which can play miscellaneous biological roles. Hemoglobin is a tetramerous protein with two α- and two β-globular chains. Each globular chain possesses a prosthetic group i.e. heme portion and basically acts as oxygen carrier in oxidation-reduction reactions and in tissues. In addition to tissues such as RBCs, α- and β-globular chain encrypt in the brains and peripheral tissues of humans and rodents, many different novel bioactive peptides were isolated from variants of hemoglobin which have significant physiological functions (Gomes et al. 2010).

Neokyotorphin as a bioactive peptide is a globular chain of Hb with (137–141) sequence at C terminal presenting originally the analgesic action like that of Leu-enkephaline (Fukui et al. 1983; Kiso et al. 1983). Neokyotorphin peptide (137–141) isolated from various sources i.e. bovine brain, heart of rat, lung and brain. Moreover, it also exists in hibernating ground squirrel with variant biological actions. Except for hemorphins, neokyotorphin has been imparted a series of role like regulation of body temperature, modulation of antibacterial action, regulation of vagus nerve action on heart, provide safety from convulsion in epileptic cases, regulation of brain function of ground squirrel and multiplication in cancerous cells and in lipocytes (Hazato et al. 1986; Ueda et al. 1987; Kolaeva et al. 1990; Sazonova et al. 2003).

Kyotorphin, a non-opioid, BAP is also a globular chain fragment of Hb with (140–141) sequence at C terminal is a potential analgesic agent mainly isolated from different sources such as bovine brain, spinal cord of rat and brain of rat. Until now, the target for its biological activity is still unknown (Takagi et al. 1979).

A BAP, Hemorphins, (Tyr-Pro-Trp-Thr) derived from β globin of hemoglobin at N-terminal mainly reduces electrical contraction in GPI bioassay. Extended hemorphin peptides have been obtained from a variety of sources such as bovine and human tissues. It has been found that hemorphin plays an antagonist role for receptors of opioids (Nyberg et al. 1997). Extended hemorphin-7 is considered as a powerful ligand for the IV receptor of angiotensin (Moeller et al. 1997; Moeller et al. 1999). LVV-hemorphin-7 was found to provide a protection to BAP from degradation process by inhibiting peptidases like insulin-regulated aminopeptidase (IRAP) and minimize the Bp level by giving injection into the peritoneal cavity. Besides these, in supersensitive rat, LVV-hemorphin 7 was noted to lower the heart rate (Cejka et al. 2004). In insensible rats, it heightened the action that caused reduction in Bp in bradykinin (Fruitier-Arnaudin et al. 2002). It is noteworthy that LVV-hemorphin-7 has significance in terms of reminiscence and acquisition of knowledge. As in rats, learning is boosted by administrating the injection of LVV-hemorphin 7. It is thought that LVV-hemorphin-7 depicts undeviating action on other focal points which causes the delivery of acetylcholine followed by depolarizing effect, heightening the cholinergic dionifus leading to upgraded reasoning level (Herbst et al. 1997; Lee et al. 2001). Finally, the ability of LVV-hemorphin 7 is to provide safety to the surface of IRAP followed by blocking the action of IRAP that is consistent with a major function in remembrance and in knowledge acquisition (Herbst et al. 1997) (Kovács and De Wied 1994; Engelmann et al. 1996; Matsumoto et al. 2001).

Hemopressin (PVNFKFLSH) is a BAP fragment extracted from rat brain. It has a sequence of (96–104) isolated from a globular chain of hemoglobin noted to have a major role as anti-nociceptive and anti-hyperalgesic (Dale et al. 2005; Lippton et al. 2006). It is thought that, Hemopressin acts as a substrate for many and different metal peptidases such as neurolysin and oligopeptides (Piot et al. 1992; Moisan et al. 1998). Extended hemopressin obtained from a globular chain of hemoglobin at N-terminal known as RVD-Hpα and VD-Hp-α. Extended Hp shows the antagonistic action at the reception site of cannabinoid (Gomes et al. 2009).

A peptide with sequence of (33–61) isolated from α-globulin of bovine haemoglobin showed antibacterial action in the digestive tract of tick *Boophilus microplus*. It was considered that, it's most powerful action was against the bacteria *Micrococcus luteus* A270, and could also be used for identification of antibodies (Froidevaux et al. 2001).

Definisms BAP derived from β globin of vertebrate, leucocytes and epithelial cells, have importance in natural immunity. Another BAP Magininns derived from alpha chain showed antimicrobial action and their helix like structure helps in connection and is permeable for target point (Kosciarz et al. 1999).

5.2.5 Bioactive Peptides from Egg Sources

Egg is a valuable component of a healthy and balanced diet. It consists of protein and has been considered as a source of multiple peptides that has found application against diabetes, microbes, adhesiveness, oxidants, high blood pressure, and may act as an immune modulator, and a mineral binder. Hydrolysis of proteins can be utilized to release potentially bioactive fragments depending on the enzyme activity that have miscellaneous medicinal value (Yu et al. 2014).

Hydrolysis of egg protein have been shown to produce antioxidizing agents that can be used for peroxidation either in the presence or absence of relevant enzymes (Mine 2007). The bioactive peptides KIVSDGNGM, Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu and VSDGNGM primarily released from egg protein were noted to possess antioxidant properties due to its configuration, hydrophobic nature and strength of its amino acid unit. The antioxidant property were measured using different methods like oxygen radical absorbance capacity and Trolox equivalent antioxidant capacity (TEAC) etc. (Miller et al. 1993).

The egg of hen comprising of protein has medicinal value in terms of their action as a antimicrobial agent involving lysozyme and ovotransferrin (Mine et al. 2004; Kovacs-Nolan et al. 2005). Ovotransferrin, BAP, as a member of transferrin and monomer of glycoprotein comprising of only one polypeptide chain with 686 amino acid that was found to be capable for binding to iron. It is proposed that, in addition to functioning as iron carrier, it can also protect marketed food or nutrient from contamination as an antimicrobial agent against multiple bacteria (Mine 2007). Crippled Ovotransferrin and fully iron Ovotransferrin exhibiting the same biological action as antibacterial agent, in its turn, that representing antibacterial action may not be owing to its iron carrier capability but also the interaction between bacterial cell and protein reason for it (Ibrahim 1996). Antimicrobial peptide, Ovotransferrin OTAP-92, also has application to approach membrane of gram-negative bacteria and found to cause destruction of cytoplasmic membrane that results in death of bacterium. Another bioactive peptide called defensin that has similarity with Ovotransferrin in reference to peptide sequence penetrating into the cytoplasm or in ion channel that result in bacterium destruction (Ibrahim et al. 2000).

Additionally, multiple BAPs with antimicrobial activities were also liberated from various parts of the egg such as albumin, vitelline membranes and eggshell (Mine et al. 2004; Abdou et al. 2007; Hervé-Grépinet et al. 2010; Thammasirirak et al. 2010). Majority of these peptides have both polar and non-polar region, and overall referred as positive charge carrier, but peptides of α helical chain with hydrophobic end are also present and are remarkable in their action against microbes. However, multiple categories of peptides with structure and configuration dissimilarity have been found to be useful for antimicrobial activities (Epand and Vogel 1999).

Biological active phospho-peptides isolated from hydrolysis of phosvitin and their calcium-binding property are considered to be due to their ability to form chelates (Young et al. 2011). Phospho-peptides present in phosphorylated protein consisting of multiple phosphoserines unit found to be useful for efficient binding with calcium, as a consequence, increases calcium access to the body followed by retarding the generation of calcium phosphate that is insoluble (Jiang and Mine 2001; Feng and Mine 2006).

It was suggested that egg peptides are of considerable interest because of their potentially beneficial biological activities, such as antitumor, antithrombotic antibacterial, and anti- adhesiveness (Nelson et al. 2007). Egg peptides IRW acts as antidiabetic agent and have importance in food and nutrition. Egg white is used as a purifier to separate solid particulates or suspended solids from drinks like alcohol. Biologically active peptide, Lysozyme, has antibacterial action, thereby used to preserve food. Furthermore, egg protein and its fragments as a peptide have received increasing attention in food because of its health developing value (Yu et al. 2011).

5.2.6 Bioactive Peptides from Plant Sources

BAPs released from vegetables and plant sources have been isolated, characterized and studied frequently because of their benefits for human kind. Different BAPs have been produced from extracts of protein, produced by seeds of soyabean or soya bean milk which showed biological action against microbes. Another way to obtain peptides is through endogenetic protein hydrolysate (Singh et al. 2014). Peptides from milk of soya-bean are obtained when food is processed (Capriotti et al. 2015).

Another BAP called oligopeptides are discharged mainly from a soy protein i.e. glycinin, or hydrolysis of soy and the fermented products of soy i.e. tempeh and natto via many and different endogenetic protein such as trypsin, pronase, Glu-C protease, protein hydrolysis of kidney membrane and plasma proteases. Hydrolysis of natto via pronase yields another peptide that biologically acts as an angiotensin-converting-enzyme inhibitor. Moreover, it presents some characteristics of a surface active agent. Different sources like cooked coffee, cocoa, grilled grain and other fermented products like alcoholic drink also provide BAPs. A bioactive peptide, 2, 5-Diketopiperazines obtained from alcoholic beverage i.e. awamori has antioxidant potential (Kumar et al. 2012).

In industry food processing, a huge amount of waste is produced. For example, production of oil such as olive oil generates a variety of by products involving solid product, which is a collection of pulp and stone, and liquid stuff formed by olive tissues. All these by products called pollutants are not certainly perishable. Peptides with antioxidizing and antihypertensive abilities have been obtained from hydrolysis of proteins, that are released from seeds of olive (Esteve et al. 2015).

Vegetables and fruit processing liberate high amounts of waste products. Some of which are used as fodder and majority of them are useless. Fruit stone (plum) roughly known as seed, is full of protein with biological importance in medicine and the food industry. They have been considered as potential source of BAPs with low cost. High intensity focused ultrasound has been used for the production of protein derived from waste products (González-García et al. 2014). Hydrolysis, via enzyme like flavourzyme, thermolysin and Alcalase, of these proteins yields peptides that act as antioxidizing agents and possess inhibitory action against ACE. Among them, hydrolysis via alacase enzyme generate BAPs with antihypertensive and antioxidant activities. Furthermore, it is analyzed that, hydrolysis via alacase release variety of peptides such as HNLPLL, HLPLLR, YLSF, DQVPR, LPLLR, MLPSLPK and VKPVAPF that exhibit more potent biological action i.e. antihypertensive and antioxidant. At the global level, *Hordeum vulgare L*, barley grain is the fourth most abundant cereal food. It is also known as fodder. It has less nutritional value because of lower concentration of lysine and thereby, is less expensive (Bamdad et al. 2011).

In Mexico, some years ago, maize has been considered as a highly grown cereal bran next to wheat and rice which is rich in protein. It is a grain full of protein that helps satisfy world's protein demand (Malumba et al. 2008; Pechanova et al. 2013). Depending on the solubility, protein food can be divided into saline soluble, water soluble, alkaline soluble and alcoholic soluble (Uarrota et al. 2011). Several studies indicate that breakdown of corn kernel releases corn gluten meal that is a byproduct consisting of protein. It is observed that corn protein on hydrolysis yields corn peptides (CPs) with anticancer properties particularly against breast cancer. In HepG2 cells, CPs persuade cell death and used as a potent anticancer agent by retarding cancer causing agents by improving the immune system (Li et al. 2013).

Hordein is a barley prolamin and considered to be the main protein byproduct of barley. It is abundant with multiple amino acids such as, Tyr, Leu Pro, Val, Phe and Glu, majority of them present antioxidant action when they are either in protein or in peptide form. Hordein is a complex consisting of three fragments; first one is B hordein i.e. full of Sulphur, C hordein with lack of Sulphur and D hordein having greater molecular mass. C hordein is considered to possess powerful antioxidant property. Hydrolysis via enzymes, is the most efficient way to increase antioxidant potential of barley hordein (Chanput et al. 2009).

Recently, it is thought that lunasin like peptide resides in barley, with effective biological action. This knowledge pushed researchers to search and identify lunasin in other cereals (Jeong et al. 2002). Currently, lunasin is found in seed crops like amaranth, soybean, rye and wheat. Other bioactive compounds (except for lunasin) like peptides are under observation in these grains (Jeong et al. 2010).

Avena sativa L, an oat, is considered as a familiar cereal because of its food value and multipurpose properties. Oat and oat by-products have been used as a supportive agent for the therapy of diabetes and heart ailments. In food, presence of oat grain make the genes more effective that play a role in the development of β cell, synthesis of protein and secretion of insulin (Yamaguchi et al. 1997). It is interesting that lunasin like peptides are also observed in oat bran and are noted with greater antioxidant ability (Nakurte et al. 2013).

Besides wheat, *Secale cereale L.,* commonly known as rye, is the most useful bran for bread especially in Europe and considered to be an important source of roughage. In northern Europe, rye is regularly used as a staple food. Recent data indicate that the use of rye bran is related to lowering the chances of degenerative diseases such as heart disease, diabetes and cancers. Besides the roughage, minerals, different phytochemicals, vitamins have been recommended as a health promoting compounds. Lunasin-like peptides are also found in rye bran and their concentration vary from one rye to another. Each of them possesses active biological activities (Bondia-Pons et al. 2009).

At a global level, rice as a cereal bran is considered to be a basic food and has importance because of its nutraceutical value and easily digestible property. Rice is also biologically important because of its contents. Rice contains a large variety of proteins like glutelin, globulin, albumin and prolamin. Prolime protein fragment has found potential application in developing and improving the immune system against leukemia. Rice protein isolate (RPI) derived from rice protein can minimize brain tumor in rats induced by 7,12-dimethylbenz[a]-anthracene (Yu et al. 2006).

5.2.7 Bioactive Peptides from Marine Sources

Marine products are now considered internationally for their diversity in the production of biologically active substances.

Peptides obtained from marine organisms have revealed a novel outlook for medicinal progress. In a study it was found that BAPs isolated from marine species can serve as potential blockers of ion channel, toxic effect on cells as well as possess antimicrobial action (De Vries and Beart 1995).

Sponges belonging to phylum porifera consist of 1000 miscellaneous species which extend from the surface to the depth of the ocean. A focus has been given to seek biologically active peptide from sponges. Tetradecapeptide Discodermins was the first bioactive peptide obtained from sponges showing inhibitory action for cell growth. Discodermins A–H, a BAP, isolated from genus sponges, Discodermia, consist of 13–14 linearly-aligned amino acid forming a ring by a cyclic ester of carboxy end with threonine element. These BAPs are characterized by their cytotoxic ability and were used for analysis against A549 cell line of human lung and P388 cancerous cells of rodents. In cell membrane of smooth muscles, Discodermins A can potentially act as a permeabilizing agent (Aneiros and Garateix 2004).

Geodiamolides A–G, is a BAP obtained from a specie of Geodia, Caribbean sponge. Another peptide jaspamide, similar to Geodiamolides, has cytotoxic property and consist of a cyclic ring of three amino acid. *Arenastatin A*, desipeptide, is a cyclic ring isolated from a powerful cytotoxic agent that is profoundly used against KB cells. Phakellistatins is a heptapeptide ring with abundance of proline. Phakellistatin 2 is particularly considered as valuable owing to its powerful biological action against various skin cancer cell lines and P388 cancerous cells of rodents (Pettit et al. 1993).

Biologically active antifungal peptides, Discobahamin A and B, were isolated from the Bahamian marine sponge living in deep water belonging to Discodermia sp. that have inhibitory action against the growth of *Candida albicans* (Gunasekera et al. 1994).

Antifungal and cytotoxic depsipeptides Halicylindramides A– E was isolated from the Halichondria cylindrata, a Japanese marine sponge. Halicylindramides A–C are tetradecapeptides that form a cyclic ester with threonine residue while Halicylindramide E is a trimmed straight peptide having an amide group at C-end (Li et al. 1995, 1996).

C–E cyclic peptides obtained from the sponge species, Microscleroderma Theonella and Microsclerodermins were also noted to have biological action against fungi. Other bioactive peptides, Theonegramide and Aciculitins A–C isolated from sponges were also noted to have effective anti-fungal action (Schmidt and Faulkner 1998).

Linear and ring BAP Dolastatins derived from *Dolabella auricularia* mollusk, have the ability to retard the cell growth. A large number of Dolastatin BAPs have been isolated from mollusks. Dolastatin 10, in particular is an apentapeptide derived from mollusk has found an application in the treatment of cancer against the cell line P388. In addition, Dolastatin 10 also retarded the polymerization of tubulin protein and hydrolysis of Guanosine-5′-triphosphate (GTP) that rely on tubulin. On the other hand, Dolastatin 11 induced huge alignment of the actin filament of the cells followed by fixing the cells at cytoplasmic division and also caused more and more polymerization of actin. When compared to depsipeptide Jasplakinolide (derived from sponges), Dolastatin 11 showed a three-fold higher cytotoxicity than Jasplakinolide against the studied cells (Bai et al. 2001).

Depsipeptide such as Tamandris A and B derived from the ascidian belonging to family Didemnidae are efficient in cytotoxic action. Analysis of Tamandris and Didemnin as anticancer agent have shown that Tamandris was a little bit more powerful than Didemnin (Vervoort et al. 2000). Depsipeptide such as didemnin, have been found useful against tumors, viral diseases and can also act as immune-suppressants. This peptide is primarily derived from the Caribbean tunicate of Trididemnum (Rinehart et al. 1990).

It is noteworthy that, Didemnin B is a well-known member of the family because of its powerful antitumor action (Rinehart et al. 1990). It is noted that, Didemnin B possesses excellent ability as producers of antiproliferative compounds along with antitumor properties. It is used widely for cure of prostatic cancer (Geldof et al. 1999). Didemnim A and B, otherwise known as bioactive substances, may be effective for dissimilar varieties of virus such as parainfluenza, herpes simplex, and dengue virus (Canonico et al. 1982). A BAP Styelin D bearing 32-units with amide group at C-terminal, derived from hemocytes of *Styela clava*, an ascidian, was shown to have antibacterial action against gram positive and negative bacteria residing in salty condition (De Vries and Beart 1995; Taylor et al. 2000).

5.2.8 Bioactive Peptides from Fungi Sources

Penicillium algidum, a fungus, on hydrolysis liberates two known cyclic peptide i.e. cycloaspeptide A and cycloaspeptide D and cyclic nitro peptide psychrophilin D. Biological analyses indicate that they are biologically active against cancer, malaria, certain viruses and other microbes. Nitropeptide, psychrophilin D showed moderate action against blood and bone marrow cancer P388 in rodents, while peptides cycloaspeptide A and D showed moderate action against malaria caused by *Plasmodium falciparum* (Dalsgaard et al. 2005).

Fungi release multiple peptides and cyclopeptides such as cephalosporins and penicillins (Kozák et al. 2018). Emodepsin, a semisynthetic Emodepsin, also known as depsipeptide, may have use against parasitic worms among animals (Woods et al. 2018). Structural diversity of different peptides shows different biological action (Wang et al. 2018). A fungus-derived peptide known as Gliotoxin, reside in subject of aspergillosis affecting their cell by retarding their binding capacity. Moreover, it reduces the immune capability and persuades the apoptosis action in affected cells (Bertuzzi et al. 2018).

It is observed that BAPs from Apicidin F, JM47, chlamydocins and FR235222 have biological action against protozoans and can retard the growth of cells and inhibit immune capacity, eventually causing cell death. In addition to the above-mentioned properties, these peptides also show antiplasmodial potential (Yang et al. 2018).

A depsipeptide called Aspergillicin obtain from fungi used to regulate immune system. Cyclodepsipeptides, Stevastelins have a lipotropic terminal. It has the ability to inhibit the activity of human T-cells but has less toxic effect in mice (Wang et al. 2018).

A cyclic depsipeptide called Verticilide was isolated from Verticillium species can make ryanodine less effective in binding with receptor and acts as a biological agent against insects. Cycloaspeptide A and D derived from fungi are considered to be most potent biological agents against parasites (Anke and Laatsch 2018).

5.3 Conclusion

BAPs are organic substances of immense importance that are concealed between structures of proteins from various natural sources. Enzymatic actions coupled with other physical techniques can liberate these biologically active peptides during certain processes like digestion or fermentation. The BAPs have wide ranging biological activities like anti-thrombotic, antimicrobial, opioid, antihypertensive, immuno-modulatory, antioxidative and mineral binding. Owing to these and several other biological activities, BAPs are being used extensively in pharmaceuticals and food industry. They are also being employed for cure and prevention of several medical conditions. BAPs are being isolated from several natural sources such as animals, plants, marine life, fungi etc. Natural sources are the major sources for isolation of bioactive peptides. This chapter provides a descriptive study on the sources from which BAPs have been isolated. Different natural sources of BAPs like meat, collagen, dairy, hemoglobin, plants, marine and fungi along with their potential biological importance were also discussed.

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Chapter 6
Fats and Oils as Sources of Bioactive Molecules

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6.1 Introduction

A biomolecule or biological molecule can be regarded as molecules that are present in organisms to carry out biological process such as development, cell division, and morphogenesis (Horton et al. 1996). A large number of biomolecules are present inside the living cells. These comprise amino acids, proteins, enzymes, monosaccharides, disaccharides, polysaccharides, fatty acids, fats and oils, nucleotides, nucleic acids, histones, acidic proteins, chlorophyll, hemoglobin and several other constituents of cell structure (Horton et al. 1996). These biomolecules are complex organic molecules in nature. They form the basic structural constituents of living cells. Among biomolecules, nucleic acid (DNA and RNA) carry out distinctive function of containing genetic code of an organism which is the sequence of nucleotides that controls the sequence of amino acids constituting a protein. There are 20 different amino acids that can be found in structure of a protein. Proteins perform various tasks inside a cell such as transporters, involve in transport of nutrients and other molecules, and as enzymes to catalyze chemical reactions which takes place

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inside the cell. Proteins also constitute hormones and antibodies, and they influence gene activity as transcription factors.

Carbohydrates are constituted by molecules comprising carbon, hydrogen, and oxygen (Horton et al. 1996). They are essential energy source and structural components of cell. Carbohydrates are among the most abundant biomolecules on earth. Lipids are another key biomolecule inside the cell. They perform a variety of functions, such as serving as a source of stored energy and as chemical messengers in signal transduction. They are also key component of biological membranes, which serves as a boundary between cells and their environment and compartmentalizes cell interior such as the nucleus and the mitochondrion, in eukaryotic organisms.

6.2 What Are Lipids and Oils?

Lipids are basically composed of carbon, hydrogen and oxygen. They are composed of fatty acid and glycerol. The glycerol backbone and variation in fatty acid chains as well as their natural modifications resulted into various forms of structural and functional lipids. These are one of the most important biologically active molecules that are insoluble in polar solvents but are soluble in non-polar solvents like ether and chloroform. Lipids are important constituent of living cells. Oils are generally ester derivatives of glycerol along with three fatty acids. They are important source of energy, essential fatty acids and fat-soluble vitamins, which have the tendency to associate with fats. There are various types of lipids and oils that are discussed below.

6.2.1 Phospholipids and Glycolipids

Phospholipids (CH_2OH-CHOH-CH_2OH) are amphiphilic molecules that contain hydrophobic tails (fatty acid chains) and hydrophilic (phosphate group) heads. The occurrence of a wide variety of phospholipids is due to the variations in head group, aliphatic chain, and alcohols. Phospholipids, are divided into glycerophospholipids and sphingomyelins based on the presence of alcohol groups (Baer and Pavanaram 1961). It plays a vital role in the synthesis of lipid bilayer, in drug delivery system, as components in cosmetics, in biomedical engineering and polymer science, etc. (Mashaghi et al. 2013).

Glycolipids ($C_{58}H_{95}NO_{12}P_2^{-2}$) are glycosyl derivatives of lipids, which consist of one or more monosaccharide units bound with glycosidic linkage to a hydrophobic moiety, e.g. sphingoid, ceramide, and acylglycerol (Malhotra 2012). Glycolipids are produced in the golgi apparatus and widely distributed in lysosomes, nuclear membrane, mitochondria, and endoplasmic reticulum (Gillard et al. 1993). Glycolipids have been shown to modulate intracellular activities in signal transduction, cell proliferation, and programmed cell death in lymphoid cells mediated by ceramide.

Gangliosides help in synaptic transmission through binding with calcium ions (Spoeckner et al. 1999).

6.2.2 Cholesterol and Steroids

Cholesterol ($C_{27}H_{46}O$) is an organic molecule and biosynthesized in all animal cells. It is an important part of cellular membranes, bile acids, steroid hormones, and human brain (Dietschy and Turley 2001). It is essential for normal development of brain cells (neurons and glia) and also required for synapse and dendrite formation. Cholesterol deficiency can cause degeneration of the synaptic and dendritic spine, decrease synaptic plasticity, and failure of neurotransmission (Koudinov and Koudinova 2005).

Steroids ($C_{27}H_{45}OH$) is an organic compound (Cholesterol derived lipophilic) synthesized by sterol lanosterol or cycloartenol in the animals, plants and fungi cells. It is the main constituent of some hormones (hormones of the adrenal cortex and gonadal), bile acids and sterol (Pattenden et al. 2004). Steroids are used as *anti*-inflammatories, anesthetics, *anti*-biotics, *anti*-asthmatics, *anti*-cancer agents and as contraceptive drugs Lopez et al. 2014).

6.3 Bioactive Molecules from Plants

Plants are known to produce various substances which are indirectly associated with their growth and reproduction. These substances are known as secondary metabolites. Plant derived biologically active fats and oils are a type of secondary metabolite having complex chemical structures. These plant derived fats and oils have important ecological functions as they protect plants against harmful microbes and herbivores. Moreover they are also reported to have herbicidal properties, strong antioxidant, and antibacterial activity. Most of the plant derived fats and oils are in the form of fatty alcohols. Fatty alcohols are produced via the process of transesterification. Transesterification is the process in which a fat or oils reacts with an alcohol to form esters or glycerol. Some of the important bioactive plant derived fats and oils are discussed below.

6.3.1 Perillyl Alcohol

Perillyl alcohol or POH ($C_{10}H_{16}O$) is naturally found in essential oil extracted from lavender, peppermint and caraway, spearmint, cherries, sage, and cranberries plants (Crowell and Elson 2001). Traditionally, POH used in cleaning and household products, cosmetics, and fragrance in toiletries (Laszlo 2008). It has biological potential for glioblastoma treatment and chemo preventive agents used in veterinary clinic for cancer treatment mainly in lung, breast, pancreas, colon, and skin cancer (Ong et al. 2017).

6.3.2 Geraniol

Geraniol ($C_{10}H_{18}O$) is acyclic monoterpene alcohol; it is a mixture of two isomers termed as geraniol (trans) and nerol (cis). Geraniol richly found in Palmarosa oil while nerol obtained from neroli (Clark 1998) and possesses biological activities as antifeedant properties and pest control agents (Papachristos et al. 2004). Besides, it has a potential to inhibit the growth of parasites against zoonosis disease (Hierro et al. 2006) also shows chemotherapeutic activity towards pancreatic and other cancers (Burke et al. 1997).

6.3.3 Alpha-Pinene

Alpha-pinene ($C_{10}H_{16}$) is bicyclic compound distributed in the diversity of medicinal plants and coniferous trees. Pinene act as insect repellent properties to protect the plants (Huang et al. 2013) Also, α-pinene has been used in pharmaceutical industries as an antioxidant, anti-inflammatory, antibiotic, bronchodilator, hypoglycemic, antiulcerogenic, and gastroprotective agent (de Almeida et al. 2015).

6.3.4 Terpinen

Terpinen-4-ol ($C_{10}H_{18}O$) is a secondary metabolite, mainly found in essential oil extracted from aromatic plants such as tea, mandarins, oranges, black pepper, and some vegetables. It is a most active ingredient and shows the potential as an antifungal agent, bactericidal, antitumor, anticancer, and anti-mites (Tighe et al. 2013).

6.3.5 Camphene

Camphene ($C_{10}H_{16}$) is a bicyclic monoterpene compound, volatile and soluble in common organic solvents. It is mainly found in essential oil of *Zingiber officinale, Chrysanthemum*, *Liquidamar* species, *Rosmarinus offinialis*, and other plants. It has a great potential to prevent the cardiovascular disease by control of hyperlipidemia (Vallianou et al. 2011).

6.3.6 α-Santalol

α-Santalol ($C_{15}H_{24}O$) is a naturally producing sesquiterpene (hydrophobic alcohol) phytochemical derived from oil of sandalwood (*Santalum album* Linn). It has biological activities as anticancer, anti-inflammatory, and antihyperglycemic properties (Misra and Dey 2013).

6.3.7 β-Elemene

β-Elemene ($C_{15}H_{24}$), are a group of natural compound present in oil of aromatic plants such as turmeric oil extracted from *Curcuma zedoaria* and classified as sesquiterpene (Jiang et al. 2017). It possesses multiple biological activities such as antiangiogenic activity, anti-proliferation, antineoplastic effects, and susceptibility for MDR due to the presence of three unsaturated bonds (Zhang et al. 2013).

6.3.8 Eugenol

Eugenol ($C_{10}H_{12}O_2$) is considered as phenolic compound and core ingredient (70–90%) of clove essential oil obtained from *Eugenia caryophyllata* buds and leaves (Barceloux 2008). It is used as pesticide and fumigant in agricultural field to protect foods against bacteria *Listeria monocytogenes* and *Lactobacillus* during storage (Kamatou et al. 2012). In additions, eugenol shows biological activity as an antioxidant, Anti-inflammatory, anticancer, and also protect the DNA damage (Sarpietro et al. 2015).

6.3.9 β-Caryophyllene

β-caryophyllene ($C_{15}H_{24}$) is a volatile compound and member of bicyclic sesquiterpene, it is chief ingredient of essential oil derived from spice and food plants such as cinnamon, basil (*Ocimum* spp.), cloves (*Syzygium aromaticum*), black pepper (*Piper nigrum* L.), cannabis (*Cannabis sativa* L.), lavender (*Lavandula angustifolia*), and rosemary (Singh et al. 2006). It possesses biological potential *viz.* anticarcinogenic, anti-inflammatory, antimicrobial, analgesic activities and antioxidant activity (Scolnik et al. 1994).

6.3.10 Citral

Citral ($C_{10}H_{16}O$), is a naturally occurring compound containing aliphatic aldehyde, main constituent (80%) of lemongrass oil and also present in citrus fruit. Lemongrass (*Cymbopogon citrates*) is the prime commercial source of this oil. It is widely used as an anti-inflammatory, antiseptic, detergent, carminative, antimicrobial, diuretic, and stimulation of the central nervous system. Also, citral inhibits the growth of tumour in the prostate gland of rats (Albert-Puleo 1978).

6.3.11 β- Thujone

β- thujone ($C_{10}H_{16}O$) is a ketone containing bicyclic monoterpene compound found in two isomeric forms as α- and β- thujone, it occurs naturally in wormwood oil (40–90%) (Olsen 2000). Thujone is toxic to the brain and works as inhibiting the activation of the GABA receptor which cause muscle spasms and convulsions (Baby et al. 2009).

6.3.12 Zerumbone

Zerumbone ($C_{15}H_{22}O$) is a dietary compound reported as 76.3–84.8% in rhizome oils of *Zingiber zerumbet* plant belongs to Zingiberaceae family (Abdelwahab et al. 2012). It possesses a diversity of biomedical properties, such as anticancer activities, antiproliferative, antioxidant and anti-inflammatory (Lawrence 2006).

6.3.13 Menthol

Menthol ($C_{10}H_{20}O$) is cyclic monoterpene alcohol known as mint camphor which is abundant in oils extracted from aromatic plants like tobacco, cornmint, and peppermint (Morice et al. 1994). It displays various biological activities such as anti-inflammatory, anticancer, antimicrobial, fumigants, antianalgesic, antipruritic activity, and antitussive effects (Kolassa 2013). Menthol also used in pesticides, candies, cosmetics, shampoos and chewing gum as a cooling and flavor enhancing ingredient (Simmons et al. 2015).

6.4 Biological Roles of Lipids and Oils

6.4.1 Structural Building

Lipids are considered as the bioactive molecules synthesized in living body. It is working in different way such as multiple signaling pathways and main constituents of the lipid bilayer. If any types of disturbance happened in the lipid metabolism can leads to create disruption in biological pathways *vis-à-vis* cancer, metabolic disease and neurological disorders (Misurcova et al. 2011; Stancu and Sima 2001). Fats and oils are esters of glycerol contain three carbon sugar and three fatty acids which creates various fatty acids on the bases of cis or trans positions of double bond. Fats are solid at room temperature, while oils are usually liquid (Compher et al. 1997).

Fatty acids is a simple lipid and composed of long chain of hydrocarbon (R) and hold carboxylic acids group (RCOOH) (IUPAC-IUB, 1977). There are two types of fatty acid are found in nature saturated and unsaturated fatty acids. Saturated fatty acid contains single bond and possesses biological potential includes stimulation of chloride and bicarbonate ion in colon, colonocyte proliferation, mucus production, and control the growth of saprophytic bacteria in colon (Uauy and Dangour 2006). Unsaturated fatty acids contain two or more double bond in their hydrocarbon chain, it has great potential to living being, present in vegetable oils shows antiallergic properties also used in cosmetology, medicine and pharmacy (Riccardi et al. 2004). Oleic acid shows increase the HDL/LDL cholesterol ratio and decrease thrombocytes (Zak et al. 2007).

Phospholipids considered as biological molecules that display both structural and functional roles in the human body. The utmost phospholipid present in the plasma membrane is phosphatidylcholine or lecithin (60–70%), followed by sphingomyelin (10–20%). Minor phospholipids in plasma are phosphatidylserine (1–2%) and phosphatidylinositol (1–2%) (Fless et al. 1984). Phosphatidylcholine shows important role in the protein synthesis, cholesterol homeostasis, secretion, and triacylglycerol storage (Havel et al. 1955).

Glycolipids are presents in all prokaryotic and eukaryotic cells and considered as a amphiphilic molecules and heterogenous group of membrane-bound compounds. It is crucial constituents of plasma membrane and performs various functions such as signaling receptor, cell aggregation or dissociation, and immune response.

6.4.2 Lipoproteins

Lipoprotein (LP) characterizes as a class of structurally and functionally diverse lipoprotein particles owing to the transport of cholesterol in blood plasma Lipids along with protein circulate in the bloodstream called as lipoprotein, it consists cholesteryl esters (60–70%) and triacylglycerol and considered as transport form of lipids (Kontush and Chapman 2010). High-density-lipoprotein (HDL) is commonly distributed lipoproteins that possess anti-oxidant, anti-atherosclerotic properties (Li et al. 1999), anti-inflammatory, vaso-regulatory, anti-thrombotic properties (Shapiro and Fazio 2017), Apolipoprotein-B considered as one of the important lipids which consist activity for atherosclerotic cardiovascular disease (Stamler et al. 2000). Cholesterol and triglycerides considered as crucial components of lipids, clinically the level of plasma lipoprotein may be increase or decrease leads to hyperlipidemia or hypolipidemia due to abnormal concentration.

Low-Density Lipoproteins (LDL) consists of cholesterol, triglycerides and protein, and considered as bad cholesterol because its high level can lead to the development of cardiovascular disease.

Very Low-Density Lipoproteins (VLDL) contains triglycerides, some cholesterol molecules, and less protein, it is less dense due to high lipid contents and responsible for the transport of triglycerides to cells in the body.

High-Density Lipoprotein (HDL) considered as good cholesterol, it is dense lipoprotein due to less cholesterol and more protein. HDL is made in the liver and in the intestines; it carries cholesterol from cells back to the liver.

Chylomicrons is a type of lipoprotein and dense in nature due to more triglycerides and less protein, responsible for transporting lipids molecules from the intestine to cells in the body (Kuske and Feldman 1987).

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Chapter 7
Alternative and New Protein Sources

Barbara Sawicka, Krishnan Umachandran, Noori Abdul-nabi Nasir, and Dominika Skiba

7.1 Introduction

Alternative sources of protein have been utilized to a limited extent by certain sections of the population but its universal use and acceptability to the rest of mankind remains a challenge. The commonly known alternative sources of proteins are single-cell organisms (yeast), aquatic organisms (krill, algae) (Doshi et al. 2007; Deng and Chow 2010; Ohikere and Ejeh 2012; Piasecka-Kwiatkowska and Stasińska 2016), and insects, which have amino acid composition similar to that of the traditional animal proteins consumed as diet with protein 77.9–98.9% as digestible (Bukkens 1997; Shockley and Dossey 2013). The alternative protein sources in food production have benefits associated to social, economic, health and environment (Woció́r et al. 2010; Udenigwe and Aluko 2012; Yadav et al. 2017). The progression of the biofood concentrates on the feedstocks facilitates more of technology innovation with biopolymers and biocomposites (Ayadi Rosentrater and Muthukumarappan 2012; Buckhalt 2016). The combination of new applications by biotechnology on agriculture has altered the agricultural economics to benefit the people (Ohikere and Ejeh 2012). Genetically modified organism (GMO)/crop varieties also address the food security requirements and boost exports. It does this by reducing the cost of farming, improve output, decrease in the use of pesticide, reduction in deforestations, ease low-till or no-till cultivation and thus cut the emissions of greenhouse gases to offer better food for the consumers (Stewart 2009; Udenigwe and Aluko

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2012). Solid development in biotechnology offered in addition cutting-edge expansions in genomics and bioinformatics made it possible to develop and discover drugs, diagnosis and early treatment of diseases and disorders affecting animal health, livestock and agricultural crop therefore to improve their well-being, production and conserve the use of existing resources to sustainability (UN ECA 2015). However, encouraging results of novel sources of nutrients such as insects, algae and in-vitro meat, though are unconventional to animal proteins for human consumption and animal feed production, still required to enhance in quantity and quality as nutrient sources that could face upfront the potential encounter on food security an inordinate stress thereupon. New technologies are emerging that allow for the upgrading of wastewater treatment plants to 'factories' in which the incoming materials are deconstructed to units such as ammonia, carbon dioxide and clean minerals, and followed by a highly intensive and efficient microbial re-synthesis process where the used nitrogen is harvested as microbial protein (at efficiencies close to 100%), which can be used for animal feed and food purposes (Shockley and Dossey 2013; Matassa et al. 2015; Wiza 2019). Bioavailability process follows food element consumption, including digestibility and solubility of the food element in the gastrointestinal tract, absorption/assimilation of the food element across the intestinal epithelial cells and into the circulatory system, and finally, incorporation into the target site of utilization (Bleakley and Hayes 2017). The enzymes which improve weight gain and feed conversion in monogastric animals, have been engineered for improved activity and deliver high levels of thermal stability to enable direct use of the grain in feed pelleting processes (Matassa et al. 2015).

7.2 Daily Demand of Human Organism to Protein

For an adult, the daily dose of protein is 50–80 g (including 1/3 is animal protein). In a span of 1 year, a man takes 27.4 kg of protein, which is calculated on the basis of nitrogen at 4.38 kg/kg/year. For the world's population, approximately 30.8 million tons of nitrogen per year is needed. It is estimated that around a billion people are starving all over the world. This hunger particularly affects third world countries attributed in part from political instability and very low agricultural production combined with over-population. Currently, about 40,000 people die every day in Africa due to hunger (Fig. 7.1).

Hence, the demand for protein in the world is enormous. Proteins provide the raw material compulsory for constructing additional muscle fibers and rebuilding broken fibers with thicker composition resulting to greater muscular strength and endurance (Schoenfeld 2010). Adding protein to an already balanced diet promotes healthy living. Animal protein is a complete protein that provides all nine essential amino acids, while some plant proteins may lack one or more essential amino acids. Animal products are high in protein but also contain high amounts of saturated or trans fats. Not everything from animals is good to our health. Consuming animal blood for instance can cause a number of problems. The human body can dehydrate

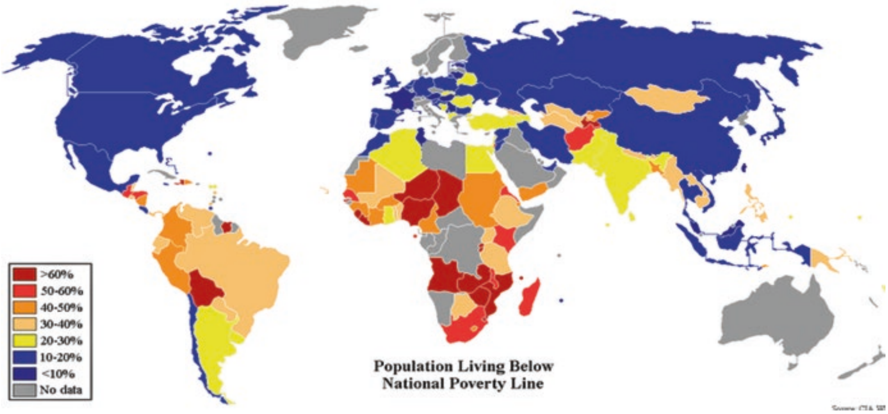

Fig. 7.1 Part of society (%) below the abundance of poverty. (FAO 2016)

when drawing its own water reserves to digest the protein contained in the blood. Furthermore, animals frequently carry diseases in their blood which may also cause infections among humans. On the other hand, tofu and tempeh are great non-animal protein sources which when consumed as meals are a healthier protein boost without the saturated fats found in animal proteins (Smith 2009).

7.3 The Need and Sources of Alternative Proteins

Economic, health and environmental demands stress the need for alternative sources of proteins in the production of food. Large areas of grassland and cropland are already needed to meet the demand of meat and dairy consumption. Agricultural sector development is essential to ensuring food security and good nutrition for all, but are additional environmental challenges (emissions and energy trends continue, global warming). There are concerns over the depletion of fish stocks and impacts on marine biodiversity (Westhoek et al. 2011). Shared fresh water resources and food security are critical to human security concerns, while worldwide, cropland is being lost to cities, industry, erosion, flooding. Salinization that accompanies big irrigation schemes, is described as one of the gravest threats to irrigation agriculture and food security. Water withdrawals are met at rate of natural groundwater recharging. Increasing reliance on nonrenewable groundwater is resulting in falling water tables being recorded on every continent. Soils when degraded towards agricultural production, the biodiversity of the region are reduced, leading to a loss of genetic resources that could contribute to a diverse and sustainable agricultural system. These losses jeopardize the food security of the local populations and ultimately result in high economic and social costs. Alternative proteins are estimated to make up 33% of global protein consumption by 2054, with algae and insects accounting for about 18% and 11% of the alternative protein market, respectively. In part, sales of insects and algae are expected to each account for 2% of the alternative protein market share by 2024 (European Commission 2018). Alternative proteins must be evaluated for nutritive value, safety, and economic considerations before mass production are undertaken. There are good alternative protein sources which include spray dried plasma, wheat protein, fish protein, chicken products, eggs, and processed red blood cells serve many functions, including provision of essential and nonessential amino acids which enhance immune and are also highly palatable. Reproductive biotechnologies changed molecular genetics were sequenced and annotated in various animals such as cattle, pigs, chicken, sheep, poultry, and bee (Odongo et al. 2010; United States Department of Agriculture 2016; National Institutes of Health 2016; Raab 2016).

7.3.1 Proteins of Unicellular Organisms

From a nutritional point of view, microorganisms can be a valuable source of protein with a high biological value. Depending on the type, strain and conditions of microbial growth and composition of the medium, the protein content in the dry mass of unicellular biomass may vary within 40–80%. Most proteins contain bacteria (50–80%), then algae and yeast (30–75%), and least molds (20–45%). Single-cell proteins are a rich source of lysine; however, they are deficient in some essential amino acids, mainly sulfuric (methionine and cysteine) (Table 7.1).

The large-scale process of obtaining Single Cell Protein (SCP) proteins from dried cells of microorganisms such as bacteria (*Cellulomonas, Alcaligenes*), yeast (*Candida, Saccharomyces*), algae (*Chlorella, Spirulina, Scenedesmus*) and molds (*Trichoderma, Fusarium, Rhizopus*) (Becker 2007; Deng and Chow 2010), generates numerous opportunities in food production technology. The diversity of methods, raw materials and microorganisms used, high substrate conversion efficiency and efficiency associated with the rapid growth of microorganisms and lack of dependence on seasonal and climatic factors (Nasseri et al. 2011) attach great importance to these microorganisms from a nutritional point of view as a protein source of high biological value (Table 7.1).

The nutritional value of these proteins is higher than those of vegetable origin, and when supplemented with methionine, become comparable to proteins of animal origin (Nalage et al. 2016). It has also been proven that SPC obtained from microorganisms are a rich source of B vitamins and minerals, such as: zinc, phosphorus, magnesium, selenium, chromium (Adedayo et al. 2011). In addition, some yeast species, e.g. Saccharomyces cerevisiae, have a probiotic effect in the human body (Muszyńska et al. 2013). Nucleic acid component (2–18% dry matter) is also a component of biomass, which is a potential danger for the human body, because it may lead to the accumulation of uric acid crystals in the kidneys or joints, leading to gout. Their content is highest in bacterial cells and the lowest in algae cells (Nasseri et al. 2011). It was also proved that SPC obtained from microorganisms are a rich source of B vitamins and minerals, such as zinc, phosphorus, magnesium,

Table 7.1 Amino acid profile of proteins derived from alternative sources (g/100 g)

Amino acids	Sources of protein						
	Algae	Bacteria	Yeast	Mushrooms	Krill	Insects	FAO pattern
Isoleucine	4.7	3.3	2.5	1.8	2.5	3.8	2.8
Leucine	8.6	5.4	3.6	2.9	4.0	6.5	6.6
Valine	6.2	4.2	2.7	2.2	2.6	5.2	3.5
Lysine	6.3	4.3	3.5	3.0	4.4	5.1	5.8
Phenylalanine	9.0	5.8	4.1	3.1	5	9.7	6.3
Metionine	3.1	2.2	1.5	1.0	2.4	3.5	2.5
Tryptophan	0.9	0.8	0.6	0.3	0.7	1.2	1.1
Threonine	5.4	3.3	2.5	2	2.2	3.7	3.4
Arginine	6.9	3.7	2.3	2.7	3.8	4.5	nd
Histidine	2.1	1.5	1.2	1.0	1.1	2.2	nd
Aspartic acid	9.7	nd	nd	nd	5.3	nd	nd
Glutamine	10.9	nd	nd	nd	6.7	9.7	nd
Glycine	6.2	nd	nd	nd	3.4	5.1	nd
Proline	4.3	nd	nd	nd	2.3	4.6	nd
Serine	4.3	nd	nd	nd	1.9	4	nd
Alanine	nd	nd	nd	nd	2.9	5.9	nd
Total protein (% DM)	40–60	50–65	44–55	30–45	60–65	5–77	nd

Source: Becker (2007), Nalage et al. (2016), Nasseri et al. (2011), Piasecka-Kwiatkowska and Stasińska (2016), Rumpold and Schlüter (2013), Tou et al. (2007), and Zielińska et al. (2017)
DM dry mass, *nd* no data

selenium, chromium (Adedayo et al. 2011). Some yeast species, e.g. Saccharomyces cerevisiae, have a probiotic effect only in the human body (Muszyńska et al. 2013). Nucleic acid component (2–18% of dry substance) is also a component of biomass, which is a potential danger for the human body, as it may lead to the accumulation of uric acid crystals in the kidneys or joints, leading to gout. Their content is highest in bacterial cells and the lowest in algae cells (Nasseri et al. 2011). Nucleic acids are also present in conventional foods and constitute a permanent component of the diet, both meat and vegetarian (Głazowska et al. 2016). The content of nucleic acids in the diet should not exceed 2 g per day, so the consumption of SCP cannot exceed 30 g per day (Piasecka-Kwiatkowska and Stasińska 2016). Yadav et al. (2017) proved that the use of a combination of two chemical substances: N-Lauroiol sarcosine and NH_4OH allows to reduce the content of nucleic acids in biomass to the desired level (<2%). The proteins of unicellular organisms may also adversely affect the human immune system, causing allergies (Nasseri et al. 2011). An obstacle in the use of microbial protein in human nutrition is the presence of anti-nutritive substances, such as nucleic acids, as well as residues of atypical biomass components and the possibility of microbial contamination difficult to eliminate during the production process (Nasseri et al. 2011; Bueschke et al. 2017). SPC on an industrial scale is mainly produced as an additive to animal feed, because the cost as production costs of microbiological proteins that could be a component of human food are too high (Nasseri et al. 2011, 2016).

7.3.2 Proteins of Marine Organisms

Algae do not compete with traditional food crops for space and resources, they are seaweeds and microalgae, rich in nutrients and a sustainable source for protein. Macroalgae (seaweed) and microalgae are examples of underexploited crops. Macroalgae are a diverse group of species as oxygen-producers, photosynthetic, unicellular or multicellular organisms excluding embryophyte terrestrial plants and lichens. Exploited for human consumptions are *Undaria pinnatifida* (wakame), *Hizikia fusiformis* (hijiki), and *Laminaria japonica* (kombu) (Van der Spiegel et al. 2013). Seaweeds can be consumed directly as whole algae, whereas microalgae are mainly used for the extraction of food and feed complements. Certain species of seaweed and microalgae contain protein levels similar to those of traditional protein sources, such as meat, egg, soybean, and milk. Even though microalgae are unicellular microscopic organisms they are still considered as a viable alternative protein source. Seaweeds are a valuable source of protein, amino acids, minerals and vitamins, negligible fat and cholesterol content, hence they accompany many health benefits when consumed, such as to lower blood pressure and prevent strokes, while microalgae are extracted mainly for high-value food/feed supplements and colorants, compared to its protein. In terms of nutrition, marine algae are excellent an alternative to conventional vegetable proteins. However, the popularity of protein from algae in the food sector is still under development, among others due to high production costs and technical difficulties in developing products acceptable in terms of taste (Becker 2007). Currently, algae are found mainly used as supplements on the market of food with pro-health effects. The consumption of algae is safe for humans when the correct breeding conditions are maintained. Although the algae are high ability to bind heavy metals, breeding in appropriate quality and purity of water, does not pose a threat to consumers (Doshi et al. 2007; Tang and Suter 2011).

7.3.3 Sea – A Potential Source for Alternative Protein

Meat and fish are partly interchangeable, in culinary as well as in nutritional terms, both being suppliers of protein (Westhoek et al. 2011). Fish remains to be accepted for high presence of protein and omega-3 fatty acids. Popular fatty fish include Cod, Tuna, Salmon (wild), Tilapia, and Halibut (US Food and Drug Administration 2008; Haslett and Smith 2009). Krill, or species of crustaceans living in the oceans around the world, is an important link in the food chain, as a food for marine animals and birds, and to a much lesser extent for humans. Krill in appearance resembles shrimp, it reaches mass from 0.01 to 2.0 g and a length from 0.8 to 6.0 cm. It creates large clusters in its natural environment, over million individuals/m^3. For the best studied krill species for consumption by humans are Antarctic Krill (*Euphausia superba*) and Kryl Pacificaly (*Euphausia pacifica*). This species is a rich source of full-value protein in the amount of 60–65% in dry matter (Nicol et al. 2000), whose content in muscle tissue depends on the species and the season and varies between 15–17%. This protein has in its composition the majority of essential amino acids (Table 7.1). The main problem that is associated with the use of these raw materials on an industrial scale in human nutrition is the low stability of protein preparations and the presence of anti-nutritive substances, such as chitin and other small molecules in products made of krill (Jakubiec-Puka 1987). Nutritional studies have proven that algae are also a source of high quality, comparable to conventional plant proteins (Becker 2007). Algae are found in the aquatic and terrestrial environment, with high humidity, all over the world. Algae, such as Chlorella sp., And Spirulina sp. (Tang and Suter 2011; Becker 2007) are used for large-scale production. The nutritional value of Spirulina varies and depends on the growth conditions. The bioavailability of nutrients from Spirulina may be greater than from other sources of vegetable origin, due to the construction of the cell wall, which consists of protein, carbohydrates and fat. Spirulina is characterized by a particularly high protein content (60–70% in dry matter) with high digestibility (90%). It contains all the essential amino acids in significant quantities (Table 7.1). Spirulina is also a rich source of vitamin B12 and carotenoids. The health-promoting properties of Spirulina are also known, among others in hypercholesterolemia, hyperglycemia, cardiovascular diseases, anemia, inflammatory diseases, cancer and viral infections (Deng and Chow 2010; Selmi et al. 2011; Tang and Suter 2011). From algae with high protein content, you can also distinguish between chlorella (about 60% of protein in dry matter). Chlorella is also a source of many vitamins, including vitamin A, B12 and folates and minerals, such as iron. Its cell wall is rigid and indigestible, therefore, chlorella cells, in order to increase the nutritional value, require special preparation to remove or destroy indigestible cell walls. A number has also been shown health-promoting properties of chlorella, including hypotensive, antioxidant and immunosuppressive effects and reducing the onset of anemia (Halperin et al. 2003; Merchant et al. 2002; Nakano et al. 2010; Tang and Suter 2011).

7.3.4 Edible Insects in Human Nutrition

Harvesting insects is like hunting and collecting activity which is becoming a threat to both the target species and the environment and therefore consuming edible insects has become marginalized. Insect breeding requires which requires no secondary energy hence certain countries have imposed austere breeding methods of insect species categorically to be gathered from free living insects in order to protect the endangered species, biotopes and biodiversity as a whole (Jiri et al. 2014). The influence of globalization has weaned away a large number of protein-hungry people of the third world from animal protein towards insects as an alternative (Heinrich and Prieto 2008). Harvesting insects in nature and solving the problem of famine are followed in some cultures as a seasonal source of proteins, not only an emergency resource but are valued as appetizing and tasty among substantial part of human population (Shockley and Dossey 2013; Jiri et al. 2014). Insects are cooked, roasted or boiled or with other culinary techniques (Yen 2010), while in some parts insects are a gourmet dish which are attractive and savory (Nonaka 2009). An alternative source of wholesome protein, often currently discussed by the FAO and the European Commission (2018), are edible insects (Shockley and Dossey 2013). The number of insect species living on earth is estimated at about two million, which are an important element of the natural environment, but mainly serve as food for many species of animals (Boczek and Pruszyński 2013). Insects and their products are used in the food, pharmaceutical and chemical sectors, and also textile. In the industry are used, among others honey, putty, wax, natural silk, halantine, cochineal, shellac, gels. In many regions of the world, e.g. in China, Japan, Thailand, South Africa, Mexico, insects are also a component of the diet. About 2000 species are edible for humans (Rumpold and Schlüter 2013). It is estimated 1.9 thousand species of insects are consumed by about two billion people in about 80 countries (FAO 2013). Almost all insect groups are used for food purposes, i.e.: beetles, caterpillars, wasps, bees, ants, crickets, locusts, termites, bugs, dragonflies, flies and other, both adults, but also chrysalis, larvae and eggs (Boczek and Pruszyński 2013). Until recently, insects in Europe have not been seen as a part of the diet, and their consumption is limited to the unconscious consumption of products in which they are used as a food additive (e.g. cochineal). The most commonly consumed edible insects include: crickets and Orthoptera, bee brood (eggs, larvae and bee chrysalis), beetles (*Coleoptera*), mealworm mill (*T. molitor*) used for the production of fodder for fish and poultry and termites (*Isoptera*) commonly eaten in African countries (Bąk and Wilde 2002; Boczek and Pruszyński 2013; Nonaka 2009; Resh and Cardé 2009). Insects are a significant, underrated alternative to nutrients supplied from conventional animal sources. They are characterized by high nutritional value, being a source of energy, protein, carbohydrates, fat and vitamins and minerals. The chemical composition of edible insects shows high variability between species, developmental stages, and type of food (Rumpold and Schlüter, 2013). The protein content in insects ranges from 5–77 g to 100 g (Rumpold and Schlüter 2013). In many species, the protein constitutes over 60% of dry matter, and its highest content was recorded for Orthoptera species (crickets, locusts, grasshoppers) (Yi et al. 2013). The insect protein has a digestibility comparable to egg white (77–98%) and is considered to be of full value, comparable to proteins, milk and beef (Shockley and Dossey 2013). Insect proteins are a good source of threonine, valine, histidine phenylalanine and tyrosine, and for some species also tryptophan, lysine and threonine (Table 7.1) (Rumpold and Schlüter 2013; Zielińska et al. 2015a). Edible insects are also a source of fat, the content of which varies from 10 to 50% (Zielińska et al. 2015b). For Orthoptera species it is on average 13%, from the order Coleoptera (beetles) 33% (Rumpold and Schlüter, 2013), and in larvae of *Rhynchophorus phoenicis* beetles belonging to the *Curculionidae* family, the fat content is 67% of dry matter and is greater than in the majority conventionally consumed high-protein products, such as beef, poultry or eggs. The composition of fatty acids in insects is comparable with the composition of poultry fat and fish in terms of their unsaturation, however, insects are characterized by a higher content of polyene fatty acids (Rumpold and Schlüter 2013). Jang et al. (2006) showed that the fatty acid composition can be modeled by applying appropriate modifications to the insect diet, and in the process of defatting meal from insects, it is also possible to obtain an oil that can be widely used in human nutrition (FAO 2013). Insects are characterized by low content of carbohydrates (0.1–5.3% of dry matter) and high content of fiber, most commonly in in form chitin (Ekpo and Onigbinde 2005; Finke 2007; Zielińska et al. 2015a). Finke (2007) estimated the content of chitin in species of edible insects at 2.7–49,8 mg per kg of fresh insects and 11,6–137,2 mg per kg of dry matter. Zielińska et al. (2015b) asset also folic acid and in smaller amounts of retinol and β-carotene (Bukkens 2005; Finke 2002; Rumpold and Schlüter 2013). In addition, edible insects are a source of peptides with properties antioxidant. In an experiment conducted by Zielińska et al. (2017) investigated the antioxidant activity of peptides obtained by gastrointestinal digestion in vitro derived from edible insects, belonging to five species. The authors showed that the consumption of edible insects can bring potential health benefits due to the strong antioxidant effect of the peptides derived from them. The obtained results showed that the insects subjected to digestion have a higher antioxidant activity than other protein hydrolysates obtained from animal and vegetable products. However, the amount of fiber found in crickets (*G. sigillatus*), beetles (*T. molitor*) and locusts (*S. gregaria*), which shrank at 1.97–3.65% on a dry matter basis. The high nutritional value of edible insects is also influenced by the presence of vitamins and minerals, mainly iron and zinc (Bukkens 2005), but also copper, manganese, magnesium and calcium (Ekpo and Onigbinde 2005). Insects are also a source of thiamine (0.1–4.0 mg per 100 g dry matter), riboflavin (0.1–8.9 mg), cobalamin (0.5–8.7 μg per 100 g), and some species. Insect production, according to FAO experts (2008, 2009, 2011, 2013, FAO et al. 2015), is characterized by:

1. low greenhouse gas emissions such as ammonia, methane or nitrous oxide, and insect droppings can be used in agriculture in the form of fertilizer. Insect farms produce 10 to 100 times less gas per kg body weight than pig farms, which has a beneficial effect on global warming (Shockley and Dossey 2013).
2. lower requirements as to farming and low costs associated with saving agricultural land, feed and drinking water consumption. Insect farming is not required to have arable land or high feed and drinking water consumption. The economic costs of breeding insects are significantly lower than the costs of raising farm animals (Smith 2009). Cold-blooded insects can use plant biomass to increase their body weight (Van Huis et al. 2013);
3. easy distribution, high diversity factor and short reproduction cycle (Tan et al. 2015; Wiza 2019).

7.3.5 Prospecting Insects in Food Sector

Insects offer sufficient quantity of energy, protein, amino acid requirements for humans and are high in unsaturated fatty acids, minerals and vitamins. Freeze-dried, sun-dried or boiled edible insects can be consumed after being wild-harvested or reared, can be processed as fried and ground into a paste form, or as an extract of protein, fat or chitin for enriching food and feed products. Entomophagy causes numerous nutritional benefits with high protein content, minerals and vitamins, they have comparable properties to traditional feed components such as fishmeal. The spinal fluid, eyes and the fluid in the meat of the sea fish, turtle blood contains protein, and many types of seaweed are edible and contain carbohydrates (Smith 2009; Haslett and Smith 2009; Wiza 2019). In addition to nutritional and health benefits, there are a number of other advantages associated with the use of edible insects in the food sector. One of the most important is the protection of the environment, which is why the protein of edible insects is often called "ecological protein". Breeding of insects is associated with aspects that are important in terms of environmental protection, as smaller than in the case of farm animals use of drinking water, feed, which can constitute waste of the agri-food industry, meeting the requirements for ensuring food and feed safety, which solves another problem related to their utilization. In addition, breeding of insects is associated with lower greenhouse gas emissions (methane, nitrous oxide, ammonia) and faces. Insects can be used in agriculture as a fertilizer (Boczek and Pruszyński 2013; Krzywiński and Tokarczyk 2011; Rumpold and Schlüter 2013; Zielińska et al. 2015b). Saving agricultural land, fodder and drinking water also means significantly lower farming costs (FAO 2013). Another positive aspect is certainly increasing the economic potential of insect breeding is the ease of distribution, high fertility rate and short reproduction cycle (Schabel 2010). The main obstacle to using insects as food in European countries is cultural barriers and the associated lack of acceptance among potential consumers, and even reluctance and fear. Consumers in European countries react with disgust at the perspective of consuming organisms that are not commonly known as food and as pests (Yen 2010; Tan et al. 2015). However, their introduction into the diet does not have to involve insect intake in the form in which they occur naturally. There are many possibilities of processing insects to a form more acceptable to society in developed countries, e.g. flours, crisps. Tan et al. (2015) argue that the method of preparation of this type of products has a big influence on sensory acceptance among consumers who have not eaten insects until now. Given in a form resembling products known to consumers or used in processed form, as an addition to traditional products, they have a positive effect on organoleptic sensations (Hartmann and Siegrist 2017; Tan et al. 2015). Similar results were obtained in studies carried out in the Netherlands. Consumption of insects in total was associated with rejection by the respondents, while the form resembling conventional food gave rise to acceptance. it was mostly accepted (House 2016). According to House (2016), the key to increasing the motivation for repeated consumption by consumers of western countries containing insects is therefore the form of the product, but also the taste, price and ease of integration with individual nutritional practices. One of the few studies on the attitudes of the Polish population towards consuming edible insects, as an alternative source of food, showed a positive or neutral attitude of consumers towards entomophagia (Bartkowicz 2017). Powdered insects can also be used in food processing as a functional additive that binds water or creates emulsions, and the development of methods of processing insects for food purposes enables isolation of pure protein or extraction of fat. Another factor hindering the introduction of insects into the food sector is microbiological safety, toxicity and the presence of other organic pollutants (FAO 2013; Zielińska et al. 2015b). In the United Nations Food and Agriculture Reports for several years, discussions on the use of edible insect proteins in the aspect of solving the growing problem of hunger in the world (FAO et al. 2015; FAO 2009, 2013). In European countries, there are no legal regulations that allow insects for human consumption. Pursuant to Regulation (EU) No. 2015/2283 of November 25, 2015, insects belong to the so-called "New food", in other words according to Regulation (EC) No. 258/97 of the European Parliament, which was not consumed on a large scale in the European Union before May 15, 1997, and registration of such food is difficult, time-consuming and requires a lot of research. One of the few countries in Europe that introduced some species of insects into consumption is Belgium (FASFC).

7.3.5.1 Safety of Insect Consumption

Food and feed security are prophesied to be under siege, owing to the sharp increase in the population besides the resultant upsurge in animal protein demand. Promotion of food security can influence the progress of local food structures and trade, the encounters that avert local foods from enjoying a larger share of the per capita food consumption, therefore they must comprise the guard of livelihoods, provide safety net for food insecure people and to inspire then join in mainstream participation (US JES COC 2012). In the case of new food sources, which are edible insects, safety is of particular importance (Van Huis 2016). From research the safety of insect consumption shows that they may, like conventional food, pose a potential threat to humans, due to the presence of endo- and exogenous allergic and toxic substances, anti-nutritional substances and pathogens in them (Rumpold and Schlüter 2013). Risk factors of origin endogenous can be, among others, allergens and anti-nutritive substances. Nishimune et al. (2000) describe an African silkworm containing thiaminase (thiamine-degrading enzyme) that is resistant to high temperatures. Ingestion of insects may be associated with the occurrence of reactions allergic, caused by the presence of inhalant, contact or food allergens in them. The greatest danger is therefore farmers and people employed in production (Rumpold and Schlüter 2013; Mlcek et al. 2014). Ekop et al. (2010) found low content of oxalate, phytate and tannin products in these products, below the levels considered to be toxic to humans. External factors can also affect the safety of insect consumption. For example, there are information about botulism, parasitic diseases and food poisoning caused by insect consumption. It is therefore important to maintain adequate conditions for the preparation of insects for consumption (Schabel 2010). In addition, some insects may sequester toxins from feed and synthesize them, e.g. cyanoglycosides, steroids or highly toxic amides, produced as chemical defense against other insects (Rumpold and Schlüter 2013; Zagrobelny et al. 2009). It was also observed that insects feeding on areas covered by the action of pesticides also contained them in their composition (Schabel 2010). Investigations of bacterial microflora of insects revealed the presence of pathogens such as: Bacillus cereus, Pseudomonas aeruginosa and Staphylococcus aureus, as well as non-pathogenic Bacillus species (Banjo et al. 2006). In addition, as in the case of dishes prepared from other raw materials of animal and vegetable origin, the storage of insects and their products after heat treatment at reduced temperatures prolongs their microbiological durability (Klunder et al. 2012; Mlcek et al. 2014). Insect microflora can be a potential danger for humans, therefore it is important to develop optimal processing conditions for each species intended for consumption insects so as to ensure the highest possible microbiological purity of the finished product (Rumpold and Schlüter 2013).

7.3.6 Animal Proteins

Animal proteins are wholesome proteins that contain all amino acids in the body's proportions. Zoonoses include: milk and dairy products, animal meat (including fish), eggs. In most developed countries, animal protein is the main source of dietary protein, not vegetable protein. Therefore, there is often a differently increased choice when the total protein intake increases. However, the consumption of animal products, especially red and processed meat, is associated with an increased risk of diseases such as cancer, T2D and cardiovascular diseases (Abete et al. 2014; Demeyer et al. 2015; Malik et al. 2016). The optimal ratio of vegetable protein to animal protein in the diet has not yet been determined. However, can higher protein intake be associated with lower glycemic hemoglobin (HbA1c). We do not know that yet. HbA1c levels measure mean blood glucose levels above 6–8 weeks. High hemoglobin is a risk factor for T2D. The long-term positive effect of a diet with higher protein content on the body, weight management, may in turn lead to a reduction in HbA1c (Clifton et al. 2014). Dietary studies have also shown that higher doses of protein may reduce HbA1c directly, especially in patients with T2D (Frank et al. 2009; Gannon et al. 2010). However, the question remains whether higher protein intake may be associated with an increased estimation of glomerular filtration rate (eGFR), an unfavorable indicator of renal function, because it is carefully controlled. Dietary studies also show that higher doses of protein may increase this rate of renal function (Brenner et al. 1982). Caring for kidneys in a diet with higher protein content results from the harmful effect of the induced glomerular hyperfiltration effect (Nothlings et al. 2008). The quality of animal protein is also problematic. Meat *in vitro*, in other words meat for breeding in cells or pure meat, is an animal product produced after isolation and identification of cells, cell culture according to tissue engineering protocols (Langelaan et al. 2010). Several cell sources may be used, including embryonic cells prior to implantation or adult stem cells. Proponents of this meat argue that an in-vitro meat bioreactor with a pool size could feed 40,000 people a year and that it would use 99% less arable land than the average for beef. The first hamburger from in vitro cultures was created by Mark Post from Maastricht University in 2013. It cost £ 200,000, and it took 2 years to set

(WRAP 2018). Mosa Meat, a Dutch start-up, is still developing, focusing on improving the taste by co-culturing fat cells and reducing costs. Other companies, both in the US and other countries of the world, are pursuing similar goals. It is estimated that current costs are around USD 11. Innovative financing mechanisms for research, for example public financing, will support further technical development of farmed meat. An alternative source of animal protein is whey protein. Its chemical composition is shown in Table 7.2. Whey protein is a by-product of residues from the cheese making process. Currently, both proteins, both casein and whey are considered viable alternatives to animal proteins (Table 7.2). However, they are not compatible with the diets of vegetarians and vegans who try to avoid products derived from animals.

Whey protein is complete, contains a lot of cystine (GSH), increases the function of the immune system, and has a high content (branched chain carbohydrates (BCAA) Whey concentrate, in addition to the isolate, contains more biologically active ingredients. Rapid digestion and transport to cells and biostimulation of biosynthesis of proteins (Colgan 1993).

7.3.7 Plant Proteins

Animal products are not able to meet the global demand for protein and energy in a sustainable way – on the Earth there will be a shortage of water and soil suitable for pasture and for growing crops for feed. Animal husbandry causes higher greenhouse gas emissions than transport, water pollution from wastewater from piggery and sheds, and desertification of soils used as pastures and fields (Henchion et al. 2017; Shewry and Halford 2002). Transport itself will also be significantly reduced, because we focus on local food production. What's more, recently, the need to constantly use antibiotics in animal husbandry on a scale several times greater than the therapeutic demand for humans is a growing topic (Anonymous 2018a). Currently, vegetable sources of protein dominate in the supply of protein in the world (57%), with meat (18%), dairy (10%), fish and shellfish (6%) and other animal products (9%) constituting the remainder (FAO et al. 2015). The protein of plant origin is preferred in relation to animal protein from the point of view of environmental protection, because it is associated with a lower requirement for land use, the production of a smaller amount of greenhouse gases (GHG), than food of animal origin (Anonymous 2018a). Due to the high production costs and the limited availability

Table 7.2 Chemical compositions of Whey

Specification	Whey powder	Whey concentrate	Whey isolate
Protein	11–14.5	25–89	90+
Lactose	63–75	20,363	0.5
Milk fat	1.0–1.5	43,141	0.5

Own research based on: Colgan (1993), and Geiser (2004) of animal protein, increased attention is focused on the use of vegetable proteins as potential sources of cheap, dietary protein.

7.3.8 Cereal Proteins

Cereal proteins are the most important part of protein intake in diets all over the world and they constitute an important source of protein for animals as well as for humans (Bradley and Folkes 1976; Bleakley and Hayes 2017; Boczek and Pruszyński 2013), especially in the diet of developing countries. Common wheat (*Triticum aestivum* spp. *vulgare*) and durum wheat (*Triticum durum*) constitute the largest group of vegetable protein sources in the European diet (Van Spiegel et al. 2013; Shewry and Halford 2002). Wheat bread is a key element of protein in Europe with a typical loaf of 8 grams protein per 100 g. The protein content in wheat grain varies from 10% to 15% (Shewry and Halford 2002). These spare proteins include prolamins, globulins and germins (Cunsolo et al. 2012). Maize (*Zea mays* sp.), rice (*Oryse* sp.) and wheat (*Triticum aestivum* ssp. *vulgare*) are the main protein products consumed around the world. In some regions, such as in West Africa, millet is the most important protein product. In turn, in southern India, where malnutrition of infants and children is common, rice and millet are eaten regularly, and in Ethiopia the preferred cereal is Teff, with a protein-like amino acid profile. A typical Ethiopian diet consists of 65 g of protein, of which 41 g comes from Teff, and only 6 g from the consumption of animal protein (Jansen et al. 1962). Maize (*Zea mays* sp.), as a source of food, represents respectively 25% and 15% of the total consumption of maize in developing countries and around the world. This species is very similar in terms of share in calorie consumption worldwide: from 61% in Central America, 45% in East and South Africa (ESA), 29% in the Andes, 21% in West and Central Africa (WCA), to 4% in South Asia (Shiferaw et al. 2011). Oat protein (*Avena sativa* sp.) is of very high quality, and the content and quality of amino acids in the seed is comparable to soy protein (Cavazos and de Mejia 2013). It contains much more lysine, an essential amino acid, compared to other types and species of cereals, but has a lower content of proline and glutamic acid. This means that after digestion, oat protein can be tolerated by people with gluten intolerance and sensitive to allergies (Klose et al. 2009). The rice, in turn, does not contain large amounts of protein, but the rice protein flour is prepared beforehand using enzymatic treatment with carbohydrate-hydrolyzing enzymes to obtain products containing up to 91% of protein (Shih and Daigle 2000; Cavazos and de Mejia 2013). Cereals can bring great health benefits as a rich source of BIOPEP bioactive peptides (Cavazos and de Mejia 2013). Bioactive food-derived peptides gained increased interest as control factors for chronic diseases and reduced the risk of side effects resulting from the use of synthetic drugs (Colgan 1993; Gobbetti et al. 2004; Hernández-Ledesma et al. 2011; Pihlanto and Mäkinen 2013). Bioactivity associated with cereal proteins is: antioxidants, anti-inflammatory effects, cholesterol lowering, satiety, antidiabetic and other (Cavazos and de Mejia 2013). They affect the physiological effects demonstrated on animal models. However, prolaminates from some cereals, including wheat, barley and rye, cause biologically active anti-nutritive peptides after proteolysis that are able to adversely affect in vivo the intestinal mucosa of celiac patients, while prolaminates from other cereals, such as corn and rice is not. Bioactivities associated with peptides derived from oat, barley and wheat proteins include opioid activity, while rice protein contains the RGD sequence and no visceral toxicity, which may be beneficial for health and nutrition (Colgan 1993; Iwaniak and Dziuba 2009; Buczyńska and Szadkowska-Stańczyk 2010; Deng and Chow 2010; Cunsolo et al. 2012; Malaguti et al. 2014; Demeyer et al. 2015; Gangopadhyay et al. 2016; Dietary Guidelines for Americans 2015–2020). From an agricultural point of view, the use of varieties, environmental conditions and agricultural practices can affect the bioactive content of peptides in cereal proteins.

7.3.9 Productivity of Legumens

Some plants have unique advantages, e.g. legumes have a unique ability to bind nitrogen of the legumes, soy is a very important source of protein, but 85% of its production is used to produce animals and fish (Sawicka et al. 2000; Anonymous 2018b). However, the desire to increase soy production in response to increased demand for animal protein is associated with deforestation and habitat loss, especially in South America. Ethical issues are connected with this, because about 12 million hectares of arable land, outside Europe, is necessary to ensure feed for European livestock production (Klunder et al. 2012; Kalembasa et al. 2014; Szymańska et al. 2017; Symanowicz et al. 2018).

The amount of nitrogen from the biological reduction process in the yellow lupine biomass harvested in the flowering phase was lower after using a larger dose of this component, while in the phase of full maturity this relationship was reversed (Kalembasa et al. 2014) proofs that. The amount of nitrogen from the fertilizer increased with increasing nitrogen dose. The percentage share of nitrogen from the biological reduction process in the yellow lupine biomass was similar in the flowering phase (53.4%) and full maturity (51.6%). However, the share of nitrogen from fertilizer was higher in the first day of the lupine harvest than in the II period, while in the case of nitrogen collected from the soil the dependence was reversed. Diversified nitrogen fertilization therefore does not significantly affect the percentage of nitrogen from biological reduction in yellow lupine (Tables 7.3 and 7.4). This species fertilized with a higher dose of nitrogen contained a higher percentage of this element originating from fertilizer, and a smaller one from soil stocks than after using a smaller dose. It is possible, therefore, to increase the nitrogen content of seeds not only through the natural binding of nitrogen from the air. However, an increase in the outlay on industrial means of production in higher-intensity technologies caused a decrease in the gross agricultural income value (Kalembasa et al. 2014; Szymańska et al. 2017).

Table 7.3 Seed and biomass yield (tons · ha^{-1}) and nitrogen and protein content (kg · ha^{-1}) in yellow (*Lupinus luteus*) and narrow-leaved lupins (*Lupinus angustifolium*), depending on the soil cultivation system

Specification	Yellow lupinus (*Lupinus luteus*)			Narrow-leaved lupins (*Lupinus angustifolius*)		
	Cultivation systems					
	Traditional	Simplified	Direct sowing	Traditional	Simplified	Direct sowing
Yield						
Seeds	2.83	2.94	2.76	3.2	3	2.51
Whole biomass	12.73	13.48	11.97	9.86	9.71	7.61
The total amount of nitrogen						
In seeds	160.2	162.5	156.2	132.8	109.2	99.8
In biomass	245.2	251.5	238.5	181.5	152.4	136.9
Amount of nitrogen from the atmosphere						
In seeds	96.1	110.8	108.4	66.5	55.9	55.2
In biomass	137.3	149.2	147	85.5	62.4	63.8
The amount of protein in seeds	600.6	692.5	677.5	415.6	349.3	345

Source: Sawicka et al. (2000), and Kalembasa et al. (2014)

Table 7.4 The yield of seeds, biomass and vegetable protein in 3 species of legumes

Specification	Beans (*Vicia faba* ssp. *minor*)	White lupine (*Lupinus albus)*	Soya bean (*Glycine max*)
Yield (tons · ha^{-1})			
Seeds	5.89	4.16	2.57
Biomass	12.74	11.85	9.73
The amount of total nitrogen (kg·ha^{-1})			
Seeds	289.7	213.8	123.1
Biomass	354.1	248.8	168.2
The amount of biologically reduced nitrogen (kg·ha^{-1})			
Seeds	208.9	131	99.3
Post-harvest remnants	31.1	11.9	10.4
Total	240	142.9	109.7
The proportion of biologically reduced nitrogen in the total nitrogen content (%)			
Seeds	72.1	61.3	80.7
Post-harvest remnants	48.3	34.1	23.1
Weighted average	67.8	57.4	62.5
The amount of vegetable protein in the seed yield – 6,25 × N (kg · ha^{-1})			
	1305.6	818.7	620.6

Source: Sawicka et al. (2000), and Kalembasa et al. (2014)

Despite the favorable agricultural income generated from the production of leguminous plants, e.g. pea and yellow lupine, in relation to cereals, in practice, this does not encourage agricultural producers to become more interested in growing pulses as commodity production. So far, legumes for grain have been grown mainly for their own needs, and the commodity of their production was only a few percent. An important limitation of the use of legume seeds in the feed industry is the lack of the possibility of providing a larger supply of raw material with standard parameters, as their production is fragmented. Purchase from numerous small producers, for example in Europe, is cost-intensive and increases the price of the raw material (Sawicka et al. 2000; Florek 2017; Symanowicz et al. 2018). In order to increase the possibility of using native protein in the production of feed, it is also necessary to integrate entities operating on the market, both horizontally and vertically. Establishing closer cooperation between farmers producing legume seeds (creating producer groups, clusters) would facilitate making joint purchases, organizing consultancy, selling seeds to the processing plant. Cooperation between the producer and the recipient within the framework of vertical integration would also bring great benefits. Farmers could produce seeds in accordance with the needs of feed plants, according to a uniform technology required by the recipient, while ensuring their sales. Processing plants they would receive large batches of a single raw material with the required quality standard. On the basis of the analysis, it can also be stated that the current financial support from the state encouraging the production of leguminous plants only fulfills the function of stabilizing the income of producers, without translating into the commodity of seeds. Positive changes should be seen in linking subsidies to leguminous plants with the size of the production, and not the area of cultivation (Reckling et al. 2016; Florek 2017; Szymańska et al. 2017).

7.3.10 Productivity of Perennial Butterfly Plants

The productivity of perennial legumes is one of the important features determining their utility value. This term, understood as the intensity of the production of dry matter from the surface unit during the growing season, in the case of fodder legumes refers to their yield. The genetic determinants, natural and agrotechnical factors and methods of use have a significant impact on the productivity of plants in this utility group. Genetic factors influence the diversity of yields between species, forms and cultivars, and the habitat elements that most modify the productivity of the species are mainly soils and weather conditions, whereas from cultivation operations they are primarily: the quantity, the method and date of sowing, fertilization, care and frequency of mowing. Among the many species, the greatest economic importance on arable lands is red – meadow clover (*Trifolium pratese* L.), cross-linked lucerne (*Medicago media* Pers.) and sowing (*Medicago sativa* L.) and locally sainfoin (*Onobrichis viciaefolia* Scop.). The area of cultivation of these species amounts to million ha and covers about 95% of the area of cultivation of all legume plant species (FAO 2016). It is estimated that alfalfa and esparceta constitute 1/3, and red clover 2/3 of this area. Other leguminous plants, such as: Persian clover (*Trifolium resupinatum* L.), shamrock (*Trifolium incarnatum* L.), white melilot (*Melilotus albus* Desr.), Serradella (*Ornithopus sativus* Brot.) Have a negligible share in the field of fodder production, and white clover (*Trifolium repens* L.), hornbeam (*Lotus corniculatus* L.), lucerne hoppeas (*Medicago lupulina* L.) and white-leaf clover (*Trifolium hybrydum* L.) play an important role as components of meadow and pasture mixtures. Butterfly small-seedlings are a group of plants with a very large diversity of species in terms of: biological (durability, regrowth, and multicellularity), morphological (plant height, degree of foliage, habitat of shoots), habitat requirements (type and type of soil, humidity, temperature, insolation) and use (cattle, pasture) (Sawicka et al. 2000).

The basic species are characterized by the highest productivity in field cultivation, and the yields of their dry matter are on average 8–12 tons ha^{-1}. The first year of cultivation (sowing year) decides about the productivity of these species, although the main yield in full years of use. The biological properties of red clover enable our 2-year exploitation in most of our conditions (year of sowing and full year of use), whereas in the case of alfalfa this period can be 2–3 years, not including the sowing year, and depends mainly on the number of cuts per year. The main method of sowing red clover is sowing in a protective plant, and alfalfa both sowing clean, as well as under sown. Sowing red clover into a protective plant (spring barley for grain or oats for green) is used primarily for economic reasons. Regrowth of the undercapture on the equipment of the protective plant is called a stubble, and its yield, collected most often at the end of September, depends mainly on the course of weather conditions in the summer months. Drought and high air temperature after the harvest of the protective plant limit the yielding of the abrasive, while high and proportionally distributed precipitation at high temperature stimulates the crop. Under favorable conditions one can get about 1 tons of dry matter, very good quality, from 1 ha. The abundance of alfalfa abundance cultivated as a spring barley yield is about 1–2-3 tons ha^{-1} of dry matter, while grown in pure sowing about 5 tons per ha. Sowing the legume plants into a protective plant, an additional 3.5 tons are obtained ha^{-1} of barley grain. Lucerne, cultivated as a disgrace, reacts worse than red clover to unfavorable development conditions, and especially to the lack of light. In such conditions, it germinates less, grows more slowly and more plants die. In addition, early mowing of alfalfa with grain before the flowering phase results in weaker root system development and weakens plants that are worse in winter and lower yield in the next year compared to alfalfa sown in pure sowing. Also, summer sowing without a protective plant that can be performed by mid-July is usually less favorable for the development and yielding of legume plants. In addition to annual plants (Persian clover, red clover, seradella), most butterfly species, yields best in the second year, and species with greater durability, such as: alfalfa and seed alfalfa, sainfoin and hornbeam, can also yield well in second and third year of full use. In crop for fodder, the productivity of plants depends on the structure of crop density, which is formed by the planting of crops and shoots on the surface unit. The relationship between the planting density and the density of the shoots is not directly proportional, because in practice from a different number of plants on a specific surface grows a similar number of shoots. Thus, within certain limits of the amount of sowing seeds, there is a similar crop density structure and similar yield. In addition to the sowing rate, the number of shoots per m^2 was differentiated by swaths and the age of the plantation. In addition to the density of plants and shoots in the field, the productivity of perennial legumes is influenced by their individual (genetic) characteristics, such as: height and stalk size, branching degree, leaf arrangement on the stem, which determine the intensity of light penetration in different parts of the field. The LAI leaf index, responsible for the intensity of photosynthesis and the productivity of biomass, also depends on these elements (Sawicka et al. 2000; Sawicka and Kalembasa 2013).

In the cultivation of lucerne and red clover, a marked decline has recently been noted. In relation to the eighties, in recent years their production for fodder decreased by about 30%, comparing the efficiency of feed units (JP) from 1 ha. In turn, the results of varietal experiments indicate that the potential production possibilities of alfalfa, for example, are high and amount to 18–28 thousand. JP, and red clover 16–24 thousand. JP from ha We see very large production reserves inherent in genetic material and agrotechnics and exploitation. The difference is over 200% and is the highest compared to other fodder plants. Small-winged butterfly plants can be harvested at different frequencies. This applies mainly to red clover and hybrid and seed alfalfa. The harvesting intensity is regulated by the quality of the feed, the yield and the durability of the plantations. Plants mown in the early stages of development (before stalking) are characterized by high protein content, low fiber and dry matter, and are mainly suitable for drought or leaf protein, while collected at later stages of development give higher yields of dry matter, but contain less protein, and more fiber. Breeding achievements affecting the productivity of field crop species mainly concern red clover and hybrid and seed alfalfa. The cultivated tetraploid varieties of red clover in comparison to diploid ones are characterized by higher green and dry matter yield, larger size of morphological body organs (stems, leaves, inflorescences, seeds) and higher protein yield. They are more durable and more useful for more frequent (3-fired) harvest in earlier development phases. Other breeding works, e.g. in the area of red clover, are carried out over increasing the resistance of clover and powdery mildew varieties and increasing their durability, while in alfalfa they include works on the durability of varieties for frequent mowing and kneading with heavy mechanical equipment, resistance to venous diseases, greater tolerance to lower soil pH and high concentration of aluminum, as well as suitability for pasture use (Sawicka et al. 2000; Sawicka and Kalembasa 2013). The disadvantage of some perennial legume plants is the presence of anti-nutritional substances that reduce the nutritive value of feed and may even be toxic to animals. Due to their role and function in life processes, plants are classified as so-called secondary metabolites. These include: cyanogenic glucosides, saponins, coumarins, tannins, estrogens. These substances, from the nutritional point of view, are considered unfavorable, but for plants they are a natural protection against pests and diseases. Plants selected to lower the level of secondary metabolites lose these protective barriers and are more exposed to the negative effects of the environment. Secondary metabolites play an important role in the nutrition of both humans and animals. It has been shown that in addition to adverse effects, such substances as: phytic acid, phenolic compounds, enzyme inhibitors, saponins, glucosinolates, etc. have the ability to lower the sugar content, cholesterol and triglycerides in the blood plasma, regulate insulin activity and the formation of excess free radicals. Therefore, the anti-nutritional substances also have a beneficial effect, which is why it is very important to set the rates of their collection so that the risk of ingestion is low, and the action is optimal. Due to the potential nutritional benefits resulting from the presence of secondary metabolites in the feed and their importance in ecology, they do not always have to be anti-nutritional, because their role is multifaceted and depends on various factors. These substances, from the nutritional point of view, are considered unfavorable, but for plants they are a natural protection against pests and diseases. Plants selected to lower the level of secondary metabolites lose these protective barriers and are more exposed to the negative effects of the environment. Secondary metabolites play an important role in the nutrition of both humans and animals. It has been shown that in addition to adverse effects, such substances as: phytic acid, phenolic compounds, enzyme inhibitors, saponins, glucosinolates, etc. have the ability to lower the sugar content, cholesterol and triglycerides in the blood plasma, regulate insulin activity and the formation of excess free radicals. Therefore, the anti-nutritional substances also have a beneficial effect, which is why it is very important to set the rates of their collection so that the risk of ingestion is low, and the action is optimal. Due to the potential nutritional benefits resulting from the presence of secondary metabolites in the feed and their importance in ecology, they do not always have to be anti-nutritional, because their role is multifaceted and depends on various factors. Particularly high intensity of the negative impact of these substances may occur in the case of feeding single-species feed in large quantities or for a longer period of time. High disposable doses of feed containing anti-nutritive substances may cause acute poisoning, whereas feeding such feed for a longer period may cause chronic diseases or disturbances in the reproduction of animals (Sawicka et al. 2000; Reckling et al. 2016).

Plant proteins are usually derived from legumes plant. Commercial acceptance of genetically modified (GM) food and plants called genetically modified organisms (GMOs), created as a result of recent innovations in agricultural biotechnology, has caused widespread controversy related to the environmental and economic benefits of food and animals in a wider social range, cultural values and ethical (Stewart 2009). Many initiatives have been taken to sensitize people to the ecological responsibility of breeding animals and to increase interest in alternative proteins. Plant-based proteins are also typically harder to digest than animal proteins, due to their high concentration of insoluble polysaccharides. Despite this, there are increasing concerns about the high levels of saturated fats and cholesterol found in foods of animal origin, which are linked to the development of cardiovascular disease and diabetes, leading the nutritionists and organizations to recommend more varied diet rich in plant-based proteins (Reckling et al. 2016; Anonymous 2018a).

Commercial adoption of genetically modified (GM) foods and crops called as genetically modified organisms (GMO) created through recent innovations in agricultural biotechnology has triggered widespread controversy over the environmental and economic benefits and risks of food crops and animals on a wider range of social, cultural, and ethical values (Stewart 2009). Many initiatives to sensitize people on the ecological liability of animal rearing and to escalate their interest in alternative proteins, is to be carried before inducting into the food cycle. There is already the first information on obtaining rapeseed protein by new, innovative methods. NapiFeryn BioTech paves the way in Poland and around the world. It implements it as part of a grant from the largest EU program financing research and development, Horizon 2020. However, scientists are measuring much higher and want to build in Poland the world's first biorefinery producing rapeseed protein. The protein from this plant will be differentiated by the method of obtaining from soy protein. The technology of obtaining soy protein is quite outdated (it comes from the 1940s). In addition, hexane, a solvent still widely used in the oil industry, is used for soybean processing. The new technology for obtaining protein from winter oilseed rape will not be based on it, and on very mild biomass processing methods – it will be a "delicate" process, without drastic changes in pH and without any solvents. It is a more modern solution, safer and giving a higher quality product. In addition, the conditions under which rapeseed protein is isolated do not damage it (protein denaturation). Thanks to this, the protein obtained by the innovative method, in Poland, will be natural and functional. The use of vegetable protein is of civilized importance – now animals are fed plants to produce protein. The authors of this project want to direct the protein to people. Animals are a kind of filter through which most of the protein is lost, i.e. they are an ineffective source of its processing. Not counting that 4 kg of plants are needed for 1 kg of pork and 1 kg of beef for 7 kg of plants. Instead of wasting calories, it is worth turning to the protein obtained from plants, in this case from rape, which is characterized by a high content of full-value proteins (Voisin et al. 2014; Anonymous 2018b). The protein is obtained from the remains of the oilseed rape oil, the seeds of which contain about 20% of protein with high nutritional values, but until now it has not been used as a food ingredient. Meanwhile, innovative Polish technology allows the recovery of about half of this protein in the form of an isolate with high qualitative and functional parameters. An innovative element of this technology is that the starting material is biomass, which is formed after extrusion of oil from rape seeds. Until now, the potential of this biomass was not fully used, it served only as animal feed, because there were no solutions that would isolate the protein for edible purposes from this biomass. New technology is able to do this, and it makes it unique in the world (Reckling et al. 2016; Anonymous 2018b).

7.3.11 Protein Diet

Determining the protein requirement is difficult because it is metabolized with other nutrients. In addition, there is a need to supply with food the right amount of essential amino acids, which the human body does not synthesize. Plant proteins are usually more difficult to digest than animal proteins due to their high concentration of insoluble polysaccharides. Nevertheless, there are growing concerns about the high levels of saturated fat and cholesterol in animal foods that are associated with the development of cardiovascular diseases and diabetes, leading dieters and organizations to recommend a more varied diet rich in vegetable proteins. A diet rich in protein, from 1.2 to 1.6 g protein /kg/day, may improve the regulation of body weight (Skov et al. 1999; Larsen et al. 2010; Austin et al. 2011). Protein-rich diets also seem to complement other strategies, such as reducing energy and physical activity, to combat the global obesity epidemic (Leidy et al. 2015). Because obesity is an independent risk factor for type 2 diabetes (T2D), higher doses of proteins for mass regulation and maintenance may also be beneficial for the prevention of T2D (Shang et al. 2016). Although the total amount of protein can be important, the source of protein affects other diet components, such as dietary fiber and micronutrients. According to Song et al. (2016) and Møller et al. (2017) protein sources will probably be an important determinant of health. In the last few years, a high-protein diet was recommended to regulate body weight. However, the potential health risks of these diets are still uncertain. In the last few years, a high-protein diet was recommended to regulate body weight. However, the potential health risks of these diets are still uncertain. Møller et al. (2017) investigated this aspect based on the amount and source of the protein and the study of the compound's result with glycated hemoglobin (HbA1c) and estimated glomerular filtration rate (eGFR). The analysis were performed on the basis of three population studies included in the PREVIEW project (PREVEMENT of diabetes through lifestyle Intervention and population studies in Europe and around the world): 'NQplus', 'Lifelines' and 'Young Finns'. The protein effect was assessed on the basis of two components: (1) percentage of total energy protein and (2) the ratio of vegetable protein to animal protein. A positive association was found between protein and eGFR results in Lifelines lines (slope 0.17–0.02 ml/min/1.73 m2, $p < 0.0001$). Thus, protein evaluation can be a useful tool to assess both the impact of the amount and the source of the protein on health parameters. However, further research is needed to confirm this newly developed protein assessment. Therefore, the source of protein will probably continue to be an important determinant of health (Reckling et al. 2016; Song et al. 2016). American nutritional guidelines suggest switching to a diet more based on foods of plant origin (Dietary Guidelines for Americans 2015–2020). Vegetable diets provide many phytochemicals that have been associated with protection against many chronic diseases. However, when compared to animal proteins, plant proteins lack sufficient amounts of essential amino acids. The optimal ratio of plants to animal protein in the diet has not yet been determined. Møller et al. (2017) developed a new protein diet using cross-sectional data from three large European population studies. It has been proven that both the amount and source of proteins (the ratio of vegetable protein to animal protein) are determinant factors affecting HbA1c and eGFR. However, further research is necessary to clarify the usefulness of the protein result in long-term population studies, as well as in other health conditions.

7.4 Research Priorities

1. Development of the possibility of increasing the process of biological reduction of nitrogen in various soil and climate zones of the world as the cheapest source of nitrogen for the production of plant protein,
2. Development of ways to increase the nitrogen utilization rate of fertilized plants in the process of vegetable protein synthesis,
3. Increasing the efficiency of protein processing of microorganisms and vegetable protein on animal protein.

7.5 Conclusion and Future Perspective

The global meat substitutes market is growing from year to year. Projections for the development of the protein substitute market are also based on data on population growth, which is expected to increase to 8.9 billion by 2050. With such a high population growth, the demand for protein will increase dramatically, however, it is much harder to increase meat production because it requires large expenditures (water consumption, fodder), and also promotes waste production, rather than crop production or other possibilities of protein production. Satisfying the nutritional needs of an ever-growing human population it requires searching for alternative sources of protein. Currently, only in some regions of the world presented in the work organisms are used in the food sector. The production of SCPs and marine organisms can contribute to improving food security in the world, although at present they are used to a greater extent in feeding farm animals as feed components. A promising alternative to conventional protein sources is edible insects, which have great potential as a component of human diet due to their high nutritional value. The breeding of insects instead of pigs or poultry is also supported by the fact that it is more beneficial for the environment, economical and efficient. The problem is the lack of acceptance of insects as a foodstuff among the population of developed countries, as well as difficulties in introducing insect food products to the market. The use of insects in the food industry on a large scale is also difficult due to the safety of their consumption, which must be confirmed by further research. However, globalization of the use of insects and other unconventional sources of protein in human nutrition requires decisive action to improve public demand and acceptance and increase consumer awareness of the benefits of their consumption. The search for new insects as a source of protein and the technology of their processing requires further research. In spite of fewer commercial approvals by limited number of countries, the international trade GMO constituents has created labeling the foods products and animal feeds in most countries in the world, to indicate the evidence of acceptance by many farming communities across the world. China, Brazil, and India, have substantial public sector GMO R&D program on new GM crops which include rice, cotton, maize and cassava, where the efforts are supported by US donors. There are uncertain conflicts which dilate the acceptance of GMOs, like the advent of domestic labeling and traceability requirements for food imports, which seriously inhibit the use of GM crops in exporting countries even where those crops are consumed internally or exported to third countries. In addition to this is the dramatic increase in food prices which has boosted the interest in the production of GMO crop varieties which led to some softening of regulatory restrictions and consumer attitudes in Europe and some developing countries. New proteins require the development of new value chains and attention to issues such as production costs, food safety, scalability and consumer acceptance. Positive impact on the environment is important and cannot be assumed only as a new source of protein. Care should be taken to ensure that the novel and existing protein source is compared. Greater convergence of political forces and involvement of broader stakeholders in the role of management, as well as the role of development/commercialization, it is required to refer to both sources of protein and ensure food safety.

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Chapter 8
Microbial Proteins: A Potential Source of Protein

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8.1 Introduction

The difference between food availability and population growth is widening in recent years and population growth is surpassing food availability. This lack of equilibrium is mainly due to the increasing growth rate of the world population, longer life expectancy, constant rate of food production, along with uneven distribution. The increase in population growth is very high in twentieth century, while the sources and production rate of food supply are nearly at a constant level (Stephens et al. 2018). This problem has prompted initiation of strategies and research in recent years to limit the population growth rate. However the results of these approaches are not very promising. Even if these approaches are successful, the desired results are not going to be achieved in a few decades and then it would be too late to resolve the looming food crisis. Additionally, some available areas of land are not yet cultivated. Techniques and assets of modern technology and agriculture can be utilized to get a maximum exploitation of land and produce high yield of foodstuff. Yet, experts agree that the difference between population growth and conventional food supply is too big to bridge (Suman et al. 2015).

The minimum requirement for survival and health does not only depend on the amount of food but also on its nutritional value. Unfortunately half of the world's population live and survive below this minimum requirement. It is not only about lack of availability of food but the nutritional value of food is also critical. Nowadays significant part of the world population is malnourished, even in regions where raw material is available for microbial fermentation. The significance of microbes as possible resources of food originates from the concept that microbial cell content is particularly high in most B-group vitamins and in proteins that comprise vital amino acids. Thus, microbes can possibly augment an undersupplied diet in that proteins

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produced from microbes are very much similar to traditional protein sources such as meat, egg, fish and milk in terms of overall presence of nutrients (Upadhyaya et al. 2016).

8.2 Microbial Protein Synthesis

Proteins synthesis is carried out by the ribosomes along with messenger RNA, produced from the transcription of DNA in the nucleus of eukaryotes however there is no nucleus present in prokaryotes so both transcription and translation occur in the cytoplasm. In the DNA, a series of codes are transcribed and translated to synthesize proteins. Transcription proceeds with the opening of the DNA double helix with the help of an enzyme called DNA helicase. Another enzyme, RNA polymerase equates new bases to the DNA joining them in a long strand of mRNA. When the enzyme completes the transcription the mRNA strand will be detached. This mRNA strand is single stranded and only one side of the DNA strand is transcribed. mRNA does not have the same base pairs as DNA, one of the base is replaced. Thymine in DNA is replaced by Uracil (U) which bonds to Adenine, like thymine. The mRNA is organized in codons. Each codon codes for an amino acid that forms the building blocks of proteins. More than one codon can code for a single amino acid. Once transcription is completed, produced mRNA is transported out of nucleus into the cytoplasm to attach with a ribosome. Ribosome is an organelle which carries out protein synthesis. Some ribosomes are free in the cytoplasm while others are attached to the endoplasmic reticulum. The endoplasmic reticulum is an organelle which helps in the correct folding of proteins and delivers it to other areas. Proteins are synthesized by the process of translation. There are three steps to translation: Initiation, Elongation and Termination.

The ribosome binds around the mRNA and then a tRNA (transfer RNA) joins to a codon referred to as the start codon. In most cases, the start codon is AUG. tRNA are molecules residing in the cytoplasm, each with their particular codon which joins to corresponding codons on mRNA. tRNAs carry the amino acids which complement to that of the codon. The ribosomes are composed of two subunits, a larger subunit and another smaller one. There are three sites present where tRNA can be found at a given time while the protein is being synthesized. Next the ribosome starts synthesizing protein. An amino acid which complements the mRNA code begins to join in a strand, the ribosome moves on the strand in a direction of 5′–3′ until a STOP codon is found. As the ribosome moves on the mRNA the amino acid from the first tRNA is released and binds to the amino acid on the next tRNA via a peptide bond. This is why a chain of amino acids is termed as a polypeptide chain. The ribosome achieves this with the aid of enzymes known as aminoacyl tRNA synthase. Once the ribosome finds a stop codon (UAG, UAA, and UGA) it will detach the polypeptide chain and fall off from the mRNA (Lewin et al. 2011).

8.3 Microbes for Microbial Protein Production

In this section, we describe the various microbes that are utilized for the production of microbial proteins (Fig. 8.1).

8.3.1 *Bacteria*

Properties which make bacteria appropriate for microbial protein production include fast growth and reproduction rate with minimum generation time. Bacteria can be grown on a variety of raw materials ranging from carbohydrates to gaseous and liquid hydrocarbons that comprise methane and petroleum fractions (Bamberg 2000) to petrochemicals like methanol and ethanol; nitrogen sources that are beneficial for bacterial growth comprise nitrates, ammonia, ammonium salts, urea, and the organic nitrogen present in wastes. It is recommended to provide mineral nutrient to the bacterial culture to fulfill shortage of nutrients that might be lacking in natural waters in amount enough to sustain growth. Generally phototrophic bacteria are suggested for microbial protein production. Some investigators also propose utilization of methanotrophic and other bacteria for microbial protein

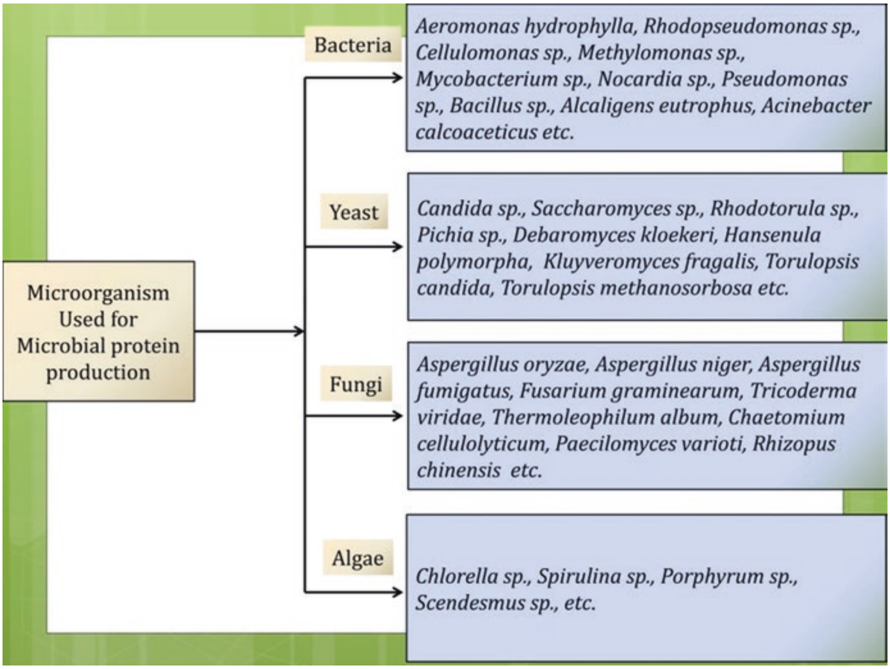

Fig. 8.1 Various microorganisms utilized for the production of microbial proteins

production. Generation time of *Methylophilusis* is about 2 h and it is utilized as animal feed and generally produces more favorable protein composition in comparison to fungi or yeast. Large amounts of microbial protein can be produced utilizing bacteria like *Methylophilus methylitropous*, *Acinetobacter calcoaceticus, Acromobacter delvaevate*, *Aeromonas hydrophilla, Bacillus megaterium, Bacillus subtilis, Brevibacterium, Lactobacillus species, Cellulomonas species, Methylomonas methylotrophu*s, *Pseudomonas fluorescens, Rhodopseudomonas capsulate, Flavobacterium species, Thermomonospora fusca* (Dhanasekaran et al. 2011; Suman et al. 2015).

8.3.2 Algae

Centuries ago *Spirulina* was used by people of Aztecs near Texcoco in Mexico and near lakechad in Africa. It was used as food after drying. *Spirulina* is the most commonly used algae as microbial protein. Even astronauts carried it to space during their space travels. Likewise, biomass gained from *Senedessmus* and *Chlorella* has been collected and utilized as food by tribal groups in some parts of the world. Algae are used as source of food in various ways and its benefits include relatively easy cultivation, efficient utilization of solar energy, high growth rate and rich protein content. The algae *Spirulina* is recommended as supplemental protein (Raja et al. 2008). It is blue green algae with strong antioxidant activity and incites a free radical scavenging enzyme system. A diet supplemented with *Spirulina* and other nutraceuticals can help to protect stem/progenitor cells. *Spirulina maxima* in particular, can help to avoid fatty liver development triggered by carbon tetrachloride. Evidence has shown that *Spirulina* can help patients with immune suppression, malnutrition, neural and hepatic compromise. Further inquiries on the antiviral effects of this alga and its clinical consequences are strongly needed (Suman et al. 2015).

8.3.3 Fungi

Various fungi are utilized as a source of protein rich food (Bhalla et al. 2007). Filamentous species of fungi are also utilized as microbial protein. In the late 1900s, filamentous fungi and *Actinomycetes* bacteria were noted to produce protein from several substrates. At the time of World War II, efforts were made to utilize *Fusarium* and *Rhizopus* grown in fermenters as a source of protein rich food. The strains of *Aspergillus oryzae* or *Rhizopus arrhizus* were chosen due to their nontoxic behavior. Saprophytic fungi can be grown on complex organic compounds and can render them into simple substrate. Large amount of fungal biomass is formed as a result of growth. Yield of mycelia varies greatly depending upon organisms and substrates. On the other hand, some species of moulds, for example, *Aspergillus niger, A. fumigates*, *Fusarium graminearum* which are reported to be dangerous to humans, must not be utilized or toxicological estimations should be performed before endorsing them as microbial protein (Suman et al. 2015). Lately, microbial protein technology using fungal species is utilized for the conversion of lignocellulose wastes. The filamentous fungi that have been used as microbial proteins comprise *Fusarium graminearum, Chaetomium cellulolíticum, Aspergillus niger, A. fumigates, A. oryzae, Penicillium cyclopium, Cephalosporium cichorniae, Rhizopuschinensis, Tricoderma viridae, Scytalidum aciduphlium, and Tricoderma alba Paecilomyces varioti* (Jaganmohan et al. 2013).

8.3.4 Yeast

Yeast cells are high in proteins and nutrients (Burgents et al. 2004). Most popular yeast species utilized as microbial proteins comprise *Torulopsis, Candida, Hansenula, Pitchia*, and *Saccharomyces*. Microbial protein production utilizing *Saccharomyces cerevisiae, grown* on various fruit wastes has been done successfully. The typical oily yeasts genera including *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces* have also been utilized as microbial proteins. Cucumber and orange peels were evaluated for the production of microbial protein using *Saccharomyces cerevisiae* by submerged fermentation (Sengupta et al. 2006).

8.4 Process of Microbial Protein Production

Microbial proteins can be produced by using waste materials as substrates, predominantly from agricultural waste like crumbs, wood cuttings, and corncobs among others. Few additional waste substances utilized as substrates are remnants from alcohol manufacturing processes, food processing, hydrocarbons, or even human and animal feces (Suman et al. 2015).

As described by Nasseri et al. (2011), the process of microbial protein production from any substrate or microorganism will necessitate some vital stages (Fig. 8.2):

(1) based on the condition of carbon source physical and/or chemical pretreatments may be needed.
(2) Together with the carbon source, nitrogen, phosphorus and few other nutrients are also needed to sustain microorganism's optimal growth.
(3) Contamination needs to be avoided by sustaining a sterile and aseptic environment. For this purpose, the constituents of the medium needs to be sterilized by heating or filtration and the apparatus used in the fermentation process needs to be heat sterilized.
(4) The desired microorganism should be inoculated in the pure form.

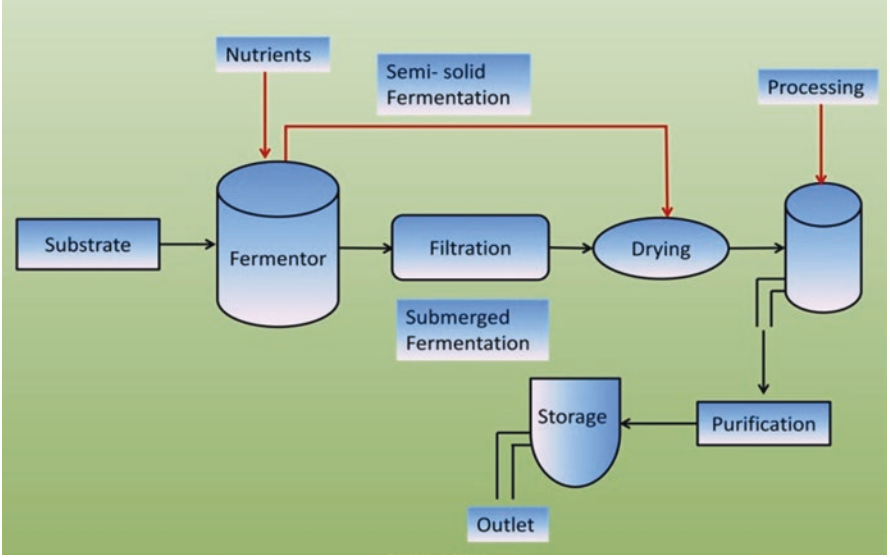

Fig. 8.2 Graphical representation of processes involved in the production of microbial proteins

(5) Procedures for microbial protein production are particularly aerobic (eliminating those using algae). Thus, adequate amount of oxygen needs to be provided. Furthermore, cooling systems are also vital as large amount of heat is produced.
(6) Recovery of the microbial biomass is achieved from the medium.
(7) In order to increase microbial protein use and/or storage time, processing of the microbiological biomass is necessary.

8.5 Nutritional Values of Microbial Proteins

The nutritional value of microbial proteins differs with the microorganisms used. The technique of drying, harvesting and processing affects the nutritional value of the end product. The composition of microbial protein depends on the microorganism and the substrate used to produce it (Suman et al. 2015).

Microbial protein generally includes proteins, fats, carbohydrates, water, and other elements like potassium and phosphorus. Aside from their nutritional benefits, microbial proteins can also be produced whole year round so supply may not be a problem. It can also help the environment as waste materials are utilized as substrates. Very less area of land is needed and microbial protein is produced in a very short time in comparison to other sources.

To evaluate nutritional value of microbial proteins, numerous factors should to be taken in consideration like composition of nutrients, amino acids and vitamins;

their effects on the gastrointestinal tract and the risk of allergies. The carcinogenic and toxicological potentials should also be evaluated so, feeding trials are also needed. The process of harvesting, drying and processing can also affect the nutritional values of the microbial protein. Microbial protein composition depends on the type of substrate and organism used. Proteins not only provide nutritional value but they are also involved in a number of other functions (Upadhyaya et al. 2016).

Microbial protein from yeast and fungi has 50–55% protein. It has high protein and carbohydrates ratio. It contains large number of lysine residues and less number of methionine and cysteine residues. It also provides good balance of amino acids and it contains high B complex vitamins. Few yeast strains have probiotic characteristics like *Saccharomyces cerisiae* and *Debaryomyces hansenii*. Microbial proteins produced by utilizing bacteria comprise more than 80% protein even though they contain a small amount of sulphur containing amino acids with high nucleic acid content (Upadhyaya et al. 2016).

Excellent nutrient profiles and the possibility of economical mass production make microbial proteins a potential solution for the ongoing food crisis. They are also used in aquaculture feeding as a replacement for fishmeal and for protection of rotifer and *Artemia.* Yeast proteins have revolutionized the aquaculture diets. Few yeast strains with probiotic characteristics, like *Debaryomyces hansenii* and *Saccharomyces cerevisiae* have the ability to boost survival of larvae either by residing in the fish gut, thus eliciting the early maturation of the pancreas, or through the immune exciting glucans derived from the yeast cell walls. The idea that microbial proteins can be the solution of food shortages in the less developed countries is gaining research interests among scientists. To assure future success of microbial proteins, first, food technology problems need to be resolved in order to make it comparable to the conventional foods and second, the production should equate favorably with other protein sources (Suman et al. 2015).

8.6 Harmful Effects of Microbial Proteins

The potentials that microbial proteins can do to human beings and the world's problem on hunger are gaining special interests but concerns regarding their acceptability, safety and potential toxicity are emerging. For one, high nucleic acid content in microbial proteins can be a problem which is noted to be beyond the acceptable level of 71%. To address this, nucleic acid can be removed or reduced with either one or all of the following methods: by the chemical treatment with sodium hydroxide, treating the cells with 10% sodium chloride, activating endogenous nucleases in last step of biomass production and thermal shock. Hueihsiung Yang developed a modest technique to reduce the nucleic acid content in *Brevibacterium NNJM98A* by incubating non-proliferating cells at a pH of 10.3 and 55 °C for 3 h (Upadhyaya et al. 2016).

Another issue is the presence of cell walls that cannot be digested. As with yeast and algae, there might be intolerable colour and flavours, and live cells of organisms need to be killed prior to consumption. There is a chance of skin reaction due to ingestion of foreign proteins and gastrointestinal reactions might occur, which may give rise to nausea and vomiting. There is high risk of contamination during the process of production and cell recovery is also problematic. All these detrimental factors lessen the acceptability of microbial protein as a potential global food (Suman et al. 2015).

8.7 Conclusion

Indeed, microbial proteins demonstrate very promising characteristics as a nutrient for humans. Microbial proteins possess various advantages in comparison to plant and animal proteins due to ease of reproduction and growth that is not seasonal or climate dependent. Microbial proteins have high protein content with wide spectrum of amino acids, low fat content and high protein to carbohydrate ratio. These can be produced using wastes as substrates and are therefore environment friendly. The utilization of microbial proteins as a nutrient source can resolve issues related with food shortage of rapidly growing population especially in underdeveloped countries. However with all of these benefits, microbial protein production has not gained much attention due to lack of popularity as a nutrition source among people. Furthermore, high nucleic acid content, non-digestible cell walls, unpleasant colors and flavors and risk of contamination further limit their acceptability as a global food. So efforts needs to be made to uncover alternate substrates and approaches which can minimize the drawbacks of microbial protein production which consequently can lead to the acceptance of this valuable nutrient source on a global scale (Upadhyaya et al. 2016).

Due to economic, political and practical issues, microbial protein has not become the major supply of foodstuff protein as initially envisioned. Developed countries do not see the need of utilizing microbial proteins because they have abundant stocks of high value proteins due to advances in agriculture. Microbial proteins can still be a supportive food source in tropical less developed countries where traditional food is high in carbohydrates and low in proteins. Continuing increase in population growth rate can eventually lead to shortage of food and nutrition even with agricultural advancement. Providing sufficient nutritious food to everyone is going to be a daunting task for future generations. Microbial proteins despite its current poor popularity can still be a promising solution for this problem in the future.

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Chapter 9
Vitamins and Minerals: Types, Sources and their Functions

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9.1 Introduction

Nutrients are grouped into two major classes: macronutrients and micronutrients. Macronutrients are nutrients that are needed by the body in large amounts while micronutrients are those needed by the body in minute amounts. Macronutrients such as carbohydrates, proteins, and lipids provide molecules for the structural and metabolic activities of the human body, while micronutrients (vitamins and

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minerals) are essential for the body's proper functioning. The need for micronutrients depends on the metabolic activities as well as on the life cycle of an individual. Even in intrauterine life, the need for micronutrients is essential for the normal development of the fetus. In particular, vitamin D, iodine, iron, and folic acid deficiencies could lead to congenital disorders or even death. The daily requirements of these micronutrients are not fixed, although many scientific papers have mentioned the daily-required allowance of various vitamins and minerals. Factors such as physical exercise, pregnancy, childhood, adolescence, old age or specific diets (e.g. Vegan) influence the need for micronutrients. Therefore, the evaluation of the micronutrients' requirements and the consequences of micronutrients' deficiencies are critical to explain their role in health and disease (Derbyshire 2018; Hans and Jana 2018).

Fortification of food is one of the most effective and safe strategies used to enhance nourishment. For example, mother milk feeding can be considered a type of fortification, which is essential for the healthy growth of babies up to 2 years of age (Allen and Dror 2018). Micronutrients play an important role in reduction in the risk of disease and the maintenance of good health. Micronutrients and vitamins are vital for the proper functioning and proliferation of all dividing cells in the body. Therefore, micronutrients are essential for growth and metabolism. Balanced diet aids in maintaining good health by increasing the body's resistance to infection and, in the case of disease, can be a useful component of the therapy (Piccardi and Manissier 2009). Nutrients provide basic molecules which humans are unable to synthesize. Vitamins and minerals are required in small quantities (<100 mg/day) and are known as micronutrients (Ormsbee et al. 2014). The two important classes of micronutrients are shown in Fig. 9.1.

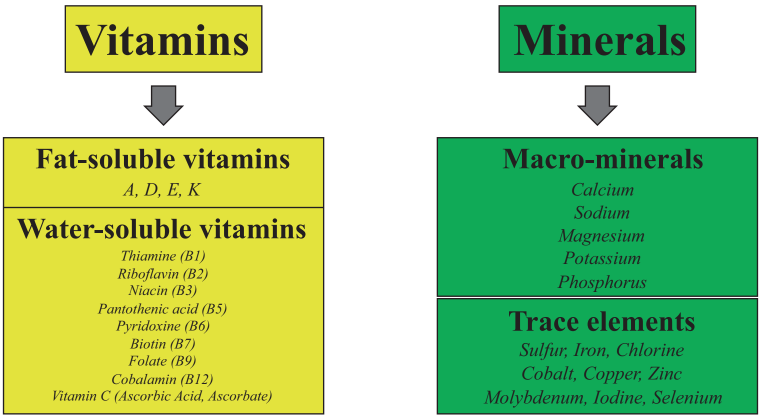

Fig. 9.1 Classification of micronutrients

9.2 Micronutrients: Health Benefits, Deficiencies and Risks

The human body needs micronutrients only in small quantities (in milligrams or micrograms) although they are as important as the macronutrients (Welch and Graham 2004). They are essential for the body's healthy development. Some vitamins for example work as cofactors or coenzymes in many metabolic processes. Trace elements like Zinc can help to improve immune function and minerals like Iron can prevent anemia among others (www.cdc.gov). As the body cannot synthesize micronutrients, therefore, they need to be supplied in the diet in adequate amounts. If their supply is not enough deficiency is possible and this can results to a myriad of diseases. Vitamin A deficiency for example can lead to blindness. A folic acid deficient pregnant woman can give birth to infants with neural tube defects. Iodine deficiency can lead poor brain fetal development. Micronutrient deficiency can increase the risk of infections and can lead to more micronutrient deficiencies (www.unicef.org). These are just the tip of the iceberg. Micronutrient deficiency may lie hidden and usually, the signs and symptoms develop late in the disease and are most often irreversible. The details of the impact of these micronutrients on health and diseases are discussed in the succeeding parts of the chapter.

9.3 Vitamins

Vitamins are essential substances for the normal functioning and development of the body. There are two classes of vitamins namely: Fat-soluble and Water-soluble vitamins (Table 9.1). The known vitamins include A, C, D, E, and K, and the B vitamins: thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), cyanocobalamin (B_{12}), biotin, and folate/folic acid.

9.3.1 Fat-Soluble Vitamins

As earlier mentioned, vitamins can be classified as either a water-soluble or fat-soluble vitamins. The fat-soluble vitamins are very vital for the smooth functioning of the body. Their deficiencies have been implicated in several health disorders. The recommended daily allowance (RDA) of the fat soluble vitamins A, D, E, and K is 8000–1000 μg/day, 8000–5000 μg/day, 8–10 μg/day, and 70–140 μg/day respectively (Kamangar and Emadi 2012). The RDA for water soluble vitamins including thiamin, riboflavin, pyridoxine, niacin, biotin, ascorbic acid, and pantothenic acid are 1 mg/day, 1.2 mg/day, 2–2.2 mg/day, 13 mili-equivalents, 100–200 μg/day, 60 μg/day, and 4–7 mg/day respectively (Kamangar and Emadi 2012).

Table 9.1 Types, functions and sources of vitamins

Vitamins	Types/Subclasses	Functions	Sources	References
Fat Soluble Vitamins	A	Vision, growth and development, Immune function Reproduction, Red blood cell formation, Skin and bone formation.		
	Preformed Vitamin A		Animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, whole milk, and other dairy products.	Rodriguez-Amaya (2004)
	Pro Vitamin A		Red palm oil, green leafy vegetables (e.g. spinach, amaranth, brocolli), yellow vegetables (e.g. pumpkins, squash, and carrots), and yellow and orange non-citrus fruits (e.g. mangoes, apricots, and papayas), red pepper, sweet potatoes. Also found in Brazilian palm fruit known as *Buriti* and the Vietenam fruit known as *Gac*.	Booth et al. (1992) and Vuong (1997)
	D (active form: 1,25-dihydroxyvitamin D)	Maintains bone health, muscle and nerve contraction and and general cellular function in all cells of the body.	Made in the skin from a cholesterol like precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet. Diets such as Fortified milk, cheese, and cereals; egg yolks; salmon.	WHO/FAO (2004) and Feldman et al. (1997)
	E (α-tocopherol)	Antioxidant, formation of blood vessels and boosting of immune function	Good dietary sources of vitamin E include nuts, such as almonds, peanuts and hazelnuts, and vegetable oils, such as sunflower, wheat germ, safflower, corn and soybean oils. Also sunflower seeds and green, leafy vegetables such as spinach and broccoli contain vitamin E.	Bellizzi et al. (1994) and WHO/FAO (2004)
	K	Helps in blood clotting, bone metabolism and regulation of blood calcium levels.		Shearer et al. (1996), Booth et al. (1994), Booth et al. (1993), Conly and Stein (1992) and WHO/FAO (2004)

	K_1 (Phylloquinone)		Green leafy vegetables like spinach, broccoli. Vegetable oils from soyabean, rapeseed and olive. Also found in peanut, corn, sunflower and safflower	
	K_2 (Menaquinone)		Animal liver, fermented foods such as cheese, fermented soyabean (Japanese *natto*), intestinal microflora	
Water-Soluble Vitamins	B_1 (Thiamine)	Cofactor for several enzymes involved in energy metabolism, plays central role in cerebral metabolism.	Beef, liver, dried milk, nuts, oats, oranges, pork, eggs, seeds, legumes and yeast.	Aviva (2010) and Makarchikov (2009)
	B_2 (Riboflavin)	Converts carbohydrates into glucose for energy production, neutralizes free radicals hence acts as anti-oxidant	Plant foods and animal sources, namely poultry, meat, fish and dairy products such as eggs, milk and cheese. Green vegetables such as collard greens, turnips as well high quality protein-rich foods.	Pinto and Rivlin (2013)
	B_3 (Niacin)	Helps lower LDL cholesterol, lowers risk of cardiovascular diseases, eases arthritis.	Animal foods such as lean red meat, poultry and liver. Pea butter is an excellent source of niacin. Other useful sources include whole grain cereals, bread tea, coffee, maize (sweet corn).	Lule et al. (2016)
	B_5 (Patothenic acid)	Pantothenic acid is a key component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier proteins, a component of the fatty acid synthase complex.	Peanut butter, liver, kidney, peanuts, almonds, wheat bran, cheese and lobster. Vast majority of is already incorporated into Coenzyme A (CoA) and as phosphopntetheine	Gregory (2011)
	B_6 (Pyridoxine)	Acts as a critical co-factor for a diverse range of biochemical reactions that regulate basic cellular metabolism.	Richest sources of vitamin B6 include fish, beef liver and other organ meats, potatoes, starchy vegetables and fruits.	Marcelina et al. (2018) and Juan et al. (2018)

(continued)

Table 9.1 (continued)

Vitamins	Types/Subclasses	Functions	Sources	References
	B_7 (Biotin)	Biotin is a water-soluble vitamin and serves as a coenzyme for five carboxylases in humans.	Biotin is found in egg yolk, almonds, sweet potatoes, mushroom, cauliflower, cheese, spinach.	Janos et al. (2009); www.nutri-facts.org/Vitamin_B7
		It helps to convert food into glucose, which is used for energy production. It also helps to produce fatty acids and amino acids (the building blocks of protein). Biotin activates protein/amino acid metabolism in the hair roots and fingernail cells.		
	B_9 (Folate)	Helps in DNA replication, metabolism of vitamins and amino acids, proper cell division. Folic acid helps to reduce risk of spina bifida (neural tube defects) in neonates when taken by pregnant mothers.	Occurs naturally in dark green leafy vegetables, spinach. Also found in liver, avocado pear, paw-paw fruits, orange, beans and nuts	Kunisawa et al. (2012)
	B_{12} (Cyanocobalamin)	Plays a significant role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism. It helps in brain function and synthesis of red blood cells.	Vitamin B12 is naturally found in foods including meat (especially liver and shellfish), eggs, and milk products.	Kelly et al. (2006)
	C (Ascorbic acid)	One of the important properties of vitamin C is its antioxidant activity. Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function Antioxidant activity of vitamin C helps to prevent certain diseases such as cancer, cardiovascular diseases, common cold, age-related muscular degeneration and cataract.	Vitamin c is abundantly available in many natural sources, including fresh fruits and vegetables. The richest sources of ascorbic acid including Indian gooseberry, citrus fruits such as limes, oranges and lemons, tomatoes, potatoes, papaya, green and red peppers, kiwifruit, strawberries and cantaloupes, green leafy vegetables such as broccoli, fortified cereals and its juices are also rich sources of vitamin C. Another source of vitamin C is animals. They usually synthesize their own vitamin C and are highly concentrated in the liver part	Sudha and Raveendran (2017) and Schlueter and Johnston (2011)

9.3.1.1 Vitamin A

Vitamin A was the first fat-soluble vitamin identified in 1913. Beta-carotene is converted to vitamin A in the liver. It protects the eyes in case of infections and contributes to the vision in dim light. Vitamin A combines with the protein opsin to form rhodopsin in the retinal rod cells. When vitamin A levels are inadequate, the lack of rhodopsin makes it difficult to see in dim light (Gräslund et al. 2008). Vitamin A is also involved in the physiological function of the epithelia and glands, and in normal cell differentiation. Vitamin A supports bone growth and the immune functions. Being oil-soluble, it is prescribed as retinol palmate every 6 months to pre-school children. The prevalence of vitamin A deficiency is 33.3% in pre-school children worldwide, while in Africa it is estimated at 44.4%. In Ethiopia, 80,000 deaths occur due to vitamin A deficiency. In this country, vitamin A deficiency prevalence is listed at 61% in preschool children (Abrha et al. 2016). Vitamin A deficiency is linked to the following diseases:

Vitamin A insufficiency leads to visual impairment, xerophthalmia, Bitot's spots (triangular, frothy, harsh and raised patches seen on the bulbar conjunctiva), keratomalacia (softening of the thickness of the cornea), follicular hyperkeratosis, anorexia, growth retardation, respiratory and intestinal infections, and the degeneration of the myelin sheaths. The excess of vitamin A may cause nausea, vomiting, and anorexia. The functions of vitamin A in the body include: development and upkeep of the epithelial tissue, support of the skin, upkeep of visual sharpness (in diminishing light), bone development and immunity. Sources of vitamin A include: green leafy vegetables, germination cereals and pulses, meat, fish, green mango, papaya, pumpkin, yellow fruits and vegetables. Other sources are eggs, spinach, cabbage, carrots, amaranth, angle liver oils, cod liver oil, liver or halibut liver oil (Abrha et al. 2016; Gräslund et al. 2008).

9.3.1.2 Vitamin D

The best-known utility of vitamin D is to control the concentration of phosphorus and calcium in the blood. This anti-rachitic vitamin occurs in a number of forms. Four crystalline D vitamins are isolated and at least 10 pro-vitamins D are known. For humans, most of the vitamin D supply comes from animal sources or synthesized following sun exposure. Vitamin D_2 is formed by exposing ergosterol to ultraviolet light. Vitamin D_3 is the natural vitamin D found in fish oil and formed in the skin of man and animals following exposure to sunlight (Nair and Maseeh 2012). It may be formed by the irradiation of 7-dehydrocholesterol. Vitamin D_2 and D_3 are known as vitamers. Vitamin D enhances the absorption of phosphorus and calcium from the intestine and their deposition in bones. Vitamin D sources include exposure to sunlight, liver, eggs, butter, cheese, fish liver oil, fortified foods, milk, and margarine. Vitamin D also stimulates the normal mineralization of bones and increases the tubular reabsorption of phosphate. It also possesses antioxidant properties (Christakos et al. 2015; Gaman et al. 2019). Ageing decreases the capacity of the skin to produce vitamin D_3. After the age of 70, vitamin D levels decrease to about 25% of the normal value in adults (Kennel et al. 2010). In the temperate zone, the cutaneous production of vitamin D_3 is limited by the reduced ultraviolet radiations availability. In the absence of solar exposure, about 400–600 IU of vitamin D seems to be required daily to maintain the normal bone metabolism. Deficiency of vitamin D can affect bone development. In children, vitamin D deficiency causes rickets, a disorder in which bones deteriorate and bend under pressure. In adults, vitamin D deficiency results in osteomalacia (soft bones) which enhances the risk of bones fractures (Kennel et al. 2010; Nair and Maseeh 2012; Christakos et al. 2015).

9.3.1.3 Vitamin E

Vitamin E is an anti-sterility factor and a natural antioxidant. Structurally related names of vitamin E include tocopherols or tocotrienols (Gheorghe et al. 2019). It is involved in the healing of wounds and in immunity. Sources of vitamin E include wheat germ oil, nuts, cereals, meat, eggs, milk, green leafy vegetables, and other vegetables. The deficiency of vitamin E is associated in humans with cystic fibrosis, ataxia and abetalipoproteinemia (disorder that interferes with the normal absorption of fat and fat-soluble vitamins from food) and with habitual abortions and testicular degeneration in laboratory animals. Also, vitamin E deficiency can lead to increased hemolysis of the red blood cells and macrocytic anaemia in premature infants. The effects of excessive vitamin E doses lead to interference with the utilization of vitamins A and K, prolonged prothrombin time, intestinal irritability, headache, fatigue, and dizziness (Schmölz et al. 2018; Birringer et al. 2019; Gomez-Pomar et al. 2018; Moisa et al. 2018; Kemnic and Coleman 2019; Reddy and Jialal 2018).

9.3.1.4 Vitamin K

The major function of vitamin K is the formation of prothrombin in the liver along with other vitamin K dependent clotting factors namely: VII, IX,, X, protein C and S which are essential for normal blood coagulation or blood clotting. It is found in fresh green leafy vegetables, lettuce, cabbage, egg yolk, soybean oil, and liver. Our body can also produce its own vitamin K courtesy of the normal bacterial flora of our intestines. Premature babies receive 0.5–1 mg vitamin K intramuscularly or 1–2 mg orally at birth to prevent Vitamin K deficiency. Vitamin K deficiency can result to generalized bleeding, the development of hemorrhagic disease of the newborn, and prolonged clotting time in adults. Excessive doses of vitamin K can lead to hyperbilirubinemia in infants and vomiting in adults (Reddy and Jialal 2018).

9.3.2 Water Soluble Vitamins

9.3.2.1 Thiamine (Vitamin B1)

Thiamine or vitamin B1 is water-soluble and acts as coenzyme known thiamine pyrophosphate. Thiamine pyrophosphate is involved in carbohydrate metabolism. Thiamine pyrophosphate is also involved in the hexose monophosphate shunt. It is a neuro-protective agent (Ikeda et al. 2016). Thiamine is found in nuts, potatoes, meat, beans, and cereals. Its deficiency leads to a disease known as beriberi. It is often observed in polished rice-eating communities because, in polished rice, the seed coat, which contains this vitamin, is removed. There are three types of beriberi: dry, wet and infantile (Romagnoli et al. 2012). Thiamine is a co-catalyst in sugar digestion and is necessary to the function of the heart, nerves, and muscles. Vitamin B1 sources include unmilled oat, wheat germ, beets, nuts, meat, lentils, potatoes, pork, eggs, poultry, dried beans, green peas, beans, green verdant vegetables. Thiamine insufficiency can cause beriberi, polyneuritis, mental disarray, ataxia or Wernicke-Korsakoff syndrome in alcoholics. Excess can lead to tachycardia, migraines or peevishness, a sleeping disorder (Wiley and Gupta 2019).

9.3.2.2 Riboflavin (Vitamin B2)

Riboflavin (or lactoflavin) is a yellow crystalline substance. Riboflavin is found in grain, milk, eggs, liver, oats, and green verdant vegetables. It is involved in tissue respiration. Its derivatives are FAD (flavin adenine dinucleotide in its oxidized state) and $FADH_2$ (FAD in its reduced form). $FADH_2$ gives two ATP in the electron transport chain. FAD and $FADH_2$ are involved in oxidation-reduction reactions. One $FADH_2$ is obtained in the TCA cycle (Barile et al. 2016). These act as coenzymes in the alpha-keto glutarate dehydrogenase and succinate dehydrogenase complexes. Its deficiency results in a condition known as ariboflavinosis. Ariboflavinosis is characterized by cheilosis (textured desquamation of the skin around the mouth), glossitis (sparkly red and sore tongue), soreness of the lips, eye disturbances and photophobia (light sensibility), oily skin the nose, scrotal dermatitis (Henriques et al. 2010).

9.3.2.3 Niacin (Vitamin B3)

It is a water-soluble vitamin essential to the human diet, but can be synthesized in the body from tryptophan. Its deficiency leads to a condition known as pellagra. It is found in some cereals, yeast extracts, and meat. In the body, niacin is converted to the NAD (nicotinamide adenine dinucleotide) coenzyme. These co-enzymes are involved in oxidation-reduction reactions. They act as coenzymes of the isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase and malate dehydrogenase complexes (Ronsein et al. 2016). Niacin has lipid-lowering effects and can be used in the treatment of diabetes mellitus (Elam et al. 2000).

9.3.2.4 Pantothenic Acid (B5)

It is a member of the vitamin B complex group. As part of the fatty acid synthase and of coenzyme A, it is involved in hormonogenesis and energy production. Patients with pantothenic acid deficit may develop adrenal insufficiency, enteritis or dermatological conditions (alopecia, dermatitis etc) (Lykstad and Sharma 2019).

9.3.2.5 Biotin (Vitamin B7)

Biotin is also considered as a member of the B-complex group. It helps in the synthesis of fatty acids, utilization of glucose, metabolism of proteins, and utilization of vitamin B_{12} and folic acid. The effects of biotin deficiency or excess have remained unknown. It is obtained from green beans, egg yolk, dark green vegetables, kidneys and liver (Hsu et al. 2016).

9.3.2.6 Folic Acid (Vitamin B9)

Folic acid is the synthesized form of folate, a water-soluble vitamin, found in green leafy vegetables, fruits and liver. Following conversion, vitamin B9 or folate becomes tetrahydrofolate, its active form. It is an essential molecule in the synthesis of nucleic acids (DNA and RNA). Folate deficiency can lead to neural tube defects, thus pregnant women should receive folate supplementations as a preventive method. Folate deficiency can also cause megaloblastic anemia, a type of macrocytic anemia, and prompts a differential diagnosis with vitamin B_{12} deficiency, which also causes megaloblastic anemia (Lykstad and Sharma 2019; Ankar and Kumar 2019).

Methylenetetrahydrofolate is involved in the synthesis of thymidine, an important component of the DNA. Neural tube defects are common in children whose mothers were deficient in folic acid during pregnancy (Hodgetts et al. 2015). Neural tube defects can be prevented in children whose mothers receive folic acid supplements during pregnancy. Methyl vitamin B_{12} mediates the reaction in which the amino acid methionine, which is required for the synthesis of myelin, is generated from homocysteine. During this process, methyl tetrahydrofolate is also converted to tetrahydrofolate. The normal generation of methyl tetrahydrofolate depends upon an adequate supply of both folic acid and vitamin B_{12}. Deficiency of either of them can produce a defect in all the tissues with rapid rate of cellular proliferation, e.g. the bone marrow (resulting in megaloblastic anaemia) and the gastrointestinal tract.

The administration of large doses of folic acid in a patient with vitamin B_{12} deficiency may alleviate the anaemia but it cannot cure or may even aggravate the neurological deficit, by increasing the tissue demand of vitamin B_{12} (Reynolds 2014).

9.3.2.7 Cobalamin (Vitamin B12)

Vitamin B_{12} (cyanocobalamin) is a water-soluble vitamin found in meat, dairy products, fish and eggs. (Hariz and Bhattacharya 2019). It is essential for the normal production of red blood cells by the bone marrow and for the growth of nervous cells. Vitamin B_{12} deficiency causes megaloblastic anemia and, *via* myelin damage, neurological deficits (ataxia, neuropathy) or even neuropsychiatric symptoms such as dementia. Vegan diets contain insufficient amounts of vitamin B_{12}. Folate deficiency can also cause megaloblastic anemia and should be taken into account when looking for the potential cause of the anemic syndrome (Lykstad and Sharma 2019; Ankar and Kumar 2019).

9.3.2.8 Vitamin C

Vitamin C (ascorbic acid) is a crystalline solid which is soluble in water (Halliwell 2001). Most animals and plants synthesize ascorbic acid; however, primates and humans lack gluconolactone oxidase, a key enzyme in the final step of ascorbate synthesis. Thus, in these species, the daily requirements of vitamin C should come from the diet. Oranges, lemons, grapefruit, leafy green vegetables and beef liver are the best sources of vitamin C (Ferraro et al. 2016). Vitamin C is needed to form collagen that gives strength to the connective tissues and required for wound healing and a normal immune function. Vitamin C deficiency causes changes in the connective, leading to the development of scurvy, a disease in which the synthesized collagen is unstable. The symptoms of scurvy include muscle pain, joint swelling, and bleeding. Vitamin C acts as antioxidant and free oxygen radical scavenger and can be used topically in skin disorders, including those caused by photo-aging (Sorice et al. 2014; Luis Gomez et al. 2018). The hyperpigmentation of the skin can be treated with vitamin C, since it inhibits the activity of melanocytes, i.e. the cells involved in the synthesis of melanin (Telang 2013).

9.4 Minerals

Minerals are inorganic elements that cannot be synthesized in the body but obtained from the diet. They are naturally present in soil and water. Some are essential to living organisms while some are very toxic. Plants absorb significant amount of minerals from the environment and usually passed them along the food chain to animals. The deficiency of such nutritionally important minerals usually proves fatal.

Minerals are key elements of the body. They are needed in the buildup and function of important biomolecules in the human body. Although, minerals are not a source of energy in the body but they are necessary for the maintenance of normal biochemical processes in the body (Zhao et al. 2016). Based on the body needs, these essential minerals can be classified as either a macro or micro (trace) minerals.

9.4.1 Macro-Minerals

Macro-minerals are nutritionally important minerals such as sodium, calcium, phosphorus, magnesium and potassium. They are classified as macro because the average adult daily requirement is greater than 100 mg/day (Prashanth et al. 2015).

9.4.1.1 Calcium

It is obtained from hard water, shellfish, fish, dark green leafy vegetables and milk (Jeurnink and De Kruif 1995). Calcium is a mineral which is essential for an adequate growth and bone development (Matkovic and Ilich 1993). It is a common mineral found in blood (McCarron and Reusser 1999). Cells require an adequate amount of calcium to perform various functions (Miller et al. 2001). Teeth and bones are rich in calcium (Vallet-Regí and González-Calbet 2004). It is also involved in blood clotting (Hall et al. 1991). Most of the calcium is found in bones (Reid et al. 2015). The total extracellular fluid space contains about 900 mg of calcium which is in dynamic equilibrium with the skeleton (Bronner and Stein 1995). About 1% of the skeletal calcium (10 g) is readily exchangeable with the calcium in extracellular fluids and constitutes a large calcium reservoir. The remaining 99% of the bone calcium is only slowly exchangeable. Nearly 500 mg of calcium are deposited in and mobilized from the bones daily in a continuous process of remodelling. Calcium is secreted into the intestine through bile, pancreatic juice, and intestinal secretion but is completely reabsorbed. As the blood level of calcium decreases, bones start to release calcium to increase the calcemia. When the calcium level increases in the blood, it is deposited in bones or excreted in urine. Calcium is involved in the nerve function, contraction of muscles and clotting of blood. Its level in blood is maintained by the parathyroid hormone and calcitonin. The daily-recommended allowance for calcium is 1 g per day. Vitamin D is involved in the uptake of calcium by the body. Its deficiency results in rickets, osteoporosis, and osteomalacia. Tetany may occur due to the deficiency of calcium in the blood. Causes of hypocalcaemia include chronic renal failure (Naveh-Many et al. 1995), phosphate therapy etc. (Välimäki et al. 1999). The excess amount of calcium in the blood can result in the development of kidney stones (Pettifor 2008; Sahay and Sahay 2012).

9.4.1.2 Sodium

Sodium (natrium) is present in most foods and its dietary deficiency is rare. Sodium is involved in the control of blood. Sodium chloride is the most common form of sodium which is marketed as table salt (Cogswell et al. 2016). Kidneys are the main regulators of body sodium and normally 98% of the body loss of sodium occurs in urine. If more sodium is ingested, its excretion in the urine increases. If less sodium is ingested or if plasma sodium falls due to any reason, sodium may totally disappear from the urine. This is usually through the adrenocortical hormone aldosterone, which increases the tubular reabsorption of sodium in the renal tubules. An increased level of sodium in the blood defines hypernatremia, and is characterized by seizures, oedema, neuromuscular excitability, irritability, weakness and lethargy (Kalogeropoulos et al. 2015; Xi et al. 2015).

9.4.1.3 Magnesium

Magnesium is obtained from hard water, spices, apricots, bananas, soybeans, nuts, green leafy vegetables, and whole grains. It aids in the maintenance of bone growth and integrity and is involved in the regulation of the cardiac cycle and the functioning of muscles and nerves. Deficiency diseases are hypomagnesaemia and neuromuscular irritability. Toxicity symptoms are hypotension, respiratory failure, and cardiac disturbances (Allen and Sharma 2019; Gragossian and Friede 2019).

9.4.1.4 Potassium

It is obtained from whole and skimmed milk, meat, bananas, raisins and prunes. A proper plasma potassium level is essential for the normal heart functioning. Potassium ions also take part in the normal functioning of skeletal muscle fibers. Potassium is needed for many enzyme reactions (Weaver 2013). Glycogenesis requires the presence of potassium. Insulin administration causes a fall in plasma potassium level because the deposition of glycogen brought about by insulin is also accompanied by the deposition of potassium. Moreover, insulin also increases protein synthesis within the cells, which by binding potassium ions can lead to a low plasma potassium level. Potassium deficiency leads to hypokalemia, paralysis, and cardiac disturbances. Excessive potassium levels lead to hyperkalemia, paralysis and cardiac disturbances (He and MacGregor 2008).

9.4.1.5 Phosphorus

It is obtained from legumes, nuts, cereals, fish, meat, cheese and poultry (Takeda et al. 2012). The bioavailability of minerals such as iron and zinc may be low in a total vegetarian diet because of the presence of substances such as phytic acid. Besides, large amounts of dietary fiber may interfere with its proper absorption.

Because humans are omnivorous, trace element deficiencies are unlikely to develop. Studies have shown that mineral deficiencies are not more frequent among vegetarians than among non-vegetarians. In fact, man's need for trace elements has not yet been precisely determined. Trace elements should not be used as dietary supplements, since excessive amounts can have injurious effects. Phosphorus is involved in the formation of bone and teeth, ATP, GTP and UTP. It is a component of DNA and RNA, it is present in phospholipids and it forms part of cell membranes. Hyperphosphatemia can occur in renal failure (Arai and Sakuma 2015).

9.4.2 Trace Elements

As the name implies, trace elements are essential group of minerals, which are needed, in small quantity for the day-to-day metabolic processes in man. They are regarded, as trace elements because their daily requirement should be below 100 mg, above which can be toxic to health. However, the deficiency of any of these trace elements can lead to serious health challenges (Prashanth et al. 2015). Trace minerals include iron, copper, zinc, iodine, manganese etc. (Table 9.2).

Table 9.2 Trace minerals, functions and sources

S/N	Minerals	Functions	Sources	References
1	Iron (Fe)	Helps in the formation of heme proteins needed for the transport of oxygen to the red blood cells, flavoproteins and other enzymes.	Fe is widely distributed in organ meats, red meats (30–70% is haem iron), egg yolks; legumes; dried fruits; dark, leafy greens; iron-enriched breads and cereals; and fortified cereals; fish; poultry; shellfish	Fairweather-Tait and Hurrell (1996)
2	Copper (Cu)	Part of many enzymes including metalloenzymes; needed for red blood cell formation, connective tissues	Foods high in copper include liver, kidney, shellfish, wholegrain cereals and nuts. Soft or acidic water passing through copper pipes can also contribute copper to the diet.	Fairweather-Tait and Hurrell (1996)
3	Zinc (Zn)	It plays main roles in the cell-mediated immunity, bone formation, tissue growth, brain function, growth of the fetus and child. It also has roles in pathogenesis of some dermatological disorders.	Meats, fish, poultry, oysters, leavened whole grains, vegetables	Bagherani and Smoller (2016)

(continued)

Table 9.2 (continued)

S/N	Minerals	Functions	Sources	References
4	Iodine (I)	Growth and development, Metabolism, Reproduction, Thyroid hormone production	Seafood, foods grown in iodine-rich soil, iodized salt, bread, dairy products	Fairweather-Tait and Hurrell (1996)
5	Selenium	Anti-oxidant; Selenium is needed for the proper functioning of the immune system, and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. It is required for sperm motility and may reduce the risk of miscarriage.	Meats, seafood, grains. Cereals, seafood and meat products are the richest sources of Se and are the main contributors to the daily Se intake, whereas vegetables, fruits and beverages are generally in Se	Combs (1988), Fairweather-Tait and Hurrell (1996) and Margaret (2000)
6	Chromium (Cr)	Chromium acts as an antioxidant. It also helps to decrease insulin resistance in diabetic patients	Dietary sources of chromium include brewer's yeast, cheese, pork kidney, whole grain breads and cereals, molasses, spices and some bran cereals. Lean beef, oysters, eggs, and turkey are sources of chromium	Anderson et al. (1992), Anderson (2000) and Tulasi and Rao (2014)
7	Manganese (Mn)	It activates numerous enzymes — such as hydrolases, transferases, kinases, and decarboxylases — and is a constituent of some enzymes. Manganese also plays a role in blood clotting and hemostasis in conjunction with vitamin K.	Manganese is present in a wide variety of foods, including whole grains, clams, oysters, mussels, nuts, soybeans and other legumes, rice, leafy vegetables, coffee, tea, and many spices, such as black pepper.	Watts (1990), Aschner and Aschner (2005) and Buchman (2014)
8	Flouride (F)	It is involved in formation of bones and teeth; helps prevent dental decay and caries.	Drinking water (either fluoridated or naturally containing fluoride), fish, and most beverages, from oral tooth pastes as well.	O'Mullane et al. (2016)
9	Molybdenum (Mo)	Functions as a cofactor for at least four enzymes: sulfite oxidase, xanthine oxidase, aldehyde oxidase, and mitochondrial amidoxime reducing component.	Legumes; nuts, breads and grains; leafy greens; leafy, green vegetables; milk; liver	Novotny (2011)

Source: Modified from https://www.uofmhealth.org/health-library/ta3912

9.4.2.1 Iron

Iron is obtained from green leafy vegetables, dried nuts, beans, peas, egg yolk, red meat, kidney, and liver. Iron is one of the most abundant metals present in the body and is essential for life. As a component of haemoglobin and myoglobin, it is involved in the transfer of oxygen between blood and tissues. In most of the cells, iron is present as a component of enzymes involved in oxidation-reduction reactions. At birth of an infant, large amount of iron (246 mg) accumulates in the body. This iron storage depends on the iron intake of the mother during pregnancy. The greatest demand for iron is during the last 3 months of pregnancy. The developing fetus requires about 20–30 mg/day of iron in the pregnant woman. The iron in diet comes in the form of haem and non-haem. Haem has a higher bioavailability and can be found in meat, fish, poultry, and milk (Abbaspour et al. 2014). Non-haem is found in varying degrees in plant products. Iron deficiency leads to hypochromic microcytic anaemia. Factors that reduce iron absorption are the surgical removal of the upper small intestine, subtotal gastrectomy, chronic infections, achlorhydria and anti-acid therapy, excess of phosphates and oxalates, diarrhoea and malabsorption. In the case of deficient intake of iron, blood loss or increased iron demand, iron deposits in the body depletes, and anaemia occurs. Symptoms include pallor, weakness, irritability, fissures of the angles of the mouth, heart murmurs and indigestion (Lopez et al. 2016). Iron excess leads to the development of cirrhosis, skin pigmentation, and hemochromatosis. In the United States of America, 5% of men and 2% of women are suffering from anaemia (Johnson-Wimbley and Graham 2011).

9.4.2.2 Chloride

Sources of chloride include cornbread, potato chips, green olives, and animal products. Chloride is necessary to maintain the composition of blood and to the formation of hydrochloric acid. It regulates the acid-base balance and the osmotic pressure. Deficiency symptoms include alkalosis and failure to thrive in infants. Toxicity symptoms include an increase in the extracellular volume and hypertension (Shrimanker and Bhattarai 2019; Sur and Shah 2019).

9.4.2.3 Cobalt

Intestinal bacteria synthesize cobalt. It takes part in the formation of vitamin B_{12}. It is stored in the body in amounts of 1–2 mg. The liver stores enough cobalt for 3–4 years as hydroxyl cobalamine and methyl cobalamine. The daily requirements are 2–3 microgram (Angelova et al. 2014).

9.4.2.4 Copper

It is obtained from organ meats, nuts, dried legumes, whole grains, and cereals. In food, it is present as copper complexes and released in the stomach due to the acidic pH of the gastric juice. It is involved in bone formation and hematopoiesis. It is absorbed in the small intestine mostly by diffusion and in small amounts using carriers. In the circulation, copper combines with albumin, reaches the liver, and incorporated into ceruloplasmin, which is distributed to the tissues. Copper is excreted through bile in faeces and in urine, skin, hair, and nails. It is transported by albumin and is bound to ceruloplasmin. It is part of certain enzymes like ferro-oxidase, catalase, cytochrome oxidase and tyrosinase (Leone et al. 2006). It is required for red blood cell production. Copper-containing proteins like ceruloplasmin contribute to the absorption of iron in the gastrointestinal tract. Copper deficiency can cause hypochromic anaemia. Copper toxicity manifests as hepatolenticular degeneration and biliary cirrhosis (Patil et al. 2013).

9.4.2.5 Zinc

It is obtained from liver, muscle, oysters, cereals, and pulses. Infants need 5 mg/day, children need 10 mg/day and adults need 35 mg/day. It is a constituent of metalloenzymes and enables cell growth and proliferation, sexual maturity, and fertility. It improves immunity, appetite, and taste. Iron and copper reduce its absorption. Zinc deficiency is rare and can be seen in patients with kidney diseases and in alcoholic patients. The growth of children with zinc deficiency is reduced. Zinc toxicity symptoms include the development of gastrointestinal diseases and a decreased immune function (Prasad 2008; Bredholt and Frederiksen 2016).

9.4.2.6 Silicon

Elevated levels of silicon are present in the connective tissue of the aorta, i.e. collagen and elastin components of the aortic wall. Silicon levels decline with ageing and with the development of atherosclerosis. Silicon seems to reduce the levels of fatty acids in the blood and the aorta. Fatty acids are associated with the formation of atherosclerotic plaques. Studies have shown silicon supplements also help in the prevention of osteoporosis (Jugdaohsingh 2007).

9.4.2.7 Manganese

It is obtained from nuts, cereals, and tea leaves. It is required for enzymes, such as succinate dehydrogenase, arginase, and glucosyltransferase. It is required for the synthesis of chondroitin sulphate which is necessary for cartilage formation (Tuschl et al. 2013). It stabilizes the DNA and the RNA. The total body content is 15 mg and the daily requirement is of 4–10 mg. It is excreted in the bile and the pancreatic juice. Its deficiency causes a defective bone formation and leads to glucose intolerance, alopecia, reddening of hair and dermatitis. Toxicity symptoms include functional abnormalities of the central nervous system (Pallauf et al. 2012).

9.4.2.8 Fluoride

It is obtained from seafood, vegetables, grains, tea, coffee (Moody et al. 1980) and fluorinated water (Buzalaf et al. 2001). It is necessary for the mineralization of bones, the development of the dental polish and the aversion of dental caries. Dental caries can be prevented by the fluoridation of water and the addition of fluorides to toothpaste. Fluoride salts can be administered to children in the form of drops, tablets, and mouthwash. Toxicity leads to dental fluorosis (Iheozor-Ejiofor et al. 2013).

9.4.2.9 Iodine

Sources of iodine include seafood, iodized salts, eggs, dairy products and water. It is an essential element for the normal growth and development of the body. Iodine is necessary for the formation of thyroid hormones. Iodine deficiency occurs in nearly 2.6 billion individuals. Various degrees of iodine deficiency affect 50 million children worldwide (Ershow et al. 2016). Iodine cannot be stored in the body and requires a lifetime intake in tiny amounts. Common salt fortified with iodine can be used as an effective intervention for a long time. Iodine deficiency can lead to the development of goiter. The daily-recommended dietary allowance of iodine is 40 microgram/day. It is used also as antiseptic, as skin disinfectant prescribed in the form of povidone-iodine (Durani and Leaper 2008).

9.4.2.10 Selenium

Selenium is a mineral with antineoplastic effects against colon cancer, breast cancer and possibly other malignancies (Hoffmann and Berry 2008). It is a natural antioxidant (Gaman et al. 2014). The amount of selenium present in food depends on how much selenium was found in the soil where the food had grown. It is obtained from animal and vegetal products. The richest food sources of selenium include muscle meat, cereals, grains, and dairy products like eggs. It partakes in the activity of metallo-enzymes and protein synthesis, prevents liver necrosis, stimulates pancreatic lipase secretion and is involved ATP generation. Selenium deficiency causes hemolytic anaemia, muscle necrosis, and cardiomyopathy. The excess of selenium causes toxicity-dermatitis, loss of hair and gives a garlic odour in breath (Rayman 2012).

9.5 Summary and Conclusion

Vitamins and minerals are essential substances for the normal functioning and development of the body. There are two classes of vitamins namely: Fat-soluble and Water-soluble vitamins. The known vitamins include A, C, D, E, and K, and the B vitamins: thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), cyanocobalamin (B_{12}), biotin, and folate/folic acid. A number of minerals are essential for health: calcium, phosphorus, potassium, sodium, chloride, magnesium, iron, zinc, iodine, sulfur, cobalt, copper, fluoride, manganese, and selenium. The essential minerals of the body are divided into two viz-a-viz: macrominerals and trace minerals. The trace minerals are those that are required in small amounts by the body but do not imply they have little significance to the body. Micronutrients satisfy both the qualitative and quantitative requirements in the human diet and are consumed in moderate amounts.

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Chapter 10
Prebiotics, Probiotics, Synbiotics and Its Importance in the Management of Diseases

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10.1 Introduction

The concept of adding living microorganisms in food is not new. Fermented milk has been in use as early as 2500 BC by Sumerians as a cough remedy. In 76 BC, Plinius, a Roman historian discussed about gastroenteritis and the administration of fermented milk products as its treatment. In the twentieth century, several authors like Metchnikoff and Tissier, introduced the oral administration of living microorganisms for diarrhea and to reduce the toxin-producing bacteria in the gut by modifying the gut microbiota. Fermented products, such as beer, bread, wine, kefir, kumis and cheese have also been used for nutritional and therapeutic purposes for

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decades (Yoo and Kim 2016). The intestinal microflora has mechanisms that alert the host to an infection. The gastrointestinal tract maintains an extensive and highly active immune system. And it is the microorganisms in the gut altogether that functions to protect this microenvironment. These endogenous microflora aid in the metabolic functions of the body, and assist in the development of the complex mucosal and systemic immunoregulator circuitry. They protect the gastrointestinal tract (GIT) against colonization by exogenous microbes and in the prevention of a potential intestinal mucosal invasion by an incoming pathogen. So any alterations in the intestinal microflora can cause dysbiosis. This dysbiosis is related to different important pathologies to which many therapeutic strategies are now implemented that aims in rebuilding and restoring balance of the intestinal ecosystem. The idea of functional food is an example of a strategy in counteracting intestinal dysbiosis (Canny and McCormick 2008). Administration of probiotics, prebiotics and its combination (synbiotics) function in the displacement of these potentially pathogenic bacteria and help in returning the balance of the microbial community. Newer therapeutic approaches like phage therapy and fecal transplantation are now also being studied (Moelling et al. 2018). All these strategies however, share a common goal that is, to restore eubiosis (i.e., to replace pathogens with more favorable microbes) (Moelling et al. 2018).

10.2 Probiotics

The word "Probiotics" is derived from the Greek word meaning "for life" (Bandyopadhyay and Mandal 2014). These are non-pathogenic live organisms when given in adequate amounts will have beneficial effects on the hosts. Kollath in 1953 was the first one to use this term. He claimed that different organic and inorganic supplements can restore the health of malnourished patients. This word was then used by Ferdinand Vergin in 1954, a scientist who found out about the beneficial effects of probiotics on the gut microflora when he was studying the effect of antibiotics and similar substances on gut microbial population. In 1962, Lily and Stillwell published an article in Science wherein they added to the definition of probiotics: "substances secreted by one microorganism which stimulates the growth of another." Parker in 1974, described "probiotic" as not only microbial organisms, but also 'other substances' that help in the intestinal microbial balance. And it was Roy Fuller in 1989 who redefined probiotics when he removed "other substances" from the definition. He stated that probiotics are "live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance", and this definition of probiotic is the basis of the one used today (Fuller 1992). Today, the Food and Drug Authority (FDA) and World Health Organization (WHO), defined probiotics as "live microbes which when given in adequate amount provides health benefit to the host."

10.2.1 Classification and Selection Criteria

Probiotics are known by genus, species, and strain. The strain designation is important because same species will have different strains, hence, different health effects. Dose should also be considered because a probiotic consumed at a higher dose will not always mean that it will have a greater health benefit than one given at a lower dose. These probiotics contain many different microorganisms as shown in Table 10.1 (Khalighi et al. 2016).

These probiotics are supplemented in food like fermented milk products, either singly or in combination. New genera and strains of probiotics are continuously emerging with more advanced and focused research efforts. Probiotic products may have either one or mixture of more than one strains. Probiotic effects are strain specific and cannot be generalized. A single strain may exhibit different benefits when used individually and in combination.

In 2002, FAO/WHO drafted guidelines which are now used as the global standard to evaluate probiotics in various food products (Gogineni et al. 2013a, b). The guidelines in evaluation of probiotics include: (1) Strain identification; (2) Strain

Table 10.1 Common probiotic microorganisms

Genus	Species
Lactobacillus spp.	*acidophilus*
	plantarum
	rhamnosus
	paracasei
	fermentum
	reuteri
	johnsonii
	brevis
	casei
	lactis
	delbrueckii gasseri
Bifidobacterium spp.	*reve*
	infantis
	longum
	bifidum
	thermophilum
	adolescentis
	animalis
	lactis
Bacillus spp.	*coagulans*
Streptococcus spp.	*thermophilus*
Enterococcus spp.	*faecium*
Saccharomyces spp.	*cerevisiae*

Source: Khalighi et al. (2016)

Table 10.2 Selection criteria of probiotic strains

Criterion	Required properties
Safety	Human or animal origin.
	Isolated from the gastrointestinal tract of healthy individuals.
	History of safe use.
	Precise diagnostic identification (phenotype and genotype traits).
	Absence of data regarding an association with infective disease.
	Absence of the ability to cleave bile acid salts.
	No adverse effects.
	Absence of genes responsible for antibiotic resistance localised in non-stable elements.
Functionality	Competitiveness with respect to the microbiota inhabiting the intestinal ecosystem.
	Ability to survive and maintain the metabolic activity, and to grow in the target site.
	Resistance to bile salts and enzymes.
	Resistance to low pH in the stomach.
	Competitiveness with respect to microbial species inhabiting the intestinal ecosystem (including closely related species).
	Antagonistic activity towards pathogens (e.g., H. pylori, *Salmonella sp*., *Listeria monocytogenes*, *Clostridium difficile*).
	Resistance to bacteriocins and acids produced by the endogenic intestinal microbiota.
	Adherence and ability to colonise some particular sites within the host organism, and an appropriate survival rate in the gastrointestinal system.
Technological Usability	Easy production of high biomass amounts and high productivity of cultures.
	Viability and stability of the desired properties of probiotic bacteria during the fixing process (freezing, freeze-drying), preparation, and distribution of probiotic products.
	High storage survival rate in finished products (in aerobic and micro-aerophilic conditions).
	Guarantee of desired sensory properties of finished products (in the case of the food industry).
	Genetic stability.
	Resistance to bacteriophages.

Source: Markowiak and Śliżewska (2017)

characterization of safety and probiotic attributes; (3) Health benefits validation; and (4) Labeling of potency claims and compositions for the entire shelf life. WHO, FAO, and EFSA (the European Food Safety Authority) suggested that probiotic strains in their selection process, must meet both safety and functionality criteria, as well as those related to their technological usefulness (Table 10.2). The characteristics of a probiotic are not associated with the microorganism's genus or species but are associated with few and specially selected strains of a particular species. A strain is safe based on the following: its origin, the absence of association with pathogenic cultures, and the antibiotic resistance profile. The functionality of a probiotic is defined by their survival in the gastrointestinal tract and immunomodulatory effect. Probiotic strains also have to meet the requirements associated with the technology of their production. Throughout the storage and distribution processes, these probiotic strains have to be able to survive and maintain their properties. Probiotics should also evidently have pro-health effects that is consistent with the characteristics of the strain present in a marketed product (Markowiak and Slizewska 2017).

Probiotic Guidelines (Anukam and Reid 2007):

1. Probiotic organisms must be living;
2. Identify the organism(s) to species level;
3. Have proven safety data;
4. Show physiological benefits when using a defined viable count of probiotics in a defined delivery vehicle (food, capsule or whatever) in a defined patient population, controlled by a placebo and/or standard therapy option if the end outcome is treating a disease.

10.2.2 Commercial Forms

Probiotic organisms can be ingested into two main forms: (a) fermented foods and (b) supplements. Both dairy and vegetable origin belong to fermented foods, with the most commonly known of each being yogurt and sauerkraut, respectively. While probiotic supplements can either be: freeze-dried (lyophilized) bacteria in powder, capsule, or tablet form. To be effective clinically, commercial forms containing probiotic organisms must contain live organisms in sufficient numbers to exert therapeutic effects and these can be achieved using both forms (Khalighi et al. 2016).

10.2.3 Mechanism of Action of Probiotics

Probiotic bacteria have many functions in the host. However, there is no proper documentation of the exact mechanism by which probiotics achieve their beneficial actions on the host cell. Probiotics affect epithelial cells, dendritic cells, monocytes/macrophages, T cells, and B cells of the gastrointestinal tract differently. This is mainly because there are different genera and species of the bacteria and therefore, their effects on cells vary. But there are several mechanisms that may explain many of their positive effects, such as: modifying gut pH, antagonizing pathogens through the production of antimicrobial compounds, competing for pathogen binding and receptor sites as well as for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase. The gastric host defense mechanisms (eg. gastric activity and bile) is a harsh environment. To reach the small intestine and colonize the host, probiotics must survive this acidic environment so it can exert its effect and be beneficial. Despite the differences between species and strains, at pH values below 3.0, these organisms generally exhibit increased sensitivity. This is why acid tolerance is one desirable property that is being used in selecting probiotic strains (Corcoran et al. 2005).

Probiotics can also activate the immune response by secreting immunoglobulins like IgA, or increasing the number of natural killer cells, and even enhancing phagocytic activity of macrophages. With an increased secretion of IgA the number of pathogenic organisms in the gut is lowered and improves the microflora. Aside from its immunomodulating effects, probiotics might also be helpful in certain medical conditions like inflammatory bowel disease (IBD). Probiotics also digest food and compete for nutrients with pathogens. Probiotics decrease programmed cell death in intestine and/or increase the production of mucin. *Lactobacillus rhamnosus* avoids cytokine-induced cell death by preventing TNF. *Lactobacillus* species augment mucin expression to block invasion and adherence *Escherichia coli*. *Lactobacillus rhamnosus* prevents inflammation and intestinal epithelial cells' apoptosis showing mitogenic effects and thus mucosal regeneration. Probiotic bacteria are also competitors of harmful bacteria on epithelial cell receptors and mucus layers. *Lactobacillus casei* increase secretory IgA levels of pathogen by activating B cell. *Lactobacillus casei* down-regulates gene transcription responsible for pro-inflammatory effectors like cytokines and chemokines. This anti-inflammatory effect is processed by the NF-κB cell pathway inhibition and I-κBα stabilization specifically. Communication among bacteria occurs with help of signalling molecules or auto-inducers called quorum sensing. This facilitates the intestinal microbes for colonisation (Markowiak and Slizewska 2017).

When probiotics compete for adhesion sites, they fight for cellular attachments. Such as when pathogenic organisms enter the GI tract, some strains of *bifidobacteria* and *lactobacilli* act as "colonization barriers" (*Lactobacillus rhamnosus* strain *GG* and *Lactobacillus plantarum 299v)*. Both of these organisms do not allow attachment of *Escherichia coli* to human colon cells because they already have adhered to the mucosal epithelium. The synthesis of antimicrobial compounds is a mechanism of action wherein probiotics modify the microbial flora. Probiotics induce host cells to release peptides directly affecting pathogens and thus prevent their epithelial attack. The following antimicrobial peptides released from the gut epithelial cells are: defensins (hBD protein), bacteriocins, hydrogen peroxide (H_2O_2), nitric oxide and short chain fatty acids (SCFA) such as lactic acids, acetic acids and cathelicidin. They exert antimicrobial activity against many bacteria, fungi, viruses by lowering the luminal pH. Many types of *lactobacilli* and *bifidobacteria* produce these bacteriocins or other antimicrobial compounds. These bacteriocins are "compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action". Upon the release of these compounds by probiotic organisms a beneficial modification of the microflora happens. But not all strains of these *Lactobacilli* or *Bifidobacteria* have antimicrobial compounds, and some produce compounds that has no specific activity, so that both beneficial bacteria and pathogenic organisms may be negatively affected (Khaligi et al. 2016).

10.2.4 Therapeutic Uses of Probiotics

To attain a successful clinical outcome, it is crucial to use the same probiotic strain that has evidently demonstrated the desired therapeutic action.

Probiotic strains like *Saccharomyces boulardi, Lactobacillus rhamnosus* GG are used in prevention of antibiotic associated diarrhea**.** *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG or both can be used for preventing Clostridium difficile infection (CDI). *Lactobacillus rhamnosus* GG*, Saccharomyces boulardii* prevent recurrence after first CDI. A mixture of *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii, Lactobacillus acidophilus* cans eradicate *Helicobacter pylori* completely. *Escherichia coli* Nissle 1917, *VSL*#3 are helpful in treatment of Ulcerative Colitis. *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus johnsonii LA1* are used successfully in Crohn's Disease. *Bifidobacterium infantis* is prescribed in Irritable Bowel Syndrome. *Lactobacillus plantarum* 299 can be taken in Acute Pancreatitis. *Bifidusbacterium* spp. and *Lactobacillus acidophilus* are used in Necrotizing Enterocolitis successfully. *VSL* is used to treat Multi-Organ Dysfunction Syndrome (MODS). *Lactobacillus rhamnosus* GG *is given in* ventilator associated pneumonia, allergy and immune response.

There are many characteristics of probiotic microbes, but it is best to follow International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus which states that: a probiotic must be alive when administered, have a health benefit and be delivered at an effective dose (Hill et al. 2014). The number of live organisms present in the product determines the dosage of probiotic foods and supplements. Based on clinical trials successful therapeutic outcomes have been attained using between 107 and 1011 viable bacteria per day. Interestingly, it appears that 100 times fewer viable bacteria need to be given in a dairy medium than in a freeze-dried supplement to achieve similar numbers of live bacteria in the lower bowel (Saxelin 1996). It is said that dairy is an ideal transport medium for the bacteria, augmenting their survival in the upper GI tract (Kailasapathy and Chin 2000).

10.3 Prebiotics

The term 'prebiotics' was coined by Gibson and Roberfroid in 1995. They defined prebiotics as "a non-digestive food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health." Probiotics do not contribute to the caloric value of the food or food ingredient. Gibson and Roberfroid, however, revised this definition and proposed a new one as a "selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson et al. 2004; Roberfroid 2007). This definition matches the words "prebiotic" and "bifidogenic" and encompasses the definition of the prebiotic index (i.e., it gives the absolute increase of the fecal bifidobacteria concentration per gram of daily consumed prebiotics). According to this definition, proposed prebiotics must fulfill the following criteria by in vitro and in vivo proven tests: (1) non-digestibility (resistance to low pH gastric acid, enzymatic digestion, and intestinal absorption); (2) fermentation by the intestinal microbiotica; and (3) selective stimulation of growth and activity of intestinal bacteria (Anadón et al. 2016).

FAO/WHO defined prebiotics as non-viable food component extending health benefits of the host through modulation of gut microbes. The prebiotics can serve as support or a valid option of probiotics. Different prebiotics are responsible for modification of different bacteria at strain or species level. These non-digestible dietary supplements impart health benefits by promoting number and activity of *Lactobacilli* and *Bifidobacteria* like probiotics. When prebiotics pass through the gut, these cannot be broken down by the digestive enzymes that is why these reach the large intestine in an intact form. In the gut, these serve as food for the probiotic bacteria that live there. Prebiotics of proven efficacy therefore, are able to modulate the gut microbiota by flourishing beneficial gut microbes and suppressing harmful (pathogenic) ones. Prebiotics maintain the optimal pH in the intestine which is essential for the existence of the probiotics. They promote the immune system because they enhance the probiotics growth. They suppress the reproduction of the pathogens so that they are not able to multiply nor exhibit their harmful effects. They stimulate the peristalsis and reduce the formation of gases. They also stimulate the growth and reproduction of the useful microflora. These prebiotics serve as food for the probiotics. Any food substrate that enters the large intestine may be a potential prebiotic, but selective fermentation is a necessary determinant. Prebiotics selectively affect the microbiota and results in improved health of the host. Prebiotics, like other low digestible carbohydrates, exert an osmotic effect in the GIT as long as they are not fermented; and if they are fermented by the native flora (i.e., at the place where they exhibit their prebiotic effect), they also increase intestinal gas production (Roberfold and Slavin 2000; Schrezenmeir and De Vrese 2001).

Most of the prebiotics that are used as food supplements are plant products like inulin, fructo-oligosaccharides, lactulose, dietary fiber and gums wherein Inulin and trans-galactooligosaccharides (TOS) are the two more common prebiotics used. These two occur naturally in foods like the garlic, onions, leeks, shallots, asparagus, spinach, Jerusalem artichokes, chicory, peas, beans, lentils, oats and bananas (Bandyopadhyay and Mandal 2014). Oligosaccharides are the best-known prebiotics. Other commonly used prebiotics are Fructo-oligosaccharides (FOS), Mannan-oligosaccharides (MOS), Inulin, Lactulose, and Xylo-oligosaccharides (XOS). Among these, xylooligosaccharides is commercially available.

10.3.1 Selection Criteria

In order to meet the definition of prebiotics, a dietary substance should have three main physiological properties: (1) The first criterion infers that since prebiotics are not digested (or just partially digested) in the upper segments of the GIT, they reach the colon and are selectively fermented by potentially beneficial endogenous bacteria (a requirement of the second criterion). This fermentation may then lead to the following: increased or modification in the relative production of different short-chain fatty acids (SCFAs), a larger stool mass, a moderate decrease in colonic pH, a reduction of nitrous end products and faecal enzymes, a better immunological system. These effects are all beneficial for the host cell (the requirement of the third criterion). (2) Selectively stimulating the growth and/or activity of the intestinal bacteria is potentially associated with health protection and wellbeing and is considered another criterion. (3) The last criterion of the classification assumes that a prebiotic should be unaffected by the food processing conditions and remains unchanged, non-degraded, or chemically unaltered and available for bacterial metabolism in the intestine (Gibson et al. 2004; Wang 2009). Potential prebiotic candidates are those that do not meet all of the specifications of definitions to date such as: selective stimulation of bacterial growth may be limited; or research is not yet complete (Hooda et al. 2012). Currently, fructooligosaccharides, galactooligosaccharides, lactulose, and non-digestible carbohydrates are the prebiotics that fulfill these three criteria (Yoo and Kim 2016).

10.3.2 Mechanism of Action

The gut microbes of the GIT are itself an ecosystem. The beneficial microbes are *Bifidobacterium* and *Lactobacillus* etc. and the harmful ones are *Salmonella* species, *Clostridium perfringens, Helicobacter pylori,* etc. Prebiotics are the non-digestible or low-digestible dietary substances that help the host by favoring the beneficial bacteria growth than harmful bacteria growth selectively in the colon. Prebiotics enhance the following: (1) formation of short chain fatty acids (SCFA) and lactic acid as a fermentation product; (2) growth of the probiotic bacteria with an increase in the level of minerals like calcium, magnesium etc. in the colon; and (3) the host immunity (IgA production, cytokine modulation, etc.) (Nagpal et al. 2012). Thus, prebiotics can be used to aid in the probiotic's mechanism of action. So far, the most popular probiotic targets for prebiotic use are lactobacilli and bifidobacteria. This is based largely upon their success in the probiotic area. And the current foodstuffs that can be fortified with prebiotics are the following: dairy products, beverages and health drinks, spreads, infant formulae and weaning foods, cereals, bakery products, confectionery, chocolates, chewing gum, savoury products, soups, sauces and dressings, meat products, dried instant foods, canned foods, food supplements, animal feeds and petfoods (Gibson and Roberfroid 1995).

10.3.3 Therapeutic Use

Prebiotics are antimicrobial, anticarcinogenic, hypolipidemic in nature with few benefits in diabetes mellitus (Gibson and Roberfroid 1995). It also possesses glucose modulatory and antiosteoporotic properties. It is successfully used for curing constipation and inflammatory bowel diseases. It exerts favorable lipid effects by absorption and balance of minerals and also may promote the absorption of minerals in colon. In supplemented infant formulas, it benefits an infant who is not receiving the mother's milk.

10.4 Synbiotics

When a product contains both probiotics and prebiotics it is termed as "synbiotics". This word implies synergy i.e., acting together (Markowiak and Slizewska 2017). Gibson introduced the concept when he postulated about the additional benefits of prebiotics when combined with probiotics. He claimed that the combination might be more active in preventing GIT disorders compared to a probiotic or prebiotic when used alone. Synbiotics therefore, were developed so that the prebiotic component helps the probiotic microorganism grow and overcome possible survival difficulties. The probiotics use the prebiotics as a food source, which allows the probiotics to survive for a longer period of time inside the GIT than would otherwise be possible. And this is based on evidences that show improvement of the probiotic bacteria's survival when it passes the upper intestinal tract. H_2O_2, pH, organic acids, oxygen, moisture and stress are among several factors that have been shown to affect the viability of probiotics especially in dairy products like yogurts (Pandey et al. 2015). Synergism also acts to provide more efficient implantation in the colon of live microbial dietary supplements as well as stimulating the growth of probiotics. A probiotic needs its food and a prebiotic fuel for its perfect survival in the GIT. But it is important that in order to have a beneficial effect on human health, prebiotic compounds should selectively stimulate the growth of probiotics and not stimulate other microorganisms. A combination of *Lactobacillus* or *Bifidobacterium* genus bacteria with FOS (fructooligosaccharides) is the most common example of a synbiotic.

10.4.1 Selection Criteria

The great challenge is to determine the best probiotic and prebiotic combination for each disease setting and each individual. If one should combine probiotics and prebiotics which have demonstrated individual benefits, the combination should show additive effects. A structured approach is warranted to be able to come up with the right combination. First, one should determine the specific properties that a prebiotic will require to be beneficial to the probiotic, then select the prebiotic accordingly. Therefore, an appropriate probiotic and prebiotic should be selected while composing a synbiotic formula; then the prebiotic used should have an enhancing role on the probiotic microorganisms; and the prebiotic should particularly augment the growth of microorganisms in probiotics.

10.4.2 Mechanism of Action

Synbiotics act in two ways: (1) by augmenting the ability of probiotics; and (2) by providing defined benefits for health. When a prebiotic is added to a probiotic, it stimulates the probiotics and results in the regulation of the metabolic activity in the intestine. This is manifested by an intact intestinal biostructure, proliferation of the beneficial microbiota, and the inability of the potential pathogens to grow in the gastrointestinal tract. Synbiotics also cause the lowering of the amount of undesirable metabolites, as well as the inactivation of nitrosamines and cancerogenic substances. Synbiotics also significantly cause levels of short-chain fatty acids, ketones, carbon disulphides, and methyl acetates to drop which is good for one's health. These products act against the decay processes in the intestine and prevent constipation and diarrhoea.

10.4.3 Therapeutic Actions

Synbiotics have many beneficial effects for humans such as acting as antimicrobials in pyogenic inflammation and anticancerous, anti-allergenic, antidiarrhoeal activities. They can also be used for prevention of osteoporosis and reduction of fat in serum and sugar in blood; for improving immunomodulative ability like immune system regulation and brain functions; reduce nosocomial infections after a post-operative procedure; and improve hepatic function in patients suffering from cirrhosis. Scientific researches have already documented these actions. One study explained that when there is translocation of the products of metabolism (eg. lipopolysaccharides or LPSs, ethanol and SFCAs), these products enter the liver and cause the synthesis and storage of hepatic triacylglycerols (IHTG) that worsens steatosis (fatty liver). When the proponents of the study gave a synbiotic containing five probiotics (*Lactobacillus plantarum, Lactobacillus delbrueckii spp. bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum)* plus an inulin as a prebiotic, this resulted to a significant lowering of IHTG (intrahepatic triacylglycerol) within 6 months among adult subjects having non-alcoholic steatohepatisis or NASH. Another study found that giving of a synbiotic product with probiotics (*Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum,* *Lactobacillus bulgaricus*) and fructooligosccharides caused the inhibition of NF-κB (nuclear factor κB) and decreased the production of TNF-α (tumour necrosis factor α) which is a factor that contributes to insulin resistance and inflammatory cell uptake in non-alcoholic fatty liver disease (Wai-Sun Wong et al. 2015; Markowiak and Slizewska 2017).

10.5 Differences Between Probiotics and Prebiotics

The differences between prebiotics and probiotics are summarized in Table 10.3 (Gibson and Roberfroid 1995; Yashoda 2016).

10.6 Health Benefits and Clinical Applications of Probiotics, Prebiotics and Synbiotics

10.6.1 Gastrointestinal System

Probiotics are effective in gastrointestinal diseases and its efficacy depends upon specific strains. Probiotics:

- are recommended for prophylaxis and treatment of acute gastroenteritis and antibiotic associated diarrhea.
- enhance growth and improve clinical outcomes when supplemented with infant formula.

Table 10.3 Differences between prebiotics and probiotics

Categories	Prebiotics	Probiotics
Content	Indigestible but selectively fermentable ingredients	Live microorganisms
Functions	Provide food for probiotics; increase number and improve activity of probiotics	Enhance the health and well-being of their host organisms' digestive tract
Health Benefits	Provide supportive function to probiotics	Reduce the number of pathogenic bacteria in the GIT and improve its function; improve immune system function; prevention of cellular damage from oxidative stress
Sources	Asparagus, Jerusalem artichokes, Bananas, Oatmeal, and Legumes	Yogurt, sauerkraut, Yakult, miso soup, fermented breakfast cereal and snack bars, soft cheeses, kombucha, kimchi, and sourdough
Side Effects	increase in fermentation, leading to increased gas production, bloating or bowel movement	Possibility of sepsis when given to immunocompromised patients

- used for conditions like inflammatory bowel diseases, irritable bowel syndrome, constipation, lactose intolerance, allergies, cancers, hepatic diseases, hyperlipidaemia, *Helicobacter pylori* infection.

The European Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) association recommends the use of established probiotics prophylaxis and treatment of acute gastroenteritis in kids. The specific probiotic strain *Lactobacillus rhamnosus GG* is effective in preventing antibiotic-associated diarrhea. *Sachromyces boulardii, Lactobacillus rhamnosus GG,* and *Lactobacillus reuteri* are probiotics of choice in treating acute gastroenteritis, IBS and antibiotic-associated diarrhea. *Bifidobacterium animalis* prevent nosocomial infections and *Bifidobacterium lactis* treats constipation in children. The efficacy of probiotics is related to ingested amount and the dosage of consumption (Wang et al. 2016).

10.6.1.1 Mechanisms of Action

The probiotics are strain-specific and show effectiveness for integrity of the epithelium and regulate immune components. The gastrointestinal tract i.e., from the oral cavity to the anus, has the largest immune interface while regulating immune responses in complex manner. That is why the epithelium of the gastrointestinal tract is mainly taken into account while studying the mechanism of action of a probiotic. Probiotic bacteria bind to a virus invader and inhibit its binding to the receptor of the host cell. The lactic acid bacteria show antiviral activity by (Tapiovaara, et al. 2016):

1. Interacting directly as an absorptive mechanism or trapping mechanism;
2. Immune system stimulation by natural killer cells, interleukin, immune response activity of Th1 and IgA production;
3. Production of antiviral agents like H_2O_2, lactic acid, and bacteriocins.

10.6.2 Respiratory System

Probiotic consumption reduces the occurrence of respiratory tract infections (RTIs) in kids. *Lactobacillus rhamnosus* is one of commonly used probiotics. The benefits of probiotics in GI disorders are similar with the effects found in upper respiratory infections. However, there is little information regarding colonization in upper respiratory tract epithelium and possible colonization of the related mucosal tissues.

Probiotics' mechanism of action in the upper respiratory tract (URT) is still under scrutiny. However, the mechanism in the URT is linked with the mechanism in the GIT.

Like in the GIT, probiotic bacteria could bind to a pathogen (virus) which results in the inhibition of that pathogen's attachment to the receptor of the host-cell. Lactic acid bacteria's antiviral activity can be by the following mechanisms: (1) direct interaction as an adsorptive or trapping mechanism; (2) stimulation of the immune system by interleukin, natural killer cells, Th1 immune response activity, and IgA production; and (3) production of antiviral agents (e.g., hydrogen peroxide, lactic acid, and bacteriocins) (Tapiovaara et al. 2016).

10.6.3 Cardiovascular System

Risks factors for cardiovascular illnesses are related to high levels of low-density lipoprotein (LDL) cholesterol, increased triglyceride-rich lipoproteins and low levels of high-density lipoprotein (HDL) cholesterol. Genetic makeup, health, body composition and states of pre-existing disease can also add on to the risk. The change in gut microbe related to a disease state is linked with an individual's diet.

Diet modification changes the gut bacterial composition which is vital for either prophylaxis or cure of cardiac diseases. An imbalance in the microenvironment of the gut can cause diseases other than in the GIT. Adding the probiotics have improved disease states specifically, the metabolic syndrome-related cardiovascular diseases like hypertension, hypercholesterolemia, obesity, cardio-arterial disease, diabetes, cardiomyopathy. Probiotics are involved in host immune modulation and affects the growth and function of organs. The cardiovascular disease state occurs through altered gut microbes affecting the metabolic components. Probiotics supplement decreased the incidence of cardiovascular diseases in studies done recently. An obesity study by Sanchet, et al. showed a significantly higher mean weight loss among women given *L.rhamosus* compared to those given placebo; Moroti, et al., have seen lowering of blood glusoce levels by 38% among Type 2 Diabetes mellitus (T2DM) subjects after giving *L. acidophilus, L. rhamnosus and B. bifidum;* Kiessling, et al., stated that HDL cholesterol increased and LDL cholesterol decreased after *L. acidophilus* and *B. longum* were given; and the study by Kawase, et al., resulted in the lowering of the systolic blood pressure by the *L. casei* with *Streptococcus thermophiles* (Antony and Ponce de Leon 2018).

10.6.4 Urinary System

In females, infection by the urinary pathogen is almost always through ascending infection from the rectum and vagina to the urethra and bladder. The Lactobacillus organisms that are normally present in the vagina of healthy women, spread from the rectum and perineum and form a barrier in the vagina to bladder entry by uropathogens. The concept of artificially increasing the amount of lactobacilli through probiotic administration has long been thought of, but only recently been shown to be possible. Not all lactobacilli are effective, and to date clinical efficacy only exists for *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* B-54 and RC-14. These strains are only commercially available in Austria, and therefore for most urologists, while some probiotic organisms may reduce the recurrences of bladder cancer or oxaluria, no probiotics can be recommended widely to prevent UTI at present (Reid and Bruce 2006).

10.6.5 Reproductive System

Reproductive health problems of women are not the same between the developed and developing countries. However, they share a common link in microbial involvement. *Lactobacillus* is the dominant type of bacteria present in the vagina. It is affected by several factors like dietary intake, menstrual status, sexual practices, socioeconomic status and genetics. The problems that are seen among females are problems concerning conception, preterm labor, sexually transmitted infections, malnutrition and environmental challenges. Probiotics (live microorganisms) in adequate amounts can offer better reproductive health benefit. The development of the fetus is related to maternal nutrition. Vaginal *Lactobacilli* produces isomers of alpha-linolenic acid and other neurochemicals like by-products which go to the uterus and placenta through bloodstream from the gut and has a vital role in fetal development. The gut-brain signaling and the effect of micro-organisms on memory, anxiety like brain functions, have been shown to influence fetal development. When Probiotic lactobacilli is given during pregnancy, this can lead to immune modulation and reducing allergic responses. These probiotic interventions though not proven clinically, can help reduce the pregnancy related risk factor.

Lactobacilli influence different membrane structures involved in pregnancy as it controls the adherence junction protein regulation which is vital for epithelial barrier function (Azad et al. 2018). This membrane integrity is needed for successful blastocyst formation, retention of the amnion, chorion and placenta. *Lactobacillus reuteri* released histamine resulted in increased cAMP levels. Evidence show that probiotics stop the downstream MEK/ERK MAPK signaling through protein kinase A, and depressed the TNF production by regulation transcription. TNF is involved in preterm labor induction (Reid et al. 2013).

10.6.6 Immune System

In 2015, the World Allergy Organization (WAO) released probiotics usage guidelines for prevention of allergy. Gut micro biota is an important factor in the increasing occurrence of immunity-related disorders like asthma, inflammation, atopy, musculoskeletal disorders and liver fibrosis. It influences the physiology of host and gives rise to new targets therapeutically. Probiotics suppresses pathogenic bacteria in the gut with the activity of macrophages of peritoneum and spleen. In malnutrition processes, the probiotic administration contributes to restore the thymus histology and stimulates the adaptive immune response.

The probiotics when consumed stimulates the induction signals of bacteria on their cell wall structure. Probiotic bacteria affect the Toll-like receptors present on the gut epithelial cells or immune cells linked with lamina propria. This leads to production of various cytokines and/or chemokines that create a microenvironment in the gut lamina propria, bronchi and mammary glands to produce IgA. At the same time, cytokines stimulated by probiotic bacteria lead to the expression of Treg cells (Foxp3+) that maintain the immune homeostasis in the gut mucosa. Intestinal epithelial cells produce chemo attractant protein 1 of macrophage. This sends signals to all other immune cells activating the immune system. There is an increase in IgA A+ cells of the mammary glands, bronchus and intestine and T cell activation. This then releases IL-10 by activating regulatory T cells. Probiotics strengthen the intestinal barrier by increasing tight junctional proteins, mucin, goblet & paneth cells. Probiotic bacteria administration primes a Th1 profile response, with high dose of IL-10 and IFN-γ that play an important role in the immunomodulation.

10.7 Health Benefits of Probiotics, Prebiotics and Synbiotics in Various Diseases

10.7.1 Diarrhoea

According to WHO, the passage of more than three times of watery or loose stools during a 24 h period can be termed as diarrhea. For the last 20 years, a lot of investigations are done on probiotic microorganisms to validate its efficacy in controlling diarrhea using different experiments and appropriate well-designed clinical studies.

10.7.1.1 Acute Infantile Diarrhea

Acute infantile diarrhea caused by rotaviruses is the most studied gastrointestinal condition and rapid oral rehydration is the primary treatment. Probiotics are useful as adjunct to rehydration therapy. *Saccharomyces boulardii* prevented disease recurrence in individuals having numerous *Clostridium difficile* sequential infection. The enzyme Protease from *Saccharomyces boulardii* blocks the intestinal receptor by reducing the toxin produced by *Clostridium difficile.* It was also found to stimulate specific intestinal antitoxin.

10.7.1.2 Antibiotic Associated Diarrhea (AAD)

The use of antibiotics lead to destruction of the local microflora and this is associated with clinical symptoms such as diarrhea and crampy abdominal pain. This diarrhea consists of defective resistance to pathogens and thus interruption of microbial flora in the gut leading to change in metabolism of carbohydrate, short-chain fatty acids, and bile acids. The probiotics that are effective in antibiotic associated diarrhea include *Lactobacillus rhamnosus GG, Lactobacillus acidophilus, Lactobacillus delbruckii, Lactobacillus fermentum* etc. and *Saccharomyces* boulardii. A meta-analysis done for probiotics and AAD in pediatric subjects suggested that *Lactobacillus rhamnosus* and *Saccharomyces boulardii* at a high dosage of 5–40 billion CFU/day may prevent the onset of ADD, with no serious side effects documented in otherwise healthy children (Johnston et al. 2011)

10.7.1.3 Traveler's Diarrhea

Nearly 20–60% of travelers around the world are affected by this type of diarrhea. It particularly affects people who travel from one country to another. The prevalent causes are mostly bacteria (60–85% of cases) and most responsible bacterial pathogen is *Escherichia coli* followed by *Campylobacter jejuni, Shigella* spp. and *Salmonella* spp. Parasites account for about 10% and viruses for 5% of infections. *Saccharomyces boulardii* is more effective on bacterial diarrhea and *Lactobacillus GG* showed effectiveness against viral and idiopathic diarrhea. *Lactobacilli, Bifidobacteria, Enterococci* and *Streptococci* are used to prevent traveler's diarrhea and thus given prophylactically. There are various mechanisms of amelioration of diarrhea by probiotics: (1) activation of the immune system; (2) competition for receptor of epithelial cells on intestine; and (3) through the secretion of bacteriocins like nisin. A meta-analysis concluded that a lot of probiotics like *Saccharomyces boulardii* and a mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were significantly effective for travele's diarrhea. There were no serious adverse reactions that were reported in the 12 trials (McFarland 2007).

10.7.1.4 Irritable Bowel Syndrome (IBS)

This common chronic gastrointestinal disorder is characterized by recurrent spells of abdominal discomfort, bloating, pain and a changeable bowel habit, without mucosal abnormality and flatulence. At present, no curative treatment is available and treatment is aimed to reduce only its symptoms. Many probiotics strains including Lactobacillus plantarum decreased flatulence and relieve abdominal bloating. Reduction in pain was observed with *Lactobacillus rhamnosus GG*. Different probiotics strains such as *Bacillus infantis, Lactobacillus rhamnosus LC705, Bacillus breve Bb99* and *Escherichia coli nissle1917* were found effective in IBS treatment. Soluble, non-viscous fibers as prebiotic like guar gum may also be useful in IBS.

10.7.1.5 Inflammatory Bowel Disorder (IBD)

IBD is a chronic, relapsing, multi-factorial disorder causing inflammation of the gastro-intestinal tract that causes severe watery and bloody diarrhea accompanied by abdominal pain. IBD affects the large and small intestine, particularly the colon.

IBD includes Ulcerative colitis (UC), Crohn's Disease (CD) and pouchitis. The other precipitating factors include genetic, environmental factors, dysregulation of immune system, type of intestinal microbes and oxidative stress. CD and UC are due to the lack of adaptation of the innate immune system to the environment.

Ulcerative Colitis (UC) UC like IBD mainly affects the large intestine and rectum lining. Chronic UC can cause colon cancer. Use of various probiotic species like *Saccharomyces boulardii, Lactobacillus casei* and *Bifidobacterium bifidum* has shown promising results (Kelesidis and Pothoulakis 2012). *Bifidobacterium bifidum, Bifidobacterium breve* and *Lactobacillus acidophilus* in fermented milk were beneficial to induce mild degree remission in patients.

Crohn's Disease (CD) Crohn's disease affects the intestine usually but it may extend in GIT anywhere starting from mouth to anus. It will ulcerate and inflame the GIT thus making the body unable to digest the food properly and eliminate waste. The causative agents can be *Clostridium difficile, Salmonella species, Campylobacter jejuni,, Mycoplasma* and *Adenovirus*. Examples of effective probiotics that offer a therapeutic effect are *Lactobacillus rhamnosus* and *Saccharomyces boulardii.* This can be due to competitive action with commensal pathogenic flora on the immune system response and by restoration of intestinal mucosa integrity.

Pouchitis After ileal pouch canal anastomosis and colectomy, the ileal pouch is usually inflamed. A probiotic mixture is one remedy for this condition. By inducing mucosal cytokines like IL-4 and IL-10 this influences the intracellular interaction of mucosa and improve the function of intestinal barrier and the modulation of the cytoskeletal and tight junctional protein phosphorylation, producing anti-oxidant enzymes like superoxide dismutase and catalase ameliorating the IBD symptoms.

10.7.2 Lactose Intolerance

Lactose intolerance is most common type of carbohydrate intolerance. This occurs due to lower levels of Beta galactosidase enzyme activity and lack of digestion of lactose. The typical symptoms include abdominal distress like diarrhea, bloating, colic, abdominal pain and flatulence. The therapeutic interventions are: treatment with lactase (tablets); and the treatment with probiotics such as *Streptococcus thermophiles* and *Lactobacillus bulgaricus*. The intake of milk with *Lactobacillus acidophilus* and *Bifidobacterium longum* cause decrease hydrogen production and flatulence and improve lactose intolerance.

10.7.3 Diabetes

Diabetes is popularly referred as a serious lifestyle disease. It is of two types:

(1) in Type 1 diabetes mellitus (T1DM) the pancreas stopped producing or produces very little amount of insulin, the hormone that regulates blood sugar level. In type 2 diabetes mellitus (T2DM) the body is not able to control sugar concentration in the blood. Probiotics benefit in normalising disturbed metabolism (altered gut flora) in diabetic patients. They minimise preprandial glucose and level of insulin depending on the species, probiotic dosage and efficacy. In diabetes, disturbance in microbiota composition, insulin resistance in the host occur through: input of energy rich diet; changed lipid metabolism in liver and adipose tissue, modulation of glucagon-like peptide and gut peptide secretion; activation of the lipopolysaccharide; and alteration in intestinal barrier rigidity through glucagon-like peptide.

In T2DM, there is a reduction of bacteria producing butyrate in the GIT. The alteration in gut microbiota occurs due to dietary intolerance involving excessive fat, refined carbohydrate or fructose which initiates an immune response due to augmented permeability of intestine resulting in bacteria migrating into the general circulation. In the hypothalamus increased insulin resistance leads to increased food intake due to decreased satiety resulting in increased body weight. This leads to inflammation and immune cell infiltration in organs sensitive to insulin precipitating insulin resistance.

In T1DM, altered thickness of microvilli and the space between microvilli facilitates entry of LPS and bacterial fragment through intestinal barrier and it binds toll-like receptors that regulate innate and adaptive immunity. Probiotics like *Lactobacillus* optimisises the gut microbiota by increasing the adhesion of proteins like beta-catenin and E-cadherin by protein kinase. The pathogen *Enterobacteriaceae* species is reduced by the probiotics *Bifidobacterium animalis* present in the mucosa of small intestine and adipose tissue reducing its adherence in the intestine and further shifting to the adipose tissue. Probiotics *Lactobacillus johnsonii* motivate T helper (Th) cell differentiation in mesenteric gland to develop immunity for protection of T1DM (Shah and Swami 2017). Probiotics *Lactobacillus* and *Bifidobacterium* species diminish preprandial blood glucose, insulin and enhance glycosylated haemoglobin (HbA1c) and insulin resistance. *Cucurbita ficifolia* (a type of pumpkin) and yoghurt probiotics alone or together can blood sugar (Campbell 2017).

10.7.4 Obesity

The gut microbes in obese and lean individuals vary. But when obese people lose their weight, the gut microflora tends to be like the microflora present in gut of lean people. High fibre diets have lower fat and energy and are quite beneficial in reducing weight by promoting satiety. In the lean and obese individuals, gut microbes influence the energy balance by affecting efficiency of calorie harvested from the diet, utilization and storage of harvested energy.

10.7.5 Cancer

Cancer is an emerging disease. An American Institute for Cancer Research examined the link across nutrition and cancer incidence in the large study on the global scale and found that approximately 30% of the cancer cases worldwide could be prevented by changing the dietary habits. Probiotics as functional foods are not the magic bullets. They do not target directly the cancer cell but target the different areas and altering the characteristics of the tissues and cells which results in reduction of infection and inflammation commonly associated with certain kinds of cancer.

The probiotic *Lactobacillus bulgaricus* induces antitumor activity by: (1) altering the immune functions associated with immune response; (2) antiproliferative effects via regulation of apoptosis and cell differentiation; and (3) suppressing the Beta-glucuronidase, urease, choloylglycine hydrolase, azedoreductase and nitroreductase enzyme production by unwanted bacteria like entero-pathogens such as *Clostridium perfringens* and *Escherichia coli*. Beta-glucosidase and urease help pro-carcinogens to be converted into potential carcinogens. *Bifidobacteria* probiotics reduces colon cancer influenced by 1,2- dimethylhydrazine when used with FOS inhibited liver and mammary tumors. GOS consumption in humans resulted in reduced activity of nitroreductase which is involved in producing genotoxic metabolites, indicating the potential of prebiotics and probiotics to reduce or prevent carcinogenesis. Dietary administration of *Bacillus longum*, Oligofructose and Inulin stops preneoplastic lesion formation and suppress mammary and colon cancer.

10.7.6 Helicobacter pylori *Infections*

Helicobacter pylori, a small curved to spiral rod-shaped bacterium, is mostly associated with duodenal peptic ulceration and it is the main biological pathogen of chronic gastritis and gastric cancer and other gastric malignancies. Proton pump inhibitors like Omeprazole and Prantoprazole used with antibiotics are most useful therapies to eradicate this bacterium. Probiotics have an antimicrobial effect and competes between *Helicobacter pylori*, inhibition of adherence and production of metabolites. Presently, probiotic supplementation with *Saccharomyces boulardii* resulted in increasing the eradication rate and reducing the infection by *Helicobacter pylori*.

10.7.7 Allergy and Immune Response

Probiotics modulate the innate immunity and pathogen induced inflammation through toll like receptor signaling pathways. Infants born through different routes (Cesarean and spontaneous vaginal delivery) show major differences in cultural microbiota up to 6 months of age. Infants harboring *Bacteroides fragilis* and

Bifidobacterium species had more circulating immunoglobulin (Ig) A-secreting and IgM-secreting cells. Bacteria in breast milk and microbes in amniotic fluid potentially may affect the composition of gut microbiota. Gut microbiota stimulates the TH1, TH3, and T regulatory cells, which can balance the IL-4, IL-5, and IL-13 secreted by TH2 cells in atopic diseases like allergic rhinoconjunctivitis, asthma and atopic eczema. *Lactobacillus GG* in combination with *Bacillus lactis* during pregnancy and breastfeeding reduced the risk of atopic eczema and allergic sensitization in child (Behnsen et al. 2013).

10.7.8 **Clostridium difficile** *Infections (CDI)*

Clostridium difficile is an anaerobic, Gram-positive, spore-forming bacterium which causes gastrointestinal infection. Main symptoms are diarrhea and pseudomembranous colitis which are often fatal. *Saccharomyces boulardii produces* serine protease for degradation of the colonic receptor site for *Clostridium difficile* and reducing *Clostridium difficile* toxin A and B. Vancomycin or metronidazole minimise infection but in combination with *Saccharomyces boulardii* reduce CDI.

10.7.9 Necrotizing Enterocolitis (NEC)

NEC is an important disease in premature babies characterised by severe intestinal inflammation and necrosis and it is inversely related to gestational age and birth weight. Pathogenesis of this disease depends upon the colonisation pattern of the bacteria. It has been revealed that *Enterobacteriaceae*, delta toxin positive methicillin resistant *Staphylococcus aureus* and *Clostridium spp* are the main causative bacteria. A combination of probiotics species like *Bifidusbacterium* spp. and *Lactobacillus acidophilus* are most effective to minimise the NEC.

10.7.10 High Blood Pressure

Consumption of milk supplemented with *Lactobacillus* strains reduces blood pressure due to the Angiotensin converting enzyme inhibitors produced during fermentation.

10.7.11 Cardiovascular Diseases

Probiotics consumption can reduce serum cholesterol levels. Probiotics involve in lipid metabolism are *Lactobacillus* bulgaricus, *Lactobacillus* reuteri, *Bacillus coagulans*. The lowering of cholesterol level by probiotics is due to reduction in hydroxyl-methyl-glutaryl-Coenzyme-A reductase in liver and conversion of cholesterol into bile acid and enzymatic deconjugation of bile acid. In this deconjugation, bile acid enters into the intestine, then excreted in the faeces resulting in decrease in the serum cholesterol level.

10.8 Conclusion

Probiotics, prebiotics and synbiotics help maintain eubiosis in the gut microflora. They play a major role in improving the health status of the host individual by modifying gut pH, antagonizing pathogens through the production of antimicrobial compounds, competing for pathogen binding and receptor sites and stimulating immunomodulatory cells. The probiotic needs food and the prebiotic fuel for its perfect survival in the GIT. Synbiotic i.e., using both prebiotic and probiotic, increases the beneficial effects on the host's cell. The use of probiotics, prebiotics and synbiotics on certain diseased states like diabetes, dyslipidemia, cardiovascular diseases, hypertension and cancer have been the subject of extensive research until now. Probiotics can be an adjuvant treatment to various types of gastrointestinal disorders like diarrhea, *C. difficile* colitis, necrotizing enterocolitis and inflammatory bowel disease. The use of probiotics, prebiotics and synbiotics hold the promise of improving outcomes in treatment of illnesses. However, an increased understanding of their role in these disease states is important for the development of medicine directed mostly to diet modification. As Hippocrates would put it, "Let food be thy medicine and medicine be thy food."

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Chapter 11
Honey as Functional Food and Prospects in Natural Honey Production

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11.1 Introduction

Honey is a superfood that has practically existed for as long as recorded human history. Evidence of bee hives and honey have been found in cave paintings in Spain during the 7000 BC, and in Egyptian tombs as old as 2400 BC (The Honey Association n.d.). Aside from being used as food ingredient and natural sweetener, honey was also valued for its healing properties. Today, after over 2600 years, honey continues to be produced by honeybees in bee farms and consumed by humans as functional food because of its antioxidant, anti-inflammatory, anti-microbial, antitumor, and anti-mutagenic effects.

Honey is defined as "*the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.*" (Codex Alimentarius Commission 1999). Honey is made up of glucose and fructose mainly (~95% of dry weight), as well as sucrose, maltose, turanose, and erlose, and oligosaccharides such as raffinose and melezitosein smaller quantities (Bogdanov et al. 2008). It also contains amino acids, proteins, organic acids and enzymes such as diastase, glucose oxidase and invertase, minerals, vitamins, insoluble matter like honey comb debris and pollen. Traces of bacteria, algae and fungi may also be found.

The use of honey as natural sweetener began to decline after the cultivation of sugar cane in Papua New Guinea in 8000 BC and the production of table sugar around 100 AD in India (Deerr 1950). But throughout human history, honey had been celebrated for its folkloric medicinal effects. In 2016, the annual global honey

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production represented less than 1% of the 2014 total sugar production (Tridge 2019). However, because of the growing preference of consumers for healthier sugar alternatives and functional food, global consumption by the year 2024 is expected to hit 2.8 million tons (Global Industry Analysts, Inc. 2019).

11.2 Classification of Honey and their Distribution

There are 2 major classes of honey based on whether they come from flower nectars (called Blossom or Nectar Honey), or if they chiefly are excretions of plants or plant sucking Hemipterans insect (called Honeydew Honey). The differences in the relative amount of the fructose and glucose is used to determine if the honey is unifloral blossom honey (Bogdanov et al. 2004).The types of minor sugars in blossom honey, however, do not vary since they are products of the enzyme invertase. Between honeydew and blossom honey, greater amounts of oligosaccharides are found in honeydew honey, including melezitose and raffinose that are absent in blossom honey.

There are over 300 types of honey in the world today. Depending on the botanical and geographical origin of the honey, there will be variations in the appearance, taste, and composition of the honey (Bogdanov et al. 2008). Blossom honey types are named based on the plant species honey is derived from. For example, the source of the Manuka honey is the manuka bush (*Leptospermum spp.*) found in Asia and New Zealand, and Heather honey from heather (*Erica spp.* or *Calluna vulgaris*) mainly from Europe and Asia. Clover honey from Europe or Asia contain nectar from *Trifolium spp*. Eucalyptus honey from America is composed of *Eucalyptus* nectar (Bogdanov et al. 2015).

Multifloral blossom honey are commonly labelled as blossom or nectar blend. Honeydew honey types are not as many as blossom honeys, and are named based on where they were taken (e.g. forest honeydew, beach forest honeydew, tree honey, bug honey, or flea honey). Honey may also be named according to the method of its removal from the comb (i.e. extracted honey, pressed honey, drained honey, comb honey, or honey with comb).

11.3 Honey as Functional Food

While the two main purposes of food are to provide nutrition and to give satisfaction to the body, a third purpose of food has emerged after mounting proof coming from scientific researches worldwide. This is functional foods, and it refers to *"foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels"* (Hasler 2002).

Honey stands among these functional foods whose natural production has not been significantly modified since its discovery. Honey is a healthier alternative to table sugar because of its lower glycemic index (Atkinson et al. 2008). Honey also contains prebiotics that encourage the growth of probiotic microorganisms, such as lactic acid bacteria. Prebiotics, probiotics, and the secondary metabolites found in honey are responsible for the medicinal properties of honey (Luchese et al. 2017; Olofsson and Vasquez 2008). The details of the health benefits of honey is explained in the following section,

11.4 Health Benefits of Honey

11.4.1 Antiseptic and Antimicrobial

Honey possesses a broad-spectrum antibacterial activity that varies in potency depending on the honey's plant origin (Molan 2006). The hygroscopic nature of honey dehydrates bacteria. It's acidic environment and high sugar concentration deters microbial growth (Simon et al. 2009). The other antimicrobial components of honey are discussed in the next section. Kynurenic acid, an intermediate in tryptophan metabolism, reported in arboreal honey, was also found to have antimicrobial properties (Beretta et al. 2007).

11.4.2 Anti-Diabetic

Diabetics are advised to consume food with low glycemic index. Albeit having a high total carbohydrate content, the average glycemic index of honey is only 55, compared to 100 for glucose, and 65 for sucrose (table sugar) (Atkinson et al. 2008), making it a healthier natural sweetener. The high levels of fructose, and the presence of oligosaccharides like isomaltulose in honey reduces hyperglycemia and prolongs gastric emptying, thus slowing down intestinal absorption in humans (Vaisman et al. 2006; Erejuwa et al. 2012; Kellet et al. 2008; Kashimura and Nagai 2007).

11.4.3 Antioxidant

The antioxidant activity of honey is largely due to the synergistic effects of phenolic phytochemicals such as flavonoids and phenolic acids, and non-phenolic compounds like ascorbic acid, organic acids and amino acids, and the action of enzymes like catalase and glucose oxidase (Sousa et al. 2016; Gorjanovic et al. 2013). The levels of these bioactive substances vary depending on the geographical and botanical origin of honey.

Two classes of phenolic acids in honey have been studied: (1) benzoic acids, and (2) cinnamic acids. For honey flavonoids, 3 classes have also been found:

(1) flavones, (2) flavanones, and (3) flavonols. These phytochemicals contribute to the flavour, color, and the distinctive taste of honey. Phenolic phytochemicals have also been explored as potential markers of the botanical origin of honey (Silva et al. 2012).

11.4.4 Anti-Inflammatory and Wound Healing

For centuries, the topical use of honey on skin wounds have been practiced because it controls wound infection, debrides the wound, produces a clean wound bed with minimal scarring, and reduces inflammation and pain (Dunford et al. 2000; Molan 2006). To date, however, only two honey brands have been approved for therapeutic use (Medihoney™ and Active Manuka Honey).

Bee pollen enzymes, essentials oils, propolis, and secondary metabolites like flavonoids, polyphenols and terpenes were found to contribute to honey's wound healing properties (Azeredo et al. 2003; Viuda-Martos et al. 2008). The mild acidity of honey, and its low levels of hydrogen peroxide also enhance tissue repair and the antibacterial activity of honey (Lusby et al. 2005). Honey can also stimulate the cytokine production of the monocytes, thus, initiating tissue repair (Molan 2006). Chestnut honey, rhododendron honey, and multifloral blossom honey from Turkey were reported to improve histopathological parameters during wound healing (epithelialisation, angiogenesis, macrophages, and fibroplasias) when compared to the control treatment (Nisbet et al. 2010).

11.4.5 Regulation of Blood Pressure and Cardiovascular Function

Honey was found to improve the lipid profile and lower the C-reactive protein (CRP) levels. Honey consumption also lowered homosycteine and the triacylglycerol levels in subjects with hypertriglyceridemia (Al-Waili 2004). The risk factors for cardiovascular diseases, such as obesity, hypertension, smoking, and chronic periodontal disease, are correlated with elevated levels of CRP (Koenig 2003). Thus, the anti-inflammatory activity of honey also relates to its potential cardiovascular function protection.

11.4.6 Anti-Cancer

In a review by Ahmed and Othman (2013), the anti-cancer activity of cancer was organized into: (1) antioxidant activity; (2) anti-inflammatory activity; (3) p53 regulation; (4) cell cycle arrest; (5) immunomodulatory activity; (6) anti-mutagenic activity; (7) estrogen modulation; (8) cyclooxygenase-2 (COX-2) modulation; and (9) tumor necrosis factor (TNF) modulation. While the complete mechanism of honey's anti-cancer activity is not yet fully elucidated, the phenolic constituents of honey have been reported to be the main actors in its anti-cancer activity (Abubakar et al. 2012). But as with the other health benefits of honey consumption or use, the anti-cancer components in honey will vary with the geographic location and the plant sources of honey.

11.5 Known Bioactive Components of Honey

11.5.1 Probiotics

Honey is not microbe-free as it is considered a good source of probiotics, such as acid-loving lactic acid bacteria and yeast. Probiotics are defined as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" (Sanders 2008). Honey acidification is a by-product of glucose oxidase metabolism. The acidic environment deters the proliferation of many spoilage microbes (Da Silva et al. 2016), naturally preserving the honey.

Lactic acid bacteria (LAB) come from the stomach of honeybees (Olofsson and Vasquez 2008), and from the flower secretions and pollen. Among the LAB symbionts found in fresh honey *Lactobacillus acidophilus, L. apis, L. alvei, L. plantarum, L. insects,* and *L. parabuchneri*, and *Bifidobacterium asteroids* and *B. coryneform*. LAB symbionts contribute to the therapeutic and antibacterial properties in honey.

11.5.2 Prebiotics

A dietary prebiotic is "*a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health*" (Gibson et al. 2010). Examples of prebiotics in honey are non-digestible polysaccharides and oligosaccharides, such as maltulose, maltotriose, turanose, panose, melezitose, raffinose, inulobiose and kestose, that encourage the growth of probiotic microbes (Leite et al. 2000; Mei et al. 2010). The osmotic constitution as well as the composition of honey also protects probiotic bacteria as they pass through the human gastrointestinal tract during digestion (Luchese et al. 2017).

11.5.3 Quercetin

Honeys abundantly contain phenolic compounds which act as natural antioxidants. Quercetin, a flavonol, has been identified as one of the most predictable elements of nectar, honey, pollen, beebread, and propolis, and as a signaling substance responsible for pollen germination stimulation and pollen tube growth (Liao et al. 2017). Studies have determined health benefits of quercetin for bees including increased response for detoxification and immunity genes in honey bees (Mao et al. 2013; Mao et al. 2013). Moreover, polyphenols identified in honeys including quercetin have been reported as "promising pharmaceutical drugs in the treatment of cardiovascular diseases" (Khalil and Sulaiman 2010). Quercetin-detection capability of honey bees, however, still remain a mystery since it is a non-volative compound. Some researchers stated that it may be detectable by gustatory receptors (Kaškonienė et al. 2015).

11.5.4 Hesperitin

Hesperitin is a common bioactive flavonoid which belongs to the chemical class "flavanone", that quickly emerges as an appealing curative agent which covers a broad spectrum of properties including antioxidant, anti-carcinogenic, hypolipidemic, vasoprotective, and other essential therapeutic properties (Chakraborty et al. 2012).

The phenolic profile of honey vary depending on the floral source and geographical location. Honey composition of phenolic compounds were identified as strongly affected by geographical location, while concentrations of phenolic compounds are reliant on the floral source. Studies have shown that geographical location and floral source have a more considerable impact on flavonoid content than on the phenolic acid profile of honey samples. Results have shown that phenolic acid compounds and flavonoids can be utilized as a botanical marker to determine the floral source and geographical origin of honey samples. Consequently, hesperitin was identified as an investigative compound that can be used in determining the floral source of various honey samples, whether it be unifloral or multifloral (Cheung et al. 2019).

11.5.5 Luteolin

Luteolin is one of the most commonly reported flavonoids in honey which has antioxidant, anti-inflammatory, antitumor properties, and has neuroprotective effects against microglia-induced neuronal cell death (Lin et al. 2008; Rahman et al. 2014). Luteolin is listed as a major component of the flavonoid fraction of mānuka honey (Yao et al. 2003). Luteolin was found to be weakly correlated with non-peroxide antibacterial activity of honey and probable marker compounds for mānuka monofloral honey (Chan et al. 2007).

11.5.6 Kaempferol

Kaempferol is a major flavonoid found in several natural products including bee pollen. Kaempferol exhibits numerous pharmacological properties which includes antimicrobial, anti-inflammatory, antioxidant, antitumor, cardioprotective, neuroprotective, and antidiabetic activities (Imran et al. 2019). Different polyphenols have been identified as floral markers in honey. For acacia honey, kaempferol rhamnosides and rhamnosylglucosides have been proposed as markers. Kaempferol was also identified in eucalyptus honey (Istasse et al. 2016).

11.5.7 Galangin

Galangin is another natural flavonoid found in honey, and is known as a marker compound of honey. Galangin has antimicrobial, anti-diabetic, anti-obesity, antitumor, anti-inflammatory, anti-mutagenic, anti-clastogenic, anti-oxidative, radical scavenging, and metabolic enzyme modulating activities (Patel et al. 2012; Ma et al. 2019). Similar with other compounds, galangin was found to have a positive effect in the treatment of cardiovascular diseases and aid in the preservation of other protective antioxidants including vitamin E, vitamin C, and other flavonoids (Khalil and Sulaiman 2010).

11.5.8 Naringenin

Naringenin is considered as one of the most important naturally-occurring flavonoid that is extensively seen in different citrus fruits, bergamot, tomatoes, and other fruits (Salehi et al. 2019). Numerous biological activities such as antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, anti-atherogenic, antiadipogenic and cardioprotective effects have been attributed to naringenin (Alam et al. 2014; Salehi et al. 2019).

Aside from the sources mentioned, naringenin was also found in many types of honey (Afroz et al. 2016). Significant difference in the content of naringenin in relation to origin of honey was identified. High amounts of naringenin were found in linden honey, chestnut honey, and acacia honey, respectively. On the other hand, the minimum content of naringenin was found in mountain honey (Kurtagic et al. 2013).

11.5.9 Isorhamnetin

Isorhamnetin is another flavonol which is present in an individual's daily diet. It has antioxidant, antiviral, anticancer, antimicrobial, and anti inflammatory effects (Settu and Manju 2017). Isorhamnetin was found to be one of the main phenolic compounds found in bee bread, "a fermented mixture of plant pollen, honey, and bee saliva used as food for larvae and for young bees to produce royal jelly" (Sobral et al. 2017). Isorhamnetin was also detected in most honeys including melon, pumpkin, cherry blossom, rhododendron, rosemary lemon, orange, dandelion, maple, and pine tree honey (Petrus et al. 2011). Isorhamnetin was identified as a complementary biomarker for the identification of the floral origin of Argentinean Diplotaxis honeys (Truchado et al. 2010).

11.5.10 Bee Defensin-1

Bee defensin-1 is the only cationic bactericidal compound curretly identified that is naturally present in honey. It was previously isolated from the royal jelly which is the "main source of food for bee queen larvae and was identified in honeybee hemolymph". Bee defensin-1 is released by the worker bees' hypopharyngeal gland into the gathered nectar with carbohydrate-metabolizing enzymes. Presumably, it aids in the protection of the royal jelly, and of honey against microbial spoilage since antimicrobial properties of honey are dependent on the hydrogen peroxide, methylglyoxal and defensin-1 content (Kwakman et al. 2010; Ilyasov et al. 2012).

Bee defensin-1 amount ranges from 0.04 to 5.17 μg/g of honey. Lesser amounts of bee defensin-1 questions the purity of honey. Defensin-1 is found in all tested types of larval jelly and honey, including Manuka honey, although its amount differes significantly (Valachova et al. 2016).

11.6 International Standards for Honey

The Codex Alimentarius Standard for Honey and European Honey Directive have been widely used worldwide as guidelines describing the standard honey quality criteria. The latest revision of the Codex Alimentarius Honey was in 1998(Codex Alimentarius Commission 1999), while the latest revision for the EU Directive for honey was in 2001 (EC 2001). Codex Alimentarius Standard is generally usable for honey trade globally. However, other regional standards like the

European Honey Regulation may also be recognized, especially when there are local quality requirements that cannot be met if the Codex Alimentarius Standard was to be followed. In New Zealand, for instance, the Unique Manuka Factor Honey Association (UMFHA) has a unique trademark UMF®, which set an independent standard to ensure that honey products have been tested for quality and purity before leaving New Zealand (UMFHA 2019). UMFHA's focus is to identify the unique signature compounds of genuine Manuka Honey to protect consumers and the industry.

The draft for the EU honey standard was found to be somehow identical with the Codex standard, except that specifics on contamination, hygiene, and sugar adulteration found in Codex was not included in the EU Directive. Conversely, there are also points contained in the EU standard that are lacking the in the Codex Standard. Examples include the definition of "industrial" or "bakery-honey" and the question of honey pollen. Codex Alimentarius stated that the quality standards set are not compulsory for governments and can be agreed upon. On the other hand, the EU Honey Directive demanded that all commercial retail honeys should abide by their standards.

Table 11.1 summarizes the criteria mentioned in the CL 1998/12-S of the Codex Alimentarius and to the Council Derivative 2001/110/EC of the EU, and the other quality criteria proposed by the International Honey Commission (IHC) (Bogdanov et al. 2015).

11.7 Challenges in Honey Production

The levels of bioactive substances in honey vary depending on its geographical and botanical origin. Thus, proper labelling of honey products must be advocated so that consumers are not misled. The Codex Alimentarius Standard for Honey is generally accepted worldwide for honey trade. In the wake of expected increase in global and local honey trade, however, issues of adulteration and sub-standard honey production must be given importance, and regulations must be in place even in the regional level.

Climate change is an on-going risk to precision apiculture. Comparing the beehive weights in the optimal beekeeping conditions and during drought in the Mediterranean areas in 2017 showed that bee populations, honey reserves, and pollen spectrum was affected greatly (Flores et al. 2019). Another risk to beekeeping is the phenomenon called the Colony Collapse Disorder (CCD), where honey bee stocks dip dramatically due to unknown reasons. Researchers point to parasites, pathogens, pesticides, or immune system disorders as the drivers of CCD (Stokstad 2007).

Table 11.1 Proposed criteria to assess honey quality and standard

Parameter	European Directive 2001/110/EC	Codex Alimentarius CL 1998/12-S	International Honey Commission (Proposed Standard)
Moisture content			
General	≤ 21 g/100 g	≤ 21 g/100 g	
Heather, clover	≤ 23 g/100 g	≤ 23 g/100 g	≤ 21 g/100 g
Industrial or bake-honey	≤ 25 g/100 g	≤ 25 g/100 g	
Apparent reducing sugars content			
Honeys not listed below	≥ 65 g /100 g	≥ 65 g /100 g	
Honeydew honey or blends of honeydew honey and blossom honey	≥ 45 g /100 g	≥ 60 g /100 g	
Xanthorrhoea pr.	≥ 53 g /100 g	≥ 53 g /100 g	
Apparent sucrose content			
Honeys not listed below	≤5 g/100 g	≤ 5 g/100 g	
Robinia, Lavandula, Hedysarum, Trifolium, Citrus, Medicago, Eucalyptus cam., Eucryphia luc. Banksia menz[a] Rosemarinus[b]	≤10 g/100 g	≤ 10 g/100 g	
*Calothamnus san., Eucalyptus scab., Banksia gr., Xanthorrhoeapr., h*oneydew honey and blends of blossom with honeydew honey	–	≤15 g/100 g	
Water-insoluble solids content			
General	≤ 0.1 g/100 g	≤ 0.1 g/100 g	
Pressed honey	≤ 0.5 g/100 g	≤ 0.5 g/100 g	
Mineral content (ash)			
General	≤ 0.6 g/100 g	≤ 0.6 g/100 g	
Honeydew or blends of honeydew and blossom honey or chestnut honey	≤ 1.2 g/100 g	≤ 1.2 g/100 g	
Acidity			
In general	≤ 50 meq/kg	≤ 50 meq/kg	
Baker's honey	≤ 80 meq/kg		
Diastase activity			
After processing and blending (diastase number in Schade scale)			
General	≥ 8	≥ 8	
Honeys with natural low enzyme content	≥ 3	≥ 3	
Hydroxymethylfurfural (HMF) content			
In general, except baker's honey	≤ 40 mg/kg		
After processing and/or blending	≤ 60 mg/kg	≤ 60 mg/kg	
Honeys of declared origin from regions with tropical climate and blends of these honeys	≤ 80 mg/kg		
Sugar content			
Sum of fructose and glucose			
Honeys not listed below		≥ 60 g/100 g	≥ 60 g/100 g

(continued)

Table 11.1 (continued)

Parameter	European Directive 2001/110/EC	Codex Alimentarius CL 1998/12-S	International Honey Commission (Proposed Standard)
Blossom honeys Honeydew honey or blends of honeydew honey and blossom honey		≥ 45 g/100 g	≥ 45 g/100 g
Sucrose			
Honeys not listed below		≤ 5 g/100 g	≤ 5 g/100 g
Alfalfa (*Medicago sativa*), Citrus spp., false Acacia (*Robiniapseudoacacia*), French honeysuckle (*Hedysarum*), Menzies Banksia (*Banksia menziesii*), red gum (*Eucalyptus camaldulensis*), leatherwood (*Eucryphia lucida*), Eucryphia milligani		≤ 10 g/100 g	
Lavender (*Lavandula spp*), borage (*Borago officinalis*)		≤ 15 g/100 g	
Banksia, Citrus, Hedysarum, Medicago, Robinia			≤ 10 g/100 g
Lavandula			≤ 15 g/100 g
Electrical conductivity			
(a) Honey not listed under (b) or (c), and blends of these honeys		≤ 0.8 mS/cm	≤ 0.8 mS/cm
(b) Honeydew and chestnut honey, except the honeys listed below and blends with those		≥ 0.8 mS/cm	≥ 0.8 mS/cm
(c) Strawberry tree (*Arbutus unedo)*, bell heather (*Erica*), Eucalyptus, lime (*Tiliaspp*), Ling heather (*Calluna vulgaris*) Manuka or jelly bush (*Leptospermum*), tea tree (*Melaleuca spp*) (exceptions listed in Codex Alimentarius)			
(d) *Arbutus, Banksia, Erica, Eucalyptus, Eucryphia, Leptospermum, Melalueca, Tilia* (exceptions listed in IHC proposal)			

[a]The European draft refers to honeydew honey and mixtures of honeydew and blossom honey, acacia, *Banksia* and *Citrus* honeys
[b]The IHC proposes also that *Rosemarinus* be included in this list

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Chapter 12
Health Benefits of Milk and Milk Products

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Peculiar Feenna Onyekere, and Chukwuebuka Egbuna**

12.1 Introduction

Besides the supply of basic nutrients for growth and development of the human body, the relevance of nutrition has extended to maintenance of health and combating of diseases (Nicoleta-Maricica 2019). Foods that provide basic nutrition as well as health-promoting benefits are known as functional foods. They contain biologically active compounds or phytochemicals that exert health beneficial effects and help reduce risk of diseases. The introduction of probiotics, prebiotics, or synbiotics into human diet is essential for the intestinal micro flora and the human health. They may be taken in the form of dairy products, raw vegetables and fruit or fermented pickles. A category of functional foods that have proven useful over time by virtue of their health benefits are probiotics (Butnariu and Sarac 2019).

The relevance of functional food has led to the publication of several articles describing studies on the constituents and biological functions of the various type of food, of which milk and milk products hold great uniqueness (Siro et al. 2008).

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Milk is a probiotic compound because it contains viable bacteria that are beneficial for human health (Roberfroid 2000). Milk is white (yellow-white or bluish-white) liquid secreted by the mammary gland of female mammals. Humans use this as food especially for the nourishment of their offspring. Milk is an important source of energy which contains water, milk sugar (lactose), fat, protein, vitamins and minerals (Soliman 2005). Quantitatively, four components are dominant: water, fat, protein and lactose; while minerals, enzymes, vitamins, and dissolved gases constitute the minor constituents (Mourad et al. 2014). The misconception that milk and milk products contain fatty content that causes obesity and heart diseases could be responsible for the less intake of milk. This chapter succinctly reviews the composition, source and health benefits of milk and milk products in clear perspectives.

12.2 Sources, Composition and Preservation of Milk

All female mammalian species act as sources of milk but cows hold the highest commercial value because 85% of milk is obtained from them (Gerosa and Skoet 2012). Other sources include buffalo milk (11%), goats (3.4%), sheep (1.4%) and camels (0.2%) (Fig. 12.1).

12.2.1 Composition of Milk

The composition of milk varies among species to species or dairy to dairy (Table 12.1).

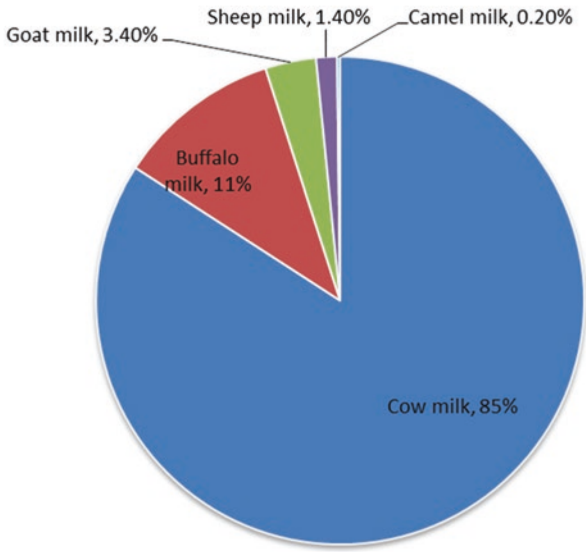

Fig. 12.1 The percentage composition and sources of milk

Table 12.1 Sources and composition of milk

Species	Water	Proteins	Fat	Lactose	Ash
Cow	87.0	3.5	3.9	4.9	0.72
Goat	87.7	3.3	4.1	3.9	0.82
Sheep	82.7	5.5	6.4	4.7	0.92
Camel	86.9	3.4	4.1	3.7	0.71

The composition of milk from different mammals in g/100 g milk (Mourad et al. 2014; Alhadrami 2016).

12.2.2 Milk Preservation

Raw milk contains different type of pathogenic bacteria including*; Listeria monocytogenes, Yersinia enterocolitica, Camphylobacterjejuni and E. coli* (Vasavada 1988). There are two major processes that could lead to contamination of raw milk: preharvest and second one is postharvest process. Several strategies are used to reduce the risk of contamination— enhanced animal health, improved milking hygiene and pasteurization. Pasteurization is a method in which pathogenic bacteria can be killed on fixed time at prearranged temperature.

12.3 Health Benefits of Milk and Milk Products

Cow milk contains high amount of protein, vitamin B, vitamin D, antioxidants, organic compounds and various minerals that affect the body in several ways. According to Karen Giles-Smith, cow milk is a rich source of high-quality protein of which 82% is casein and the remaining 18% consist of whey protein. They are rich source of peptides that helps in lowering blood pressure in hypertensive patients. Whey protein also contains essential amino acid Leucine that provides energy for boosting muscle mass and strengthens during exercise and heavy work (Giles-Smith 2013).

Vitamin B_{12} is an essential vitamin that is necessary for human health because it affects cell division. Its deficiency may lead to anaemia and neuropathy. Matte et al. (2014) described the importance of milk as a source of vitamin B_{12} for human nutrition. They reported three things mainly; ruminant animals have a natural source of vitamin B that is naturally synthesized by ruminant microflora and cobalt is a precursor in this process. Also the concentration of vitamin B12 in milk varies at different levels of heat. The absorption of vitamin B_{12} as a supplement (cyanocobalamin) varies in raw milk, pasteurized milk and micro-filtered milk in the pig's intestine. On analysis, the result shows that absorption efficiency of cyanocobalamin is 0%, raw milk is 9.2%, pasteurized milk is 7.8% and micro-filtered milk is 10.0% (Matte et al. 2014).

Milk contains high amount of calcium (most common mineral) that strengthens bones and teeth. Less intake of calcium source causes rickets in early life (Heaney et al. 2000). Grass-fed cow's milk have a rich source of omega-3 fatty acids that is good cholesterol improves heart health and prevent from different heart problems like heart attacks or strokes (Haug et al. 2007).

It is important to note that cow milk contains low amount of vitamin D. Vitamin D (vitamin D-2, vitamin D-3) is synthesized in the human skin in the presence of sunlight. It acts as a hormone and regulates the calcium and phosphorus concentration in the blood serum. It also helps in muscle development because vitamin D receptors present on muscle. Main source of vitamin D is sun exposure but now a day people do not have enough sun exposure because they live most of the time in shadowed or congested homes, working place also have less sun exposure that's why bones and muscles are weak. Vitamin D deficiency be averted by the use of vitamin D fortified milk (Schmid and Walther 2013).

Khan et al. (2019) reported that milk have antioxidant properties because milk is rich in sulfur-containing amino acids such as carotenoids, cysteine, phosphate, zinc, selenium, vitamin A, E, enzymes, superoxide dismutase, catalase. Milk products such as yoghurt and cheese have oligosaccharides and peptides that also have antioxidant property that can neutralize and act as free radical scavengers. They help protect the human body from harmful effects. These free radicals lead cancer, cardiovascular diseases, and diabetes and increase the ageing process because they cause oxidative stress which disrupts biochemical compounds like DNA, protein and lipids (Khan et al. 2019).

Goat milk is almost similar in nutrient composition as cow milk, but it differs in several characteristics. Goat milk is completely white in colour, as a result of the conversion of the beta-carotene (ingested from feed) to vitamin A. Goat milk has also been shown to have good nutritional value. It contains fat (3.8%), protein (3.5%), lactose (4.1%) unlike in cow which has higher lactose content (4.6%) but less protein (3.3%) and fat (3.6%) contents (Park 2010). Fat globule size is a factor that affects digestion value. Goat milk fat globule diameter is less than cow milk fat globule diameter which implies that its digestion rate is more due to its small size (Gantner et al. 2015).

According to Zenebe et al. (2014), the medicinal and nutritional values of goat milk are quite promising. Goat milk contains medium-chain triglycerides that helps in nutrient absorption and provide energy to the body. Goat milk also have bioactive component like gangliosides, glycolipids, cerebrosides and glycosphingolipids. These bioactive lipids act as antibodies and help detect antigens and bacterial toxins such as cholera toxin and enterotoxins. They also help to maintain cells to cell interaction. Alpha1-casein is a protein that increases the digestion time period of which goat milk has fewer amounts compared to than other sources of milk. Taurine is a free amino acid that is present in goat milk and plays an important role in brain development, growth and bile salt formation. Its deficiency causes epilepsy, retarded growth and cardiomyopathy (Zenebe et al. 2014).

Goat milk induces less allergic reaction in infants than cow milk because it is easily digestible due to its less percentage of lactose (lactose is a major carbohydrate). Several studies report that the use of goat milk resolves 30% and 40% of the cases (Haenlein 2004). Oligosaccharide is also a carbohydrate that is present in goat milk. It is beneficial to human nutrition because of their prebiotic and anti-infective properties. In animal models, goat milk oligosaccharides have been shown to have anti-inflammatory effects in induced colitis (LaraVilloslada et al. 2006).

CLA (Conjugated linoleic acid) is a bioactive lipid present in goat milk. It has anti-allergic and anti-inflammatory properties because it decreases the production of cytokines, prostaglandins and immunoglobulins that are associated with atherosclerosis, cancer and irritable bowel disease (Park 2009).

On the other hand, Sheep milk contains high amount of fat in milk than other dairy mammals (Mourad et al. 2014). Sheep milk has a high level of solids, fats and conjugated linoleic acid that makes it more suitable than other milk for producing high amount of cheese (Sinanoglou 2015).

Camel is a draught animal that has significance due to its nutritional and medicinal benefits. Its milk is called white gold of the desert (Wernery 2006). According to a prospective study conducted by Saltanat et al. (2009) aimed at assessing the influences of camel milk on the immune response of chronic hepatitis B patient, they found out that camel milk corrects the imbalance of Th1/Th2 cytokines network and inhibit the replication process of DNA by strengthening the cellular immune response, thereby improving the recovery chances of chronic hepatitis B patients (Saltanat et al. 2009).

Camel milk contains lactoferrin protein. Lactoferrin is an iron-binding glycoprotein, which plays an important role in host defense system against pathogenic organisms. In Egypt, a clinical study was performed for the role of camel milk in hepatitis C patients. The study shows that camel milk contains constituents that have the ability to decrease the level of alanine aminotransferase and aspartate aminotransferase (Redwan and Tabll 2007). Another study was performed by EL-Fakharany et al. (2012) on anti-infectivity of camel polyclonal antibodies against hepatitis C virus in Huh7.5 hepatoma. They perform three experiments on PBMCs and HuH7.5 cell. They found that camel milk-lactoferrin inhibits the HCV infectivity on direct interaction with HCV but human IgGs and casein failed to inhibit the HCV entry at any tested concentration. The concentration at which camel lactoferrin inhibits HCV replication was recorded to be at 0.25–1.25 mg/ml (EL-Fakharany et al. 2012).

12.4 Milk Products and Their Benefits

For over many centuries, Milk has been an invaluable dietary source of nutrition to humans in the production of both fresh and storable nutritious foods. In some parts of the globe more than a quarter of the milk produced is consumed as fresh pasteurized whole, low-fat, or skim milk. However, most milk is manufactured into more standardized dairy products of global economic importance, such as butter, cheese, dried milks, ice cream, and condensed milk. Milk or dairy products are the kinds of foods that are obtained primarily from or contain milk of mammals such as cattle, goats, sheep, etc. Milk can be used in different forms such as yoghurt, cheese, butter and ice cream.

Yoghurt is a fermented form of milk it contains probiotics that act as an immunomodulator and prevent inflammatory conditions such as inflammatory bowel disease (Lorea Baroja et al. 2007). Yoghurt used for hepatitis and HIV patients (Deems et al. 1993).Yoghurt is beneficial for lactose intolerance patients (He et al. 2008).

Butter is a pale yellow substance has solid texture at room temperature.it is also a milk product contains nutrients that present in milk but the fat value is high in them. It is a rich source of energy due to high-fat content, also prevent from night blindness because it contains vitamin A. great source of short-chain fatty acid (CLA) which reduce the inflammation and cancer risk (Ip et al. 1999).

Cheese is a milk product which contains almost the same nutrients present in milk such as protein, fat and minerals. It is the best source of energy for underweight people to gain healthy weight. Settanni and Moschetti studied the cheese quality improvement technique by using the non-starter lactic acid bacteria (NSLAB) to improve the quality of cheese. This bacterium (NSLAB) has the potency to produce bioactive peptides and improved probiotic quality (Settanni and Moschetti 2010).

12.5 Conclusions

Milk and milk products exert biological and nutritional effects that are beneficial to health. Use of milk and milk products according to our body requirement improves the health and nutritional status. Milk has natural ingredients such as bioactive peptides, casein or whey proteins and other ingredients that confer functional food properties to it. Further research that will harness the intrinsic nutrients in milk via quality processing techniques would help to produce viable milk products that would be beneficial to both healthy and immune-compromised patients.

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Chapter 13
Nutritional and Health Benefits of Seafoods

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Abbreviations

ATS	atherosclerosis
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CVD	cardiovascular disease
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
GLUT4	glucose transporter-4
HDL	high-density lipoprotein
HF	heart failure
Hg	mercury
hs-CRP	high-sensitivity C-reactive protein
LDL	low-density lipoproteins
PC	phosphatidylcholine
PEA	phosphatidylethanolamine

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PL	phospholipids
PS	phosphatidylserine
PUFAs	polyunsaturated fatty acids
ROS	reactive oxygen species
SCD	sudden cardiac death
TG	triglycerides

13.1 Introduction

Metabolic syndrome and the rise in other lifestyle-related illnesses in the elderly have become critical issues for the human health, both in medical terms and financially. Even in high-income countries, more and more patients suffer from obesity, hypertension, diabetes, dyslipidemia and cancer, and the burden of these conditions is growing day by day (Carrion Alvarez and Obregon Perales 2019; Iorga et al. 2019; Gaman et al. 2019a). Metabolic syndrome is associated with several types of cancer, type 2 diabetes mellitus (Cerchietti et al. 2007; Gaman et al. 2019b), nonalcoholic fatty liver disease (Byrne 2010; Epingeac et al. 2019) and cardiovascular disorders (Hwu et al. 2008; Albers et al. 2019). Most probably, the current diabetes and obesity epidemic is due to the increased consumption of dietary sugar and fat (Linseisen et al. 2009; Dobrica et al. 2019; Medina-Gaona et al. 2018). Fast food consumption and soft drinks also have negative effects on the human health. Some of the variables that influence dietary habits in humans include food accessibility, eating preferences, culture, age, and expertise in nutrition and health. A promising novel tactic to tackle unhealthy lifestyle habits is reforming the food milieu (Story et al. 2008; Manea et al. 2019; Vincek et al. 2018).

Annually, fish capture and aquaculture have resulted in 167.2 million tons: 146.3 million tons have been used for human consumption and the remaining 20.6 million tons were destined for other purposes (FAO 2016). There is a remarkable uplift in the demand for seafood products every year mainly due to their nutritional benefits (FAO 1986). The nutritional value of marine foods derives from the considerable amount of proteins, essential micronutrients and lipids contained in seafood. Marine animals, which are considered a supreme source of protein, have the highest content of polyunsaturated fatty acids (PUFAs) and a low caloric density in comparison with terrestrial animals (Tacon and Metian 2013). Seafood is now recognized as an essential human food. In the future, the demand of seafood is expected to increase, taking into consideration its elevated content in high-quality protein, vitamins, trace elements, PUFAs or minerals (FAO 2010).

Seafood possesses of myriad of benefits to the human health. For example, the vital nutrients contained in seafood are important in the development of the brain and nervous system. Apart from this, the nutrients found in seafood are known to have anticancer effects (Liao and Chao 2009). In many developing countries, seafood has helped to alleviate food shortages, offering a valuable complement to a healthy and nutritious diet. The worldwide consumption of seafood has steadily increased in recent years (FAO 2010). Since there is a clear diet-CVD correspondence (Enriquez et al. 2018; Manea et al. 2018), as evidenced both by experimental and epidemiological reports, dietary interventions containing marine food might be of aid in disorders such as atherosclerosis. Moreover, seafood has a great influence on health promotion and maintenance (Mozaffarian and Rimm 2006; Pereira et al. 2004). Seafood is healthy due to its high content of omega-3 PUFAs, mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Dyerberg et al. 1978).

Due to the abundance in PUFAs, i.e. EPA and DHA, fish oil is sold as a functional food to encourage healthier dietary habits. Many other bioactive components are also marketed and are being sold as functional foods due to their value in health, with special reference to the reduced risk of ischemic heart disease and inflammatory disorders (e.g. arthritis) and to the possible effect in cancer prevention, as hypothesized in many research papers (Harris 2004; Rudkowska et al. 2009). Fish and other seafood are also a main source of amino acids, such as taurine and choline, but also calcium, phosphorus, iodine, selenium, vitamin D_3 and vitamin B_{12} (Lund 2013).

The nutritional and health aspects of functional foods reduce the risk of lifestyle-associated diseases (Gheorghe et al. 2019; Tica et al. 2018). The bioactive moieties of seafood are essential in improving unbalanced diets and in preventing lifestyle-associated diseases. Thus, the aim of this chapter is to summarize the available evidence regarding the beneficial effects of micronutrients obtained from seafood (Fig. 13.1).

13.1.1 Major Biochemical Constituents of Seafood

In this section, we detailed the major biochemical components reported in seafoods. Table 13.1 presents summaries of these various components and their effects in human system.

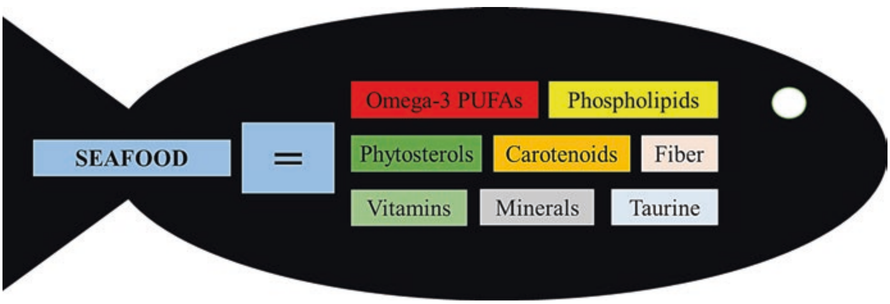

Fig. 13.1 Nutrients available in seafood. *PUFAs* polyunsaturated fatty acids

Table 13.1 Nutrients found in seafood: sources and effects in preclinical and clinical studies

Nutrient	Sources	Effects	References
Omega-3 PUFAs	Phytoplankton Mackerel Salmon Herring	Primary/secondary CVD prevention	Shahidi (2011) and Lavie et al. (2009)
		Stabilization of ATS plaques	Thies et al. (2003)
		↓ Obesity ↓ Inflammation ↑ Insulin sensitivity	Ramel et al. (2010)
		↑ Expression, affinity, membrane fluidity, protein content of GLUT4 in the adipose tissue	Peyron-Caso et al. (2002)
		↓ BMI ↓ Lipid oxidation	Couet et al. (1997)
		↑ Satiety control, improve the cytokine profile	Abete et al. (2010)
		Antiarrhythmic ↓ Inflammation ↓ Risk of congestive HF ↓ Risk of CHD and SCD ↓ Risk of ischemic stroke	Rimm et al. (2018)
		Cardio-protective and antithrombotic ↓ BP and blood lipids ↑ blood vessel function ↑ heart function ↓ inflammation and oxidative stress	Mori (2017)
		Anti-Alzheimer's disease Anti-prostate cancer	Morris et al. (2003)
Phospholipids	Krill oil Squid mantle	PC ↓ blood lipids PC ↑ brain function	Mastellone et al. (2000) and Chung et al. (1995)
		PEA and PS ↓ cholesterol PEA and PS ↑ brain function	McDaniel et al. (2003)
		↑ EPA and DHA in the plasma and cell membrane	Wang et al. (2011) and Maki et al. (2009)
		PL + omega-3 PUFAs ↓ obesity	Shirouchi et al. (2007)
		Antioxidant	Hiratsuka et al. (2008)
		↓ Inflammation	Ikemoto et al. (2001)
		Antineoplastic	Hosokawa et al. (2001)
		PL + omega-3 PUFAs ↓ TG	Hosomi et al. (2010)
Proteins	Fish oil Whole fish Fish sausages Japanese surimi Cod	Omega-3 PUFAs ↓ TG	Balk et al. (2006)

(continued)

Table 13.1 (continued)

Nutrient	Sources	Effects	References
	Sardine Jumbo squid	↓ Serum cholesterol ↑ Cholesterol catabolism ↓ Cholesterol and bile acids absorption	Hosomi et al. (2009)
		↑ Fibrinolysis	Murata et al. (2004)
		Antihypertensive	Boukortt et al. (2004)
		↓ Obesity	Oishi and Dohmoto (2009)
		Cod proteins ↓ hs-CRP	Ouellet et al. (2008)
		Cod proteins ↑ insulin sensitivity	Ouellet et al. (2007)
		↓ Cholesterol	Wergedahl et al. (2004)
		Antimicrobial	Tincu and Taylor (2004)
		↓ Lipid peroxidation	Mendis et al. (2005)
		Immunomodulation	Duarte et al. (2006)
		Valyl-tyrosine ↓ BP	Kawasaki et al. (2000)
Vitamin D	Fish Salmon	↓ risk of hip fractures Bone health	Fan et al. (2013)
Selenium	Tilapia fish Seafood	Seleno-proteins Thyroid gland activity	Holben and Smith (1999)
		↓ Hg toxicity	Ralston and Raymond (2010)
Calcium	Mollusks Crustaceans Salmon Cod bones	Bone metabolism Skeleton rigidity	Ghosh and Joshi (2008), Tacon and Metian (2013) and Malde et al. (2010)
Phosphorus	Mollusks Fish	Bone health Cell membranes Nucleoproteins Nucleic acids Organic phosphates	Martınez-Valverde et al. (2000) and Tacon and Metian (2013)
Taurine	Oysters	Regulates the actions of antioxidants Regulates Ca^{2+} levels Regulates bile acids' conjugation Immunity Membrane stability	Huxtable (1992) and Schuller-Levis and Park (2004)
		Taurine + omega-3 PUFAs ↓ ATS and dyslipidemia	Elvevoll et al. (2008)
		↓ BMI ↓ Atherogenic index ↓ TG	Zhang et al. (2004)
		Anti-hypertensive ↓ Cholesterol ↓ Inflammation	Matsushima et al. (2003) and Schaffer et al. (2010)

(continued)

Table 13.1 (continued)

Nutrient	Sources	Effects	References
Fibers	Edible seaweed	↓ Cholesterol ↓ LDL ↓ TG Interfere with micelle development ↓ Lipids and bile acids' absorption	Jimenez-Escrig and Sanchez-Muniz (2000) and Amano et al. (2005)
		Antioxidant	Heo et al. (2005)
		Antiviral	Artan et al. (2008)
		↓ Inflammation	Kim et al. (2009)
		Anticoagulant	Matsubara et al. (2000)
Phytosterols	Edible microalgae	↓ LDL-cholesterol ↓ Cholesterol	Kanazawa (2001), Malinowski and Gehret (2010) and Plaza et al. (2009)
		↓ Cholesterol uptake (gut) Bind to micelles (small intestine) ↑ luminal cholesterol excretion	Marangoni and Poli (2010)
		Antioxidant	Mannarino et al. (2009)
		↓ Inflammation	Houweling et al. (2009)
		Anticancer	Bouic (2001)
Carotenoids	Lobster Salmon Crustaceans *Hijiki afusiformis* *Laminaria japonica* *Sargassum fulvelum* *Undaria pinnatif*	Convert light into chemical energy Antioxidant ↓ ROS-induced damage	Lesser (2006) and Maeda et al. (2007)
		↓ Inflammation ↓ Dyslipidemia ↓ Oxidative stress	Cicero et al. (2007) and Woo et al. (2010)
		↓ TG ↑ HDL-cholesterol	Yoshida et al. (2010)
		↓ Fat tissue ↓ Abdominal fat ↓ Inflammation ↓ Risk of cancer and stroke	Maeda et al. (2008)

13.1.2 *Omega-3 Fatty Acids*

The importance of seafood in the human diet is due to the presence of naturally occurring fatty acids such as omega-3 PUFAs (EPA and DHA), which possess multiple beneficial health effects. The highest ratio of fatty acids, such as EPA and DHA, in the food chain is found in the marine phytoplankton. The overall content in EPA and DHA of fish is influenced by several factors, e.g. species or environment. According to Shahidi (2011), fatty fish such as mackerel, salmon or herring, have a higher concentration of omega-3 PUFAs as compared to halibut haddock or cod that are categorized in lean fish category. Shellfish such as crab, lobster or shrimp, contain low levels of omega-3 PUFAs. The most well-known metabolites of EPA are thromboxanes, 5-series leukotrienes, 3-series prostaglandins and prostacyclin (Calder 1998). Pro-thrombotic and pro-inflammatory eicosanoids resulted from arachidonic acid are not as active as EPA-derived eicosanoids. In humans, omega-3 and omega-6 levels are dependent on the dietary consumption (Lands et al. 1992). In cardiovascular health, PUFAs supplementation therapy remains a highly effective method in the prevention of cardiovascular disorders (Lavie et al. 2009).

The American Heart Association suggests that patients with ischemic heart disease should include approximately 1 g/day of EPA and DHA in their diet. This dietary intervention is helpful in the general prevention of CVD (Kris-Etherton et al. 2002). Similarly, AHA recommends that healthy people should consume fatty fish at least 2 times/week or have a dietary intake of about 500 mg EPA and DHA/day. In addition, ≥3–20 EPA and DHA g/day has effects on serum triglycerides (TG), blood vessel flexibility, endothelial function, platelet aggregation, blood pressure and inflammation (Kris-Etherton et al. 2003). In their study published in *The Lancet*, Thies et al. have demonstrated that the consumption of (n-3) PUFAs enhances the stability of atherosclerotic plaques affecting the carotid arteries (Thies et al. 2003). Moreover, Ramel et al. have shown that omega-3 PUFAs present anti-obesity effects, reduce inflammation and insulin sensitivity (Ramel et al. 2010). In obese and insulin resistant murine models, the incorporation of dietary omega-3 PUFAs into the phospholipids of the cell membrane stimulated the expression, affinity, membrane fluidity, protein content of glucose transporter-4 in the fat tissue (Peyron-Caso et al. 2002) as well as the expression of some insulin receptors (Das 1999), sensitizing tissues in the body to the action of insulin. Furthermore, in obese subjects, omega-3 PUFAs can reduce the oxidation of lipids and the body mass (Couet et al. 1997). The potential health benefits of PUFAs have also been studied in rheumatologic disorders (rheumatoid arthritis or lupus), immunoglobulin A nephropathy or gastrointestinal disorders (ulcerative colitis, Crohn's disease) (Volker et al. 2000; Walton et al. 1991; Belluzzi et al. 1996; Das 2005; Stenson et al. 1992; Donadio et al. 1994). Omega-3 PUFAs improve the control of satiety and the cytokine profile (Abete et al. 2010). Moreover, omega-3 PUFAs have antiarrhythmic effects, counteract oxidative stress and reduce the risk of heart failure and sudden cardiac death (Rimm et al. 2018; Mori 2017).

13.1.3 Phospholipids

Approximately 10% of the fat from seafood comes in the form of TG and phospholipids (PLs). Several animal studies have concluded that dietary PLs could be beneficial to the human health. Phosphatidylcholine, the major component of phospholipids (PLs), reduces the total lipids in the blood (Mastellone et al. 2000) and enhances the activity of the brain (Chung et al. 1995). Phosphatidylethanolamine and phosphatidylserine are also able to reduce lipid levels and improve the function of the brain (McDaniel et al. 2003). The abundance of effective and potential seafood derived PLs like EPA and DHA has also been included in foods as a valuable ingredient.

The administration of omega-3 PUFAs seems more effective if omega-3 PUFAs were given as PL- rather TG-formulations (Wijendran et al. 2002). The beneficial effects of dietary krill oil supplementation have also been examined in several clinical studies. Supplementation with krill oil has proven beneficial effects on the human health, mainly by increasing the EPA and DHA levels in the plasma and the cell membrane (Wang et al. 2011; Maki et al. 2009). In addition, obesity-related metabolic disorders are ameliorated under the administration of PL-containing omega-3 PUFAs (Shirouchi et al. 2007). Moreover, in several studies conducted on animals, these agents were explored for their antioxidant (Hiratsuka et al. 2008), anti-inflammatory (Ikemoto et al. 2001) and antineoplastic properties (Hosokawa et al. 2001). Previous studies have reported a decrease in serum and liver TG following the addition of PL-containing omega-3 PUFAs derived from squid mantle muscle or from soybeans to the diet (Hosomi et al. 2010).

13.1.4 Protein, Peptide, and Non-protein Nitrogen Compounds

The human diet usually includes fish oil and whole fish. Dietary omega-3 PUFAs reduce serum TG even if serum cholesterol is not reduced (Balk et al. 2006). The main micronutrients found in fish are fish proteins. Around the globe, fish proteins are essential elements in human nutrition and have been included as main ingredients in processed fish products such as fish sausages or the Japanese surimi (FAO 2010). The impressive levels of amino acids in seafood proteins can make up between 10% and 25% of their content. Seafood proteins can be classified as sarcoplasmic, myofibrillar or stromal proteins. In terms of amino acid composition and bioavailability, animal proteins are more acceptable *versus* vegetable proteins.

The potential impact of fish proteins on the lipid metabolism is another dimension of the role of fish proteins in health. In this context, researchers have shown that the administration of fish proteins in laboratory animals influenced serum cholesterol levels (Wergedahl et al. 2009). The reduction in serum cholesterol was due to the properties of fish protein to inhibit the absorption of cholesterol and bile acids. Thereby, the catabolism of cholesterol in the liver increased (Hosomi et al. 2009). Additionally, other beneficial effects of these substances include the enhancement of fibrinolysis (Murata et al. 2004), as well as their antihypertensive (Boukortt et al. 2004) and anti-obesity properties (Oishi and Dohmoto 2009). Furthermore, animal and human studies have concluded that dietary cod proteins reduce the levels of high-sensitivity C-reactive protein in the serum (Ouellet et al. 2008) and increase insulin sensitivity in subjects resistant to insulin (Ouellet et al. 2007).

Biopeptides extracted from seafood proteins have an immense influence on the promotion of healthy eating. In a placebo-controlled trial, the administration of valyl-tyrosine, a peptide extracted from sardines, has resulted in a decrease of 9.3 mmHg in the systolic blood pressure and a decrease of 5.2 mmHg in the diastolic blood pressure (Kawasaki et al. 2000). Moreover, marine bioactive peptides isolated from the jumbo squid were seen to inhibit lipid peroxidation. Their activity was similar to butylated hydroxytoluene (BHT), but much lower in comparison with α-tocopherol (Mendis et al. 2005). According to previously stated work by several authors, marine bioactive peptides have ameliorated hypocholesterolemia (Wergedahl et al. 2004) and have displayed immunomodulatory (Duarte et al. 2006) and antimicrobial effects (Tincu and Taylor 2004), both in animals and in vitro. However, the health benefits of marine proteins need further investigation in studies conducted on humans.

13.1.5 Vitamin D

In addition to its valuable protein and lipid composition, seafood also comprises considerable amounts of vitamin D (Holick 2008). Rickets, low bone density, osteomalacia and osteoporosis are health conditions associated with vitamin D deficiency (Cranney et al. 2007). Fan and his coworkers (2013) reported an inverse relation among fish consumption and chances of hip fracture in the Chinese elderly. Alongside to bone-related disorders, vitamin D deficiency seems to be also involved in the development of diabetes, autoimmune disorders, aggressive cancers, as well as CVD (Holick 2008). Sunlight exposure is a key element in vitamin D homeostasis. Limited sun exposure has been linked to vitamin D deficiency (Norman 2008). Overall, the average dietary intake of vitamin D is of at least 1000 international units (IU) or equivalent to 25 mg per day (Lu et al. 2007; Holick 2008). Vitamin D_2 (ergocalciferol) is found in plants such as mushrooms, whereas vitamin D_3 (cholecalciferol) is found in fish and is also produced in the skin *via* exposure to ultraviolet B rays (Holick 2008; Norman 2008). Ergocalciferol slightly differs in structure from cholecalciferol, as it contains one additional double bond along with methyl group. In various species of fish muscle, the vitamin D content ranges from 5 to 30 mg/100 g (Mattila et al. 1995). It was observed that wild salmon has a higher content of vitamin D as compared to farmed salmon. Moreover, the methods of preparation also affect the final quantity of vitamin D. After frying, 50% of the vitamin D is retained in the salmon (Lu et al. 2007).

13.1.6 Minerals

Selenium is an essential mineral in humans and animals. However, in high levels, it is toxic. In humans, selenium is responsible for the synthesis of seleno-proteins, as well as in the normal activity of the thyroid where it acts as a cofactor in the metabolism of thyroid hormones (Holben and Smith 1999). Selenium deficiency is associated with cardiovascular events (e.g. myocardial infarction) and a higher rate of CVD-related deaths. Likewise, selenium deficiency is also linked with a higher risk of kidney disorders and cancer (Holben and Smith 1999). Selenium also partakes in the process of reducing methylmercury toxicity (Ralston and Raymond 2010). According to the United States Department of Agriculture (USDA), seafood is an excellent source of selenium (Ralston 2008). In comparison with yeast-derived selenium, selenium obtained from fish has a higher bioavailability (Fox et al. 2004). During the analysis of seafood obtained from the South Atlantic Ocean, a beneficial selenium-to-mercury ratio (> critical level of 1:1) was observed and held responsible for the protection against methylmercury toxicity (Kehrig et al. 2013). Moreover, the selenium content of seafood can be increased by supplementing the diet of fish with selenium, as seen in studies conducted on tilapia (Molnar et al. 2012). For seafood safety, the selenium health benefit value (Se-HBV) was proposed. It takes into consideration the exposure to methylmercury and the selenium intake (Kaneko and Ralston 2007). Recently, the Se-HBV has been updated to consider its availability from fish. In order to differentiate between the two, Se-HBV is now abbreviated as HBVSe (Ralston et al. 2016). Mercury-induced toxicity seems to be related, in avid seafood consumers, to an increase in oxidative stress levels (Karimi et al. 2016).

Calcium is an essential macro-mineral in human nutrition for its role in bone metabolism. Calcium ions play a significant role in maintaining the rigidity of the skeleton. In the human body, 99% of calcium is stored in of bones (Ghosh and Joshi 2008). The average intake of calcium differs based on age groups. However, in adults, the WHO/FAO recommends a daily intake of 400–500 mg/day. In comparison with other nutrients, the absorbance of calcium in body is inadequate. Generally, the bioavailability of the total dietary calcium is estimated at 25–30% (FAO Agriculture and Consumer Protection Department 2002). Besides milk and dairy products, fish and other marine animals also contain calcium (Martınez-Valverde et al. 2000). In comparison with terrestrial meat which contains 14 mg calcium/100 g, mollusks, crustaceans and fish contain 26–68 mg calcium/100 g (Tacon and Metian 2013). Moreover, calcium from salmon and cod bones has a better absorption (Malde et al. 2010).

Phosphorus is also a key factor in bone health and the composition of cell membranes. Moreover, phosphorous is also an important component of nucleoproteins, nucleic acids, as well as organic phosphates i.e. adenosine triphosphate and creatine phosphate. Martınez-Valverde et al. (2000) reported that 700 g of phosphorous are found in the body, of which 80% is found in bones, 10% in viscera and 9% in the skeletal muscles (Ghosh and Joshi 2008). Phosphorus deficiency is related to a myriad of health disorders: metabolic acidosis, muscle disorders, variation in bone mineralization, and respiratory, cardiac or neurological disorders (Ghosh and Joshi 2008). In various studies, seafood are proposed as excellent sources of phosphorus, since 204–230 mg phosphorus/100 g are found in mollusks and fish, in comparison with terrestrial meat which only contains 176 mg phosphorus/100 g (Tacon and Metian 2013).

13.1.7 Taurine

Seafood contains taurine (2-aminoethanesulfonic acid). Taurine is abundantly found in the blood, the developing brain, the retina and the heart (Wójcik et al. 2010). Guinea-pigs and rats' diets are highly dependent on taurine. As compared to humans, the synthesis of taurine is higher in animals (e.g. Guinea-pigs and rats). Taurine (a nonessential amino acid) has a significant impact in many biological processes which regulate the actions of antioxidants, the calcium level, and the conjugation of bile acids, immunity and membrane stability (Huxtable 1992; Schuller-Levis and Park 2004). Seafood contains high amounts of taurine and a high percentage of the taurine in the human diet comes from seafood (Tsuji and Yano 1984). Marine invertebrates such as oysters contain higher amounts of taurine (>1/100 g) in comparison with plants, in which the taurine content is lower or even absent (Kataoka and Onishi 1986). Taurine decreases the risk of lifestyle-related diseases and acts as anti-hypertensive, anti-hypercholesterolemic and anti-inflammatory agent (Matsushima et al. 2003; Schaffer et al. 2010). Studies have reported that the association between taurine and omega-3 PUFAs exerts more pronounced anti-atherogenic and hypolipidemic effects in humans as compared to omega-3 PUFAs supplements alone (Elvevoll et al. 2008). Zhang et al. concluded that a 7-week taurine supplementation in a dosage of 3 g/day significantly reduced the body weight, atherogenic index and serum TG of non-diabetic obese human subjects. According to their research, an adequate amount of taurine might be helpful to decrease the risk of lifestyle-associated disorders such as stroke or atherosclerosis (Zhang et al. 2004).

13.1.8 Fiber

Carbohydrates and fibers are present in a very little amount in seafood. Edible seaweed comprises of 25–75% of dry weight of dietary fiber and also contains approximately 50–80% of water soluble fiber constituents (Jimenez-Escrig and Sanchez-Muniz 2000). Seaweeds are divided into three groups based on pigmentation: brown seaweeds, green seaweeds and red seaweeds. Brown seaweeds owe their color to the pigment known as fucoxanthin and consist of primary polysaccharides (alginates, cellulose, fucans and laminarins) (Goni et al. 2002). In green seaweed, chlorophyll and ulvan are the major polysaccharide components responsible for its color (Robic et al. 2009). Red seaweeds owe their color to phycoerythrin and phycocyanin (McHugh 2003). In terms of health, the consumption of polysaccharides extracted from edible seaweeds has been linked to a significant reduction in total cholesterol, low-density lipoproteins and plasma TG (Amano et al. 2005). Polysaccharides extracted from seaweeds showed hypo-cholesterolemic effects due to their increased interference with micelle development and absorption of lipids and biliary acids. Moreover, sulfated polysaccharides (carrageenans and fucoidan) have several important biological functions: antioxidant (Heo et al. 2005), antiviral (Artan et al. 2008), anti-inflammatory (Kim et al. 2009) and anticoagulant (Matsubara et al. 2000). Thus, their potential has been employed in cosmetics, functional foods and pharmaceuticals (d'Ayala et al. 2008).

13.1.9 Phytosterols

The structure of phytosterols and cholesterol is similar. The only difference between them is the relative positioning of the methyl and ethyl groups. In plants, phytosterols exist in the form of beta-sitosterols, campesterol and stigmasterol, while in marine invertebrates these phytosterols exist in the form of stanols, sterols and sterol esters (Kanazawa 2001). Phytosterols play an important role in the production of healthy food products like milk, fat free or low-fat yogurt, spreads, juice, bread and cereals (Demonty et al. 2009). In clinical trials, an intake of 2–3 g/day of phytosterols has significantly reduced LDL-cholesterol (Malinowski and Gehret 2010). Moreover, edible microalgae containing phytosterols have proven hypocholesterolemic effects (Plaza et al. 2009). Phytosterols reduce the uptake of cholesterol in the gut since they bind to micelles in the small intestine. Also, phytosterols enhance the action of the ATP-binding cassette G-proteins in the enterocytes *via* excretion of cholesterol in the lumen (Marangoni and Poli 2010). Other biochemical actions of phytosterols include their antioxidant (Mannarino et al. 2009), anti-inflammatory (Houweling et al. 2009) and anticancer activities (Bouic 2001). Musa-Veloso et al. (2011) have hypothesized the cardio-protective effects of phytosterols against coronary heart disease and have reported that an intake of ≥2 g/day of phytosterols significantly lowers LDL-cholesterol.

13.1.10 Carotenoids

Carotenoids (fat-soluble) contain orange and yellow pigments and have the ability to convert light into chemical energy. Carotenoids comprise antioxidants that prevent reactive oxygen species-induced damage in fungi, plankton and other photosynthetic organisms (Lesser 2006). Carotenoids (e.g. β-carotene) play important roles in many biological processes, such as the formation of vitamin A in the body (García-González et al. 2005). Other examples of carotenoids include lycopene, fucoxanthin and astaxanthin (Gaman et al. 2019c). Seafood carotenoids (e.g. astaxanthin, fucoxanthin) have many commercial applications. The xanthophyll carotenoid astaxanthin can be found in lobsters, salmon and marine crustaceans and presents anti-inflammatory, anti-hyperlipidemic and anti-oxidative stress actions (Cicero et al. 2007). Oxidative stress has emerged as a putative mechanism in the development of many health issues, including myeloid and lymphoid malignancies (Moisa et al. 2018a, b, 2019; Gaman et al. 2014, 2019d; Pascu et al. 2019). Yoshida et al. (2010) reported a significant reduction in TG and an increase in high-density lipoprotein (HDL) cholesterol in non-obese individuals after the intake of astaxanthin for 12 weeks (Yoshida et al. 2010). However, there is limited data on the effect of astaxanthin on the human health. Edible seaweeds contain the orange-colored pigment fucoxanthin (Maeda et al. 2007). Fucoxanthin decreases abdominal fat and inflammation. Also, it can be useful to reduce the risk of malignancy and stroke (Maeda et al. 2008). Furthermore, HDL-cholesterol in the plasma of mice can increase following fucoxanthin consumption (Woo et al. 2010).

13.2 Factors Affecting the Nutritional Status of Seafood

Several factors such as the production methods and the processing conditions usually influences the composition and quality of seafood. The diet of marine creatures can also influence the nutritional status of seafood (Lie 2001; Morris 2001). There are some other factors such as the ash content, the mineral composition, the protein content and other micronutrients which can alter the quality of fish (Baker 2001). Feeding, handling, transportation, storage and slaughtering methods can also interfere with the quality of fish and fish products (Robb 2001). The lipid content and configuration is affected by the aforementioned steps. The way of processing also alters the fatty acid composition of seafood *via* possible oxidation (Sampels 2015a, b).

13.3 Risk Associated with Fish Consumption

The reported increase in fish consumption might be due to their beneficial effects on health, such as their role in CVD prevention (FAO 2010). On the other hand, fish consumption might pose health risks as well. Edible marine products contain methylmercury which is metabolized by microorganisms and causes damage to the central nervous system. This effect can also occur in the fetus and in infants. It has been reported that the severity of central nervous system damage depends on the quantity of methylmercury consumed (Clarkson et al. 2003). The Food and Drug Administration (FDA) has already published several dietary guidelines addressing the type and quantity of fish which can be consumed by pregnant women and children. Additionally, some other environmental toxins found in seafood, such as dioxins and polychlorinated biphenyls, have negative effects on the human health (Arisawa et al. 2005). The health benefits of fish and fish products are only present if consumed in appropriate amounts (He 2009). The reported benefits of seafood consumption outweigh their risks, with the exception of some sea animals (sharks, swordfish) and edible plants which contain severe environmental toxins (Dewailly et al. 2007). However, the benefits and risks of seafood and other natural products require further preclinical investigation (Islam et al. 2018; Luis Gomes et al. 2018). Eventually, the evidence should be incorporated into more clinical studies and randomized controlled trials to test these findings.

13.4 Conclusion

The nutritional content of seafood (proteins, fats, minerals, vitamins etc.) has helped us recognize the value of these products in our diet. Unfortunately, the health benefits of seafood have been acknowledged only after many years. Taking into consideration the epidemic of lifestyle-related disorders worldwide, dietary interventions based on seafood might be of aid in the near future.

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Chapter 14
Fruits and Vegetables and its Nutritional Benefits

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14.1 Introduction

An increase in the consumption of fruits and vegetables has been observed in recent years due to increase in knowledge of its beneficial properties. A high amount of plant-based food consumption, at least 400 g of fruits and vegetables is recommended in dietary guidelines (Agudo 2005). Epidemiological surveys indicated a positive correlation towards a diet rich in fruits and vegetables and minimized the occurrence of degenerative diseases including certain types of cancer, cardiovascular diseases, macular degeneration, aging and others (Michels et al. 2000; Trichopoulou et al. 2003; Willcox et al. 2003; Dauchet et al. 2006; Ordovas et al. 2007; Liu 2013).

Fruits and vegetables contain a wide range of micronutrients and non-nutrient bioactive compounds such as dietary fiber, minerals (potassium, calcium, and magnesium) vitamins (A, C and E), phytochemicals (poly phenolic compounds and carotenoids, glucosinolates, organosulphur compounds, sesquiterpene lactones). Approximately there are more than 5000 individual phytochemicals have been noticed in fruits, vegetables but a large proportion of them remains unknown (Liu 2013). Bioactive compounds are the secondary metabolites of the plants, which have pharmacological and poisonous manifestations in man and animals (Bernhoft 2010). They generate important functions in the living cell such as defense against

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free radicals and prevention of disease as a result of oxidative stress and act as antioxidants (Bernhoft 2010; Kaur and Kapoor 2001). The bioactive compound biosynthesis is encouraged by light; hence they assemble in the skin and leaves of the fruits and vegetables (Bernhoft 2010). The levels of the bioactive compounds in fruits and vegetables differs based on genetic factors, environmental conditions such as light, maturity and postharvest treatments (Deepa et al. 2007; Vallejo et al. 2003). The Vitamins, minerals and bioactive compounds of fruits and vegetables are in the liquid form which makes them more bioavailable in the human diet (Yildiz and Gültekin 2006).

The advantages from the intake of fruits and vegetables, seems to be related to additive interactions between the phytochemicals that could affect different pathways such as changes in cholesterol and hormone metabolism, enhances immune response, modulation of steroid hormone concentration and detoxifying enzymes, reduction of blood pressure, stimulates antibacterial, antiviral and antioxidant activity, lowers inflammatory processes, anti-carcinogenic properties and delay of cardiovascular diseases (Yu and Ahmedna 2013). It is a fact that fruits and vegetables can be processed for economical and logistical reasons to improve their commercial shelf-life and digestibility fallowing the consumer habits of each country or to facilitate the consumption by special groups (children, pregnant women, older adults and patients with certain pathologies).

Foods that convey a specific, documented health promoting and disease preventing property beyond the basic function of supplying nutrients are termed 'functional foods.' Functional foods are an emerging science area. Currently, the functional food industry is encountering rapid growth due to the innovation of new products, and associated marketing, based upon the bioactivity of polyphenolic compounds. These products report the wellness trend and the needs of health-conscious consumers across infinite health areas (Gibson et al. 2000; Kathleen 1999). Another aspect of the success of functional foods is that they taste great and be convenient. Fruits have more health halo than vegetables and with their intrinsic sweetness, generally good taste, texture and convenience in portability; they are much more attractive to consumers. Our aim is the provision of appropriate information to plant breeding teams, to select for scientifically proven health-promoting fruits and vegetables as whole fresh functional foods.

Fruits and vegetables producing crops are classified based on their climatic adaptability (Table 14.1). They are tropical, subtropical, temperate and arctic fruits and vegetables. Tropical crops survive in the region between tropic of Cancer (23° 27"N latitude) and tropic of Capricorn (23° 27"S latitude), need a moist warm climate, resist dry weather and are evergreen. Sub-tropical fruits grow between temperate and tropical climatic conditions (either evergreen or deciduous). They are adapted to low temperatures. Temperate crops are those which survive in extreme cold. They are deciduous and become dormant in winter. The Arctic is a cold temperature region, a few crops thrive in such extreme frost conditions.

Table 14.1 Classification of Fruits and Vegetables based on climate

Type of Fruit	Fruits Examples	Vegetables Examples
Tropical	Mangosteen, jackfruit, Olive rambutan, **Syzygium**, Mango, banana, plantain, papaya, guava, pineapple passion fruit etc. (Ayala-Zavala et al. 2011)	Arugula, Asian Greens, Beans, Bell Peppers, Cabbage, Capsicum (that's the Australian name for peppers), Cassava, Chard (silverbeet, similarto spinach), Chinese Cabbages, Chilli Peppers, Cucumbers, Eggplant, Endive, Lettuce, Okra, Peppers, Pumpkins, Radish, Rocket Squash, Sweet Corn etc. (Wani et al. 2017; Litz et al. 1986; Tindall 1983)
Subtropical	Grapes, pomegranate, Pineapples, Papaya, Banana, Avocado, Litchie, Kiwi Mangoes, Guavas, Citrus fruit (sweet orange, mandarin, tangerine) (Galan Sauco et al. 2014; Litz et al. 1986)	Cherry tomato, Pumpkin, lablab bean, Choko, Ethiopian or Kenyan Cabbage, Sweet potato, Luffa (Luffa cylindrica) etc. (Wani et al. 2017; Litz et al. 1986)
Temperate	peach, pear, strawberry, craneberry, blackberry, blueberry, Apple, pear, stone fruits etc. (Kole 2011)	Endive, Escarole, Mustard, Black mustard, Kale, collards, Cabbage, Savoy cabbage, Garden cress, Watercress, Chard, Swiss chard, Spinach, lettuce, Sorrel, Asparagus, lettuce, Celery, Parsley, Rubharb etc. (Martin 1982)
Arctic	Almond and trifoliate orange, arcitic rasp berries etc. (Molau 1997)	Herbs, Braccoli (Ganong 1977)

14.2 Functional Properties of Fruits and Vegetables

Fruits and Vegetables are composed of several macro and micronutrients. Macronutrients are required in larger amounts and are mainly carbohydrates, proteins, and fats. Micronutrients are only needed in smaller quantities. Common micronutrients are vitamins and minerals. Vitamins devote the maintenance of healthy vision, immune function, bone health; cell integrity helps regulate calcium and phosphorus. Fruits are rich in vitamin C, A and E. Minerals may reduce the risk of high blood pressure and stroke. Copper and manganese are cofactors of the antioxidant enzyme, superoxide dismutase. Copper is required for the production of red blood cells. Vitamin composition of some fruits and vegetables are tabulated below (Tables 14.2 and 14.3).

14.3 Functional Bioactive Compounds and Their Properties

The list of major bioactive components of fruits and vegetables that have beneficial effects in the human body has been summarized in Table 14.4.

Table 14.2 Vitamin content of some major Fruits

Fruit	Vitamins									
	A (IU)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	B9 (μg)	C (mg)	D (IU)	E (mg)	K (μg)
Grapes	67	15	–	0.00006	0.000016	0.00009	6.5	–	–	–
Plantain	1127	0.052	0.05	0.69	0.29	22	18.4	0.7	0.14	0.7
Pineapple	58	0.08	0.03	0.5	0.11	18	47.8	0	0.02	0.7
Apple	54	0.02	0.03	0.09	0.04	3	4.6	0	0.18	2.2
Orange	225	0.09	0.04	0.28	0.06	30	53.3	0	0.18	0
Banana	64	0.03	0.07	0.67	0.37	20	8.7	0	0.1	0.5
Mango	1082	0.03	0.04	0.67	0.12	43	36.4	0	0.9	4.2
Peach	326	0.02	0.03	0.81	0.03	4	6.6	0	0.73	2.6
Papaya	950	0.02	0.02	0.35	0.03	37	60.9	0	0.3	2.6
Pear	25	0.01	0.03	0.17	0.03	7	4.3	0	0.12	4.4
Guava	624	0.06	0.04	1.08	0.11	49	228.3	0	0.7	2.6

Source: (Saini and Keum 2016; Dasgupta and Klein 2014; Płonka et al. 2012; Chun et al. 2006; Benvenuti et al. 2004; Belitz et al. 2004)

Table 14.3 Vitamin content of some major Vegetables

Vegetable	Vitamins					
	A (IU)	B1 (mg)	B2 (mg)	B6 (mg)	B9 (μg)	C (mg)
Pumpkin	0.02	0.04	0.04	0.02	0.007	16.00
Broccoli	0.2	0.1	0.3	0.21		110.00
Brussels sprouts	0.03	0.1	0.12	0.23	0.087	66.00
Cabbage	0.01	0.1	0.15	0.2	0.069	80.000
Carrot	0.6	0.01	0.01	0.03	0.016	1000
Cauliflower	0.001	0.05	0.07	0.2	0.044	80.000
Cucumber	0.034	0.03	0.01	0.03	0.005	10.000
Onion	0.001	0.03	0.01	0.17	0.01	10.000
Peas	0.3	0.06	0.05	0.05	0.022	1000
Potato	0	0.12	0.04	0.3	0.023	14.000
Radish	0.002	0.05	0.03	0.08	0.028	20.000

Source: (Saini and Keum 2016; Dasgupta and Klein 2014; Chun et al. 2006; Benvenuti et al. 2004; McCarthy and Matthews 1994; Saleh et al. 1977)

Polyphenols Polyphenols are dietary antioxidant that exists usually in fruits and vegetables. They consist of flavonoids (flavonols, flavones, isoflavones, flavonols, flavanones and anthocyanins) and non-flavonoid polyphenols (phenolic acids, lignans and stilbenes).

Flavonoids Flavonoids neutralize free radicals that damage cells and increases cellular antioxidant protection. Isoflavones- are structurally similar to estrogen but are not steroids.

Table 14.4 Bioactive compounds of Fruits and Vegetables

Functional compounds		Mechanism of action	Fruit source	Vegetable source
Flavonoids				
Flavanones	Hesperidin Hesperetin	Anticancerous, prevents viral replication eg: Polio, herpes and flu, regenerates vitamin C, protects from lymphedema, hemorrhoids, varicose veins.	Oranges (Gattuso et al. 2007)	Lettuce OnionTomato (Sanchez-Moreno et al. 2000); parsley, celery (Tomas-Barberan and Clifford 2000; Mouly et al. 1994)
	Naringenin	Anti-inflammatory, antiallergic, hypolipidemic, vasoprotective and anticarcinogenic, against bronchial asthma	Citrus fruits (Gorinstein et al. 2001; Wang et al. 2008; Harborne and Williams 2000	
	Eriocitrin	Lipid lowering, capillary permeability, antioxidant		
	Neoeriocitrin	Protect cartilage tissue		
	Naringin	For the treatment of obesity, diabetes, hypertension, and metabolic syndrome	Oranges (Chadha 2001)	
	Narirutin	May be effective in the treatment of bronchial asthma		

(continued)

Table 14.4 (continued)

Functional compounds		Mechanism of action	Fruit source	Vegetable source
Flavonols	Kaempferol	Anti-inflammatory and antioxidant	Grapes, plantain, apple (Yu and Ahmedna 2013; Pan et al. 2001; Shahidi and Naczk 1995; Radha and Mathew 2007)	Onion (Benitez et al. 2011, 2012; Gonzalez-Pena et al. 2013); Beets Cherry tomato, broccoli, (Ninfali and Angelino 2013); Cabbage, carrot, cauliflower, spinach, turnip, onion, garlic, ginger (Sultana and Anwar 2008)
	Myricetin	Reduce atherosclerosis	Grapes, apple, plantain, pineapple, straw berry, mulberry (Sultana and Anwar 2008	
	Quercetin	Anticarcinogenic, diarrhea, allergies, prevent atherosclerosis, asthma, Hay fever, hypertension, interstitial cystitis prostatitis diabetes Rheumatoid Arthritis (RA) athletic endurance	Grapes, apricot, plantain, apple, peaches, Berries (Mariaa et al. 2002; Radha and Mathew 2007)	
	Isorhamnetin	Reduce the risk of cancer, improve heart health and ease diabetes complications	Plantain, grapes, apple (Eberhardt et al. 2000)	
	Laricitrin	Prevents cardio vascular diseases, anticarcinogenic	Red grapes, apple (Castillo-Munoz et al. 2007)	
	Syringetin	Stimulates osteoblast differentiation	Red grapes (Castillo-Munoz et al. 2007)	
Anthocyanidins	Malvidin Peonidin Delphidin Peltunidin Cyanidin	Anti-inflammatory and anticarcinogenic activity, cardiovascular disease prevention, obesity control	Oranges Guava, Berries, grapes (Wang and Lin 2000)	Aubergine, red cabbage, Lettuce (Koponen et al. 2007; Wu et al. 2006)

Leucoanthocyanidins	Leucocyanidin	Protection against ulcer	Plantain (Loganayaki et al. 2010)	
Flavan-3-ols	Catechin	Anticarcinogen in lungs, stomach, esophagus, duodenum, liver, pancreas, mammary gland	cherry, apple apricot, Grapes, pineapple, Kiwi peaches, goose berry, peaches, blackberries, black grapes, strawberries, blueberries and raspberries (Zanwar et al. 2014; Mariaa et al. 2002)	beans, Broccoli, tomato, spinach, Kale (Appari et al. 2014; Pathak et al. 2018)
	Epicatechin	Reduction of diabetes, heart health	Grapes, pineapple, apple, peaches (Mariaa et al. 2002; Jose et al. 1997)	
	Gallocatechin epigallocatechin	Improve brain function, fat loss, anti-cancerous.	Banana, Grapes, apple, pear (Pathak et al. 2018)	
Flavones	Apigenin	Treatment of HIV, inflammatory bowel disease, prostate cancer and cervical cancer	Plantain (Loganayaki et al. 2010), Citrus, kiwi (Hostetler et al. 2017)	Peas, Parseley, Capsicum, onion Pepper, Lettuce (Justesen et al. 1998; Crozier et al. 1997)
	Luteolin	Antimutagenic, antitumerogenic, antioxidant, anti-inflammatory properties		
	Tangeretin	Prevents breast cancer, cholesterol lowering and in neuroprotection	Tangerines (Hung et al. 2010; Berhow et al. 1998)	

(continued)

Table 14.4 (continued)

Functional compounds		Mechanism of action	Fruit source	Vegetable source
Carotenoids	α – Carotene β – Carotene lycopene β – Cryptoxanthin Lutein Zeaxanthin Astaxanthin	Prevents cancer in endometrium, breast, lungs, liver and colon; cornea protection against UV-induced erythema	Orange, passion fruit, jackfruit, Tangerines, pineapple, Apricot, peaches, pear, guava, banana, apple, grapes, mango, Acerola (Saini et al. 2015; Lokesh et al. 2014; Divya and Pandey 2014; Chan Jr 1980)	Tomato (Sanchez-Moreno et al. 2000); Carrot (Sun et al. 2009) sweet potato, pumpkin, green beans, spinach and broccoli (Khoo et al. 2011); Broccoli, Brussels, cauliflower, Chicory, drumstick, Kale, Lettuce, Pumpkin, Squash (Saini et al. 2015).
Phenolic acids				
Hydroxybenzoic acid	Gallic acid p -hydroxybenzoic acid Gentisic acid	Reduce hypertension, atherosclerosis and dyslipidemia	Grapes, guava, pineapple, Plantain Grapes (Pan et al. 2001), pomogranate, Berries, onion, blackberry, raspberry, strawberry	Lettuce, Tomato (Sanchez-Moreno et al. 2000); Artichoke (Femenia et al. 1998; Larrosa et al. 2002); Carrot, Potato (Mattila and Hellstrom 2007). Beets (Canadanovic-Brunet et al. 2011; Ninfali and Angelino 2013)
Hydroxycinnamic acid	Caffeic acid	Chemo protective agent in oral cancer, helps in cardiac health and antihyperglycemic	Plantain, grapes (Loganayaki et al. 2010)	
	Chlorogenic acid	Reduce colon cancer, prevents hardening of arteries	Pineapple, peaches, grapes, Kiwi, cherry, plum, apple, pear (Alam et al. 2016)	
	Ferulic acid	Protect against cancer, bone degeneration, menopausal symptoms	Pineapple, grapes, apple, orange	

Stilbenoids	Resveratrol	Anti-aging, anticancer factor against colon and prostate cancers, against coronary heart disease, alleviates the risk of stroke, chemopreventive agent against melanoma, preventing Alzheimer disease and viral/fungal infections	Grapes (Niles et al. 2003; Nazar et al. 2006)	
Phytoestrogens	Glycetin	Relief from menopausal symptoms and lower risk of osteoporosis, heart disease and breast cancer	Plantain, grapes (Loganayaki et al. 2010; Cornwell et al. 2004)	
Tannins	Proanthocyanidins	Fight against tooth cavities, diarrhea, protect heart diseases and cancer	Pineapple, peaches, grapes, apple, orange (Montes-Avila et al. 2017)	Indian squash (Montes-Avila et al. 2017)
	Procyanidin B2			
Dietary fibres	Cellulose, Hemicelluloses Galactooligosaccharides, Lignin, Pectin	Maintain bowel health, lowers cholesterol levels, helps to control blood sugar levels	Grapes (Niles et al. 2003; Nazar et al. 2006)	Tomato (Sanchez-Moreno et al. 2000); artichoke (Femenia et al. 1998; Larrosa et al. 2002); Carrot (Chau et al. 2004); Onion (Benitez et al. 2012; Gonzalez-Pena et al. 2013; Colina-Coca et al. 2013, 2014)
Prebiotics	Fructooligosaccharides	Increases the growth intestinal bacteria, Resists gastric acidity	Banana, watermelon, custard apple, Gapes (Wuyts et al. 2006)	Chikory, Jerusalem artichoke, onions, savoy cabbage (Allsopp and Rowland 2009)
Others	Oleoresins, Shogaol, Gingerol, Astilbin	Reduces blood cholesterol, Suppresses arthritis	Plantain (Loganayaki et al. 2010)	Capsicum, Pepper, celery (Moyler 1991)

Flavanones These are flavonoids that are glycosylated at the seventh position to give flavanones. A variety of flavanones are present in fruits and vegetables. Hesperetin- regenerates vitamin C. It inhibits the proliferation of cancer cells, replication of viruses like polio, herpes, and flu (Divya and Pandey 2014). They have chemopreventive effects. They are used in treating hemorrhoids, varicose veins, and lymphedema Naringenin acts as antioxidant, anti-inflammatory, anti-allergic, hypolipidemic and vasoprotective (Gardana et al. 2007). Narirutin used in the treatment of bronchial asthma (Funaguchi et al. 2007).

Flavonols They occur in un-glycosylated forms. It includes catechins and proanthocyanidins. Catechins (Flavan-3-ols) It acts as an anticarcinogen in the lungs, stomach, esophagus, duodenum, liver, pancreas, mammary gland. It also prevents chronic inflammation associated with carcinogenesis and cardiovascular disease (Cheynier et al. 2000). Quercetins effective in protecting Low Density Lipoprotein from oxidation, followed by myricetin and kaempferol. It inhibits oxidation of LDL thus reducing atherosclerosis and cardiovascular diseases; inhibit colon cancer (David et al. 2016). Kaempferol It has anti-inflammatory and antioxidant properties. Proanthocyanidins. They are oligomers of catechin and epicatechin and their gallic acid esters. They form tannins.

Anthocyanins These are watersoluble plant pigments, responsible for the red, blue and purple colors of fruits (Rufino et al. 2010). Cyanidin, delphinidin, petunidin, peonidin and malvidin are the classes (Mazza and Miniati 1993). It has antioxidant properties. It enhances immune suppressive mechanisms, anti-allergic, anti-inflammatory, antimicrobial and anti-cancer (uterine carcinoma and colon adenocarcinoma) (Cheynier et al. 2000).

Flavones Apigenin, luteolin and tangeretin are important edible flavones. Apigenin-used in the treatment of HIV, inflammatory bowel disease and skin conditions (Duthie and Crozier 2000), prostate cancer and cervical cancer (Gupta et al. 2002). Luteolin exhibits anti mutagenic, anti tumerigenic, antioxidant and has anti-inflammatory properties (Kim et al. 1999). Tangeretin is a polymethoxylated flavone, stops cancer cell proliferation, breast cancer (Marc et al. 1999).

Carotenoids They inhibit the cell proliferation of human endometrium, mammary gland, and lungs. β- Carotene neutralizes free radicals, which may damage cells. β- Carotene and lutein are orange and yellow carotenoids. They minimize liver cancer and lung cancer (Pueyo and Polo 1992). B Carotene boosts the activity of natural killer immune cells. It gives cornea protection against UV light. Zeaxanthin grants to the preservation of healthy vision. Lutein may protect against colon cancer. Lycopene aids in the continuation of prostate health.

Phenolic Acids Separated into two classes- hydroxybenzoic acids and hydroxycinnamic acids.

Hydroxybenzoic acid-Gallic acid and p-hydroxybenzoic acid are common types.

Hydroxycinnamic acid- Phenols and hydroxycinnamic acid inhibit the formation of carcinogen metabolites. Chlorogenic acid prevents colon cancer, helps in the maintenance of cardiac health and prevents various tumors. It also possesses chemopreventive properties. Ferulic acid- inhibits carcinogen metabolites. Stilbenoids: They are glycosylated forms of stilbenes. Resveratrol in grapes acts as an antioxidant, anti-aging agent, reducing the oxidative damage in the DNA of neuronal cells (Nazar et al. 2006). It has an anticancer factor against colon and prostate cancers. It is beneficial in coronary heart disease by preventing vasodilation and platelet aggregation (Niles et al. 2003). It protects against age related macular degeneration (King et al. 2005). It has a role in preventing Alzheimer's disease and viral/fungal infections.

Dietary Fibers Insoluble fiber may accord to the perpetuation of a healthy digestive tract (Dreher 2018). Soluble fibre may reduce the risk of coronary heart disease and some types of cancer. Cellulose, hemicellulose and pectin are some examples.

Proteins Proteins in the form of enzymes play a significant role in free radical scavenging activities Polyphenol oxidases, peroxides, phenolase, phosphatase, proteases, pectin, Methyl esterase (PME), polygalacturonasen etc. are enzymes present in the skin (Shahidi and Naczk 1995). Bromelain of the pineapple has significant anti-inflammatory effects in acute sinusitis, sore throat, arthritis and speeding recovery from injuries. Bromelain also used in the treatment of rheumatoid arthritis, diabetic ulcers, angina pectoris and general surgery. Pineapple is an excellent cerebral toner that combats loss of memory sadness and melancholy.

Prebiotics They are non-digestible carbohydrates that cannot be broken down by the body. They are food sources for probiotic organisms.

Some vegetables and fruits can be classified as super foods, referring to foods that provide utmost nutritional benefits for minimal calories. They are packed with vitamins, minerals, and antioxidants. The term "superfood" was used as a marketing tool for selling specific foods, dietary supplements, foods with selected food additives (Fitzgerald 2014). "Super food" products were sold at a greater price than similar foods without the label.

As there is no official scientific definition of a super food by regulatory authorities in major consumer markets, such as the United States Food and Drug Administration and Department of Agriculture or the European Food Safety Authority (Brown 2010), but it is generally accepted that super foods contain high levels of vitamins and minerals. They can be a source of antioxidants, substances that protect from cell damage, preventing cancer. They also possess healthy fats that prevent heart disease, fiber thought to ward off diabetes and digestive problems; and phytochemicals, responsible for deep colors and odors which have many health benefits. Super fruits are a subset of super foods (Srinivasan 2008). The designation of a fruit as a super fruit is entirely up to the product manufacturer, as the term is primarily used to create consumer demand (Starling 2008). Following are the list of some super foods.

14.4 Dark Green Leafy Vegetables

These are rich in folate, zinc, calcium, iron, magnesium, vitamin C and fiber. They minimize the probability of chronic illnesses including heart disease and type 2 diabetes (Blekkenhorst et al. 2008; Wang et al. 2016). They also contain high levels of carotenoids (anti-inflammatory compounds), protect against certain types of cancer (Xavier and Perez-Galvez 2016). Some well-known Dark green leafy vegetables considered as super foods include Kale, Swiss chard, Collard greens, Turnip greens, Spinach. **Kale** is a good source of minerals, antioxidants and vitamins A, C and K. It also contains antioxidants lutein and beta-carotene, which prevents the risk of diseases due to oxidative stress (Lien et al. 2008). **Collard greens** are reliable sources of calcium an vitamins A, B9 (folate), C and K (Weber 2001). **Spinach** is rich in manganese vitamin K and A. It's also packed with folate, which plays a key role in red blood cell production and the prevention of neural tube defects in pregnancy (Furness et al. 2013). **Swiss Chard** is rich in the minerals potassium, manganese and vitamins A, C, and K. Swiss chard also contains a unique flavonoid called syringic acid, a compound that may be beneficial for lowering blood sugar levels (Young-Hee Pyo et al. 2004). **Turnip greens** accommodate diverse antioxidants including glucotropaeolin, gluconasturtiin, quercetin, myricetin and beta-carotene, etc., effective in lowering stress you're the risk of heart disease, cancer and inflammation (Lin and Harnly 2010).

14.5 Green Leafy Vegetables

Green leafy vegetables contain folic acid (B vitamin) that prevent birth defects, heart disease, dementia, colon cancer, vision loss and protects skin and bones. Green leafy vegetables are a source of lutein which prevents macular degeneration (a cause of age-related vision loss), cataracts and protect skin from the adverse effects of sun exposure. Lutein preserves the fats in the top layer of skin, halting dehydration, roughness and even wrinkles. Green leafy vegetables are rich in vitamin K, necessary for bone development. Examples include: **Micro greens**-Microgreens are immature greens developed from the seeds of vegetables and herbs, measure 1–3 inches. Microgreens contain up to 40 times more nutrients compared to their mature counterparts including vitamins C, E and K (Xiao et al. 2012). **Cabbage** is formed of clusters of thick leaves that come in green, white and purple colors. Vegetables in *Brassica* family contain glucosinolates may have cancer-protective properties against lung and esophageal cancer (Johnson 2002). Cabbage can be fermented and turned into a sauerkraut, which improves digestion, boosts the immune system and aids weight loss (Gupta and Garg 2009). **Beet Greens** rich in potassium, calcium, riboflavin, fiber and vitamins A and K. fiber. They also contain the antioxidants beta-carotene and lutein, which inhibit the risk of eye disorders, such as macular degeneration and cataracts (Semba and Dagnelie 2003). **Watercress** is an aquatic plant from the *Brassicaceae* family. Watercress extract is beneficial in targeting cancer stem cells and impairing cancer cell reproduction and invasion (Boyd et al. 2006). Romaine Lettuce is an excellent source of vitamins A and K which is important for good health (Nicolle et al. 2004). **Arugula** is a leafy green from the *Brassicaceae* family. It's also one of the best sources of dietary nitrates, may help increase blood flow and reduce blood pressure by widening your blood vessels (Kapil et al. 2015). **Endive** belongs to the *Cichorium* family, rich in vitamin K, A and folate. It's also a source of kaempferol, an antioxidant reduces inflammation and inhibits the growth of cancer cells in test-tube studies (Chen and Chen 2013). **Bok Choy** is a variety of Chinese cabbage. Bok Choy is popular in China and often used in soups and stir-fries. It contains the mineral selenium, which benefits brain health, immunity, cancer protection and thyroid health (Ventura et al. 2017).

14.6 Cruciferous Vegetables

Cruciferous vegetables (broccoli, cabbage and Brussels) possess sulforaphane an enzyme that detoxifies carcinogens before they damage cells. Cruciferous vegetables prevent the development of cancer cells in the breast, uterine lining (endometrium), lung, colon, liver and cervix. Isothiocyanates from cruciferous vegetables aids in the conversion of estrogen and other hormones into forms that are more easily excreted from the body. Diets rich in cruciferous and dark yellow veggies helps to defend against cardiovascular disease. Vitamin C, beta carotene and potassium are the other significant nutrients of cruciferous vegetables. **Sweet potato** is a root vegetable stuffed with a variety of nutrients, including potassium, fiber vitamins A and C, carotenoids of sweet potato inhibits the risk of cancer (Tanaka et al. 2012). Interestingly, they may improve blood sugar control in those with type 2 diabetes (Ooi and Loke 2013). The **Onions** family and related super foods including garlic and leeks have a wide range of nutritional and health benefits. Naturally controls blood sugar in diabetics, very good source of daily fiber and natural sugars (Morris 2012). Onions used as disinfectants (sulfur content), a diuretic, anti-emetic, stimulant, expectorant, and fight colds. Onions contain selenium, an important nutrient. Red onions are rich in anthocyanins, which are powerful plant pigments that may protect against heart disease, certain cancers and diabetes (Slimestad et al. 2007).

14.7 Orange Fruits and Vegetables

Beta carotene of orange vegetables and fruits such as sweet potatoes, squash, pumpkins, carrots, mangos, oranges and papayas possess beta carotene, an anti-oxidant that helps protect healthy skin cells and block sun damage (Schulz 2004). Beta carotene is necessary for the lining of membranes in the mouth, throat, lungs, stomach, intestines, urinary tract and the reproductive tract. It also helps night vision. Manganese, copper, fiber, vitamin B-6, potassium and iron are the other nutrients in orange fruits and vegetables.

Berries Berries are boosted with vitamins, minerals, fiber and antioxidants. The strong antioxidant property of berries reduce threat of heart disease, cancer and other inflammatory conditions (Skrovankova et al. 2015; Manganaris et al. 2014). Berries are effcient in eliminating digestive and immune-related disorders (Govers et al. 2018). Some of the most common berries include: Raspberries, Strawberries, Blueberries, Blackberries, Cranberries. **Blueberries** are considered to improve memory, brain function, heart function; inhibits the risks of cancer, reduce cholesterol; act as an anti-inflammatory and have anti-aging benefits etc. Blueberries are a great add-in for smoothies, yogurt, salads or just to eat alone (Seeram 2008). **Canberries** are rich in high phenol content that help to lower the oxidation of the LDL cholesterol. Proanthocyanidins in Cranberries' can protect against urinary tract infections. The antioxidants and other nutrients in Cranberries enhance immune function, helps digestion, decrease the risk of heart-problems and possess anti-aging properties.

Pomegranates are an antioxidant rich fruit used for treating diarrhea, excessive perspiring, recurrent fevers, sore throats. It also used as an astringent. Bark, rind, and seeds of pomegranates are frequently used Avocado is a good source of many nutrients, including fiber, vitamins, minerals and healthy fats, monounsaturated fats (Dreher and Davenport 2013). Oleic acid is the most predominant MUFA in avocado, which reduces inflammation in the body (Sales-Campos et al. 2013). Avocado may reduce the risk of heart disease, diabetes, metabolic syndrome and certain types of cancer (Fulgoni 3rd et al. 2013; Wang et al. 2015; Ding et al. 2007).

14.8 Conclusion

Food is a substance that we eat which provides nutrition to maintain growth and sustain life. Instead of having anything to consume, it will be good to take food which provides us both flavor and health. As the Father of Medicine, Hippocrates said more than 2000 years ago "Let food be thy medicine and medicine be thy food". Fruits and vegetables are a good source for obtaining phytochemicals with high antioxidant activity and other beneficial health properties. The utilization of these low-cost renewable resources could be prepared for the pharmaceutical, nutraceutical, and food industries with the opportunity of developing new nutraceutical and pharmaceutical products. Studies are to be focused on elucidating the possible mode of action of bioactive compounds. It is also aimed to prevent deteriorative reactions as well as identify their specific contribution to the total antioxidant capacity. Furthermore, encompassing the efforts of food technologists, nutritionists and physicians will lead to the development of programs to raise awareness of the great health advantages that fruit and vegetable consumption offers.

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Chapter 15
Cereals and Grains as Functional Food in Unani System of Medicine

Shaikh Ajij Ahmed Makbul, Nasreen Jahan, and Abid Ali Ansari

15.1 Introduction

Cereal-based food products have been the basis of human diet since ancient times (Borneo and Leon 2011). Whole grains, refined cereals, and legumes are important components of diet in many parts of the world. They are major source of energy, proteins, and other nutrients (Tayyem et al. 2016; Dias-Martins et al. 2018). The concept of functional food includes foods or food ingredients that exert a beneficial effect on health and/or reduce the risk of chronic diseases beyond basic nutritional functions. Increasing awareness of consumer health and interest in functional foods to achieve a healthy lifestyle has resulted in the need for food products with versatile health-benefiting properties. Cereal and cereal based food products offer opportunities to include probiotics, prebiotics, and fibres in the human diet (Das et al. 2012). Dietary guidelines has it that the high nutrient density of breakfast cereals (especially those that are whole grain or high in cereal fibre) makes them an important source of key nutrients in addition to providing an important source of vitamins and minerals. Breakfast cereals are also potentially important source of antioxidants and phytoestrogens and are one of the most important sources of whole grains (Williams 2014).

A whole grain is a grain of any cereal and pseudo cereal that contains the endosperm, germ, and bran, in contrast to refine grains, which retain only the endosperm (Aune et al. 2016). There are a lot of supportive evidence showing that the consumption of whole grains and whole-grain-based products are associated with the reduction of the risk of developing many diseases such as cardiovascular diseases,

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hypertension, strokes, metabolic syndrome and type 2 diabetes as well as different types of cancer. Cereals contain all the macronutrients (proteins, fats and carbohydrates) we need for support and maintenance. They are an excellent source of minerals, vitamins, and other micronutrients required for adequate health (Borneo and Leon 2011).

A food can be made functional by applying any technological or biotechnological means to increase the concentration of, add, remove or modify a particular component as well as to improve its bioavailability, provided that component has been demonstrated to have functional effect. In recent years, cereals and its ingredients are accepted as functional food and nutraceuticals because of providing dietary fibre, proteins, energy, minerals, vitamins and antioxidants required for human health. Also, cereals can be used as fermentable substances for the growth of probiotic microorganisms. Wheat, buckwheat, oat, barley, flaxseed, psyllium, brown rice and soy products are notified as the most common cereal based functional food and nutraceuticals (Das et al. 2012; Cencic and Chingwaru 2010).

Unani (Greco-Arab) system of medicine originated in Greece and passed through many centuries before establishing itself in India during the medieval period (Husain et al. 2017). The Unani system of medicine is a comprehensive medical system, which meticulously deals with the various states of health and disease. It provides promotive, preventive, curative and rehabilitative healthcare. The therapeutics in Unani system of medicine is described under the major headings of *Ilājbi'l-Tadbīr* (Regimenal therapy), *Ilājbi'l Ghidhā* (Dietotherapy), *Ilājbi'l-Dawā* (Pharmacotherapy) and *Ilājbi'l-Yad* (Surgery) (Central Council for Research in Unani Medicine: http://ccrum.res.in/writereaddata/UploadFile/Dossier_1325.pdf accessed on 30/11/2017). It was Hippocrates who famously stated "let your food be your medicine and your medicine be your food" (Jennings et al. 2015). It uses natural products of plant, animal and mineral origin as a drug in various dosage forms for the prevention and treatment of various disease of the body (Makbul et al. 2017, 2018). Coarse cereals which are used in Unani system of medicine include maize (*Zea mays*), sorghum (Jowar; *Sorghum vulgare*), barley (Jow; *Hordeum vulgare*), pearl millet (Bajra; *Pennisetum glaucum*) (Table 15.1) (Ibn al Baitar 1999; Ibn Sina 2007; Khan 2013). They are rich in dietary energy, vitamins, several minerals insoluble dietary and phytochemicals with antioxidant

Table 15.1 List of cereals, grains and millet used in Unani medicine

English name	Unani name	Botanical/Scientific name
Wheat	Gandum	*Triticum aestivum*
Rice	Chawal	*Oryza sativa*
Maize	Makka	*Zea mays*
Barley	Jau	*Hordeum vulgare*
Sorghum	Jawar	*Sorghum vulgare*
Pearl millet	Bajra	*Pennisetum glaucum*

properties (Kaur et al. 2014). This chapter emphasized on nutraceutical properties and the application of cereals and grains potentially healthy to elaborate therapeutic food products.

15.2 General Concept of Whole Grains and Cereals

Cereals are defined as wheat, coarse grains and rice. Coarse grains generally refer to cereal grains other than wheat and rice in the OECD countries (https://stats.oecd.org/glossary/search.asp, 2-10-2018). Coarse cereals are a broad sub-group of several short duration warm weather (Kharif) crops such as Jowar (Sorghum), Bajra (Pearl Millet), Maize, Ragi (Finger Millet) etc. They are used in food, fodder, fuel; value-added products and also fast food products (http://apeda.gov.in/apedawebsite/six_head_product/cereal.htm, 2-10-2018). Grains, commonly referred to as 'cereals' or 'cereal grains' are the edible seeds. There are a number of different types of grains found within the true cereal grains including wheat, oats, rice, corn (maize), barley, sorghum, rye, and millet (https://www.glnc.org.au/grains/types-of-grains/).

India is the world's second largest producer of rice, wheat and other cereals. The important cereals are wheat, paddy, sorghum, millet (Bajra), barley and maize etc. India's export of cereals stood at Rs. 52,064.52 crore/8078.85 USD millions during the year 2017–18. In India the coarse cereals are mainly grown in poor agroclimatic regions, particularly rain fed areas of the country. These crops are grown in areas with high temperature and are called dry land crops because can be grown in areas with 50–100 cm rainfall (http://apeda.gov.in/apedawebsite/six_head_product/cereal.htm, 2-10-2018).

Grains are one of the major staple foods consumed around the world and provide 56% of the energy and 50% of the protein intake. They constitute the largest component of recommended daily intake in all dietary guidelines. High intake of whole grains has been associated with a reduced risk of type 2 diabetes, coronary heart disease and obesity. Whole grains contain endosperm, germ, and bran, in contrast with refined grains, which have the germ and bran removed during the milling process. Whole grains are good sources of fibre, B vitamins, and some trace minerals such as iron, magnesium, and zinc. These nutrients are found in the outer layer of the grains or the bran that function as a protective shell for the germ and endosperm inside. The germ is nourishment for the seed and contains antioxidants, vitamin E, and some B vitamins, while the endosperm provides carbohydrates, protein, and energy. Consumption of whole grains differs considerably between populations, with the main source being whole grain bread in Scandinavian countries, whole grain bread and breakfast cereals in the United States, brown rice, unrefined maize and sorghum in some African countries, and brown rice in Asia, although most of the rice consumed in Asia is white rice (Aune et al. 2016).

15.3 Concept of Functional Food in Unani System of Medicine

In Unani System of Medicine, there is a unique concept of Food Medicine (*Dawā' Ghidhā'ī*) and Medicinal Food (*Ghidhā' Dawā'ī*). Substances used primarily for their medicinal properties but also having nutritional value are called Food Medicine. They not only treat a disease but also nourish the patient e.g. Almond (*Prunus amygdalus*), Fig (*Ficus carica*), *Halwa-e- magzsarkunjashk*, *Halwae Salab, Maul shaeer* etc. The substances which are used primarily as food but also have some medicinal properties are known as Medicinal Food like e.g. Egg, Bengal gram etc. They have the advantage of being very safe. Unani System of Medicine lays great stress on treating certain ailments by administration of specific diets or by regulating the quality and quantity of food. In addition to nutritional properties, various foods have pharmacological actions too (Central Council for Research in Unani Medicine: http://ccrum.res.in/writereaddata/UploadFile/Dossier_1325.pdf accessed on 30/02/2019). *Mizaj* (Temperament: A quality that is produced by action and reaction of opposite qualities of elements which are broken up in small particles in order to facilitate their mixing. When these components interact among themselves, a condition is produced, which is found in equal proportion in all the particles of the compound) is one of the fundamental components of Unani system of medicine. The *Mizaj* of drugs have been expressed in terms of four *kaifiyat* (qualities) viz. *har* (hot), *barid* (cold), *yabis* (dry) and *ratab* (moist) (Makbul et al. 2018).

15.4 Brief Description of Cereals and Grains in Unani System of Medicine

Diettherapy involves recommending a specific diet, which is the simplest and most natural course of treatment by a *Hakim* (traditional healer). For example, in fever Unani medicine stresses a nutrient rich, low roughage diet that might include *Dalia* (porridge) and *kheer* (a milk broth), as such type of diets are found to be very effective. Before initiation of treatment through drugs, Unani physicians as per their belief, advice restriction or alteration in daily diets, as this pattern should be adjusted according to disease. Sometimes they wait for few days, because some diseases can be cured even by diet so advised, and even during the drug therapy, specific diets are advised according to disease (National Health portal, India: https://www.nhp.gov.in/ilaj-bil-ghiza-dietotherapy-_mtl accessed on 14-12-2018). There are many classical books (literature) in Unani system of medicine that describe cereals, grains and millets such as *Al qanoon fit Tib*, *Kitabul Mansuri, Kamilu Sana*, *Al Jami lil Mufradatwal Advia wal Aghzia* (Ibn Sina 2007; Al Razi 1991; Ibn al Baitar 1999; Majoosi 2010).

15.4.1 Wheat (Triticum aestivum)

Wheat (*Triticum* spp.) is an ancient grain and also one of the leading cereal crops, ranking third in the world (Poudel and Bhatta 2017). Wheat grass has been used as traditional herbal medicine and is highly valued for its therapeutic and nutritional properties. Wheatgrass is the young grass of common wheat plant, *Triticum aestivum* belongings to the family Poaceae. It is commonly known for its high chlorophyll content which accounts for 70% of its chemical constituents. *Triticum aestivum* was found to reduce symptoms in patients with rheumatoid arthritis, to reduce severity of rectal bleeding in patients with ulcerative colitis, and to reduce the frequency of blood transfusions in patients with thallasemia major. Wheat is considered as a good source of protein, minerals, B-group vitamins and dietary fibres (Kumar et al. 2011).

In Unani system of medicine wheat is considered the best in all the variety of grains. Due to its temperament wheat amongst all the types of grains and seeds is most suitable with the temperament of human body and the most acceptable diet. Wheat has two varieties; one is turgid, bulky and reddish, the other being whitish, flaccid and light in weight. The former one is of best quality, providing more nutrition. On the other hand, the latter is less nutritive. A number of food items are prepared by wheat such as *chapati, wheat sattu, wheat nashashta* (wheat starch), *daliya* (porridge) etc. (Table 15.2). (Ibn Sina 2007; Al Razi 1991; Ibn al Baitar 1999; Majoosi 2010).

15.4.2 *Barley* (Hordeum vulgare)

Barley is an annual, erect, stout and tufted grass, reaching a height of about 0.5–1.2 m. leaves are few and linear- lanceolate; spike is terminal, 5–6 cm long and densely flowered. Glumes are two, small, narrow; short awned and enclosing three spikelets. Its fruit is caryopsis, elliptic, 9 cm long, short pointed, smooth and free or adherent to palea or both to lemma. Flowering and fruiting is during February–April (Rajesh and Mita 2015).

15.4.3 *Rice* (Oryza sativa)

Rice is a dietary staple food and one of the most important cereal crops, especially for people in Asia. It provides the bulk of daily calories for many companion animals and humans (Rohman et al. 2014). Rice is supplying as much as half of the daily calories of the world population (Abbas et al. 2011). The first attempt to cultivate rice occurred around 10,000 years ago. Rice accounts for 21% global energy, 14% protein and 2% fat supply. Rice is a good source of natural antioxidants

Table 15.2 Cereals and grains in Unani system of medicine

S. No.	Cereals and grains	Temperament	Properties	Methods of therapeutic uses	References
1	*Triticum aestivum* (wheat)	Balanced but slightly hot in nature	Nutritive, general body tonic, anti-inflammatory, concoctive.	Boiled wheat provides good nutrition and strengthens the body. Its flour is applied on the face to whiten and shine the skin. Due to its resolvent property it is used as a *zimad* (paste) in various types of swellings, when applied with butter it reduces throat inflammation.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010), Ansari et al. (2018), Zaman et al. (2013) and Bashir and Akhtar (2018), Kabeeruddin (1938)
	Roghn-e-gandum (wheat oil)		Detergent	The oil is applied on the affected area for the treatment of ring worm, blemishes, leucoderma and alopecia.	
	Wheat Sattu (it is prepared by parched roasting grains or grams, mainly barley or bengal gram. The traditional way of preparing *sattu* involves the use of an iron vessel in which the grains or grams embedded in sand are roasted. Later they are sieved and then grind to fine flour).	Hot and wet	Alterative and nutritive	The *sattu* which is prepared by soaking wheat in water has cooling effect and often prescribed by Unani physicians in *sue mizaj har* (immoderate temperament caused by excessive heat). *Sattu* prepared after boiling in water and then roasted is more nutritious and has a greater cooling effect; additionally, it is less flatulence.	
	Haleem (Haleem is made of wheat, barley, meat usually minced beef or mutton, goat meat or lamb and mutton or chicken, lentils and spices, sometimes rice is also used. This dish is slow cooked for seven to eight hours, which results in a paste-like consistency, blending the flavours of spices, meat, barley and wheat).		Nutritious, general body tonic	Boiled wheat produces thick humours, especially when cooked with meat this type of diet is a boon to physically active people. It has more nutrition and produces good quality of blood. It also provides vigour and vitality to the body.	

	Wheat Chapati It is round flat bread native to the Indian subcontinent made from stone-ground wholemeal flour and water that is combined into dough.		Nutritious	Chapati prepared from hard and heavy variety of wheat provides good nutrition than soft wheat.	
	Wheat Nashashta (starch)	Cold and dry	Dessicative, astringent, Wound healing, Hemostatic	It is used in the treatment of chest diseases viz. dry cough, chest pain, tuberculosis; its paste with saffron is applied on blemishes. It is used with dry fruits in the form of *hareera* to strengthen the brain. Its paste prepared with egg white used in the treatment of conjunctivitis.	
2	*Oryza sativa* (Rice)	Hot and dry	Nutritious	It provides balanced nutrition. It can be cooked with milk to increase its nutritional value and also to reduce its dryness. White rice has some detergent property. For this purpose, rice is soaked in water for few hours then it is rubbed with hands so that the constituents responsible for detergent activity can be taken in water. This water when used for washing the face serves as an effective catalyst in the process of vanishing blemishes and whitening of face. Due to its astringent property it heals wounds when used as a dusting powder.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999) and Majoosi (2010)

(continued)

Table 15.2 (continued)

S. No.	Cereals and grains	Temperament	Properties	Methods of therapeutic uses	References
	Rice Chapati	Hot and dry	Nutritious	It produces good quality of humours. Because of its dry nature it is effective in the patients suffering from those diseases which are caused by *sue Mizaj ratab* (altered temperament caused by excessive moist in the body) *viz.* infectious diseases.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010), Ansari et al. (2018), Zaman et al. (2013) and Bashir and Akhtar (2018)
				Rice cooked with goat milk is more effective as it produces *lateef* humour (tenus humour) in the body. It produces good quality of blood and increases the production of semen that is why it is frequently used with aphrodisiac drugs. It neutralizes the bad effect of excessive *sauda* (melancholia) especially effective in those persons who have experienced nightmares. Rice is very effective in tubercular patients. It is extremely effective if consumed with curd in summer season as it relieves thirst triggered by excessive heat. Rice is a good diet for the patient suffering from dysentery as it heals the intestinal erosion and ulcers. It is also found to be effective in bloody diarrhoea, diseases of urinary tract and hysteria.	
3	*Zea mays* (corn)	Cold and dry but prone towards hotness	Nutritious, appetizer, anti diarrhoeal, anti ulcer.	Its nutritive value is less than wheat. It is appetite stimulant; effective in gaining weight; increases the production of semen and strengthens the body. It is effective in phlegmatic and biliary diseases; resolves the viscous phlegm and coagulated blood; useful in the treatment of diarrhoea and tuberculosis.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010) and Ghani (2011)
				Paste of its flour with vinegar if applied at the affected area, very effective in itching and splitting of nails. Enema of its decoction is very useful in healing intestinal ulcers.	

4	*Hordeum vulgare* (barley)	Cold and dry	Nutritious	Barley is preferred not only for nutritional but also for its nutraceutical properties. However, it is less nutritious than wheat; it is not easily digested and causes flatulence.	Chaturvedi et al. (2011), Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010), Ghani (2011), Ansari et al. (2018), Zaman et al. (2013), Rushd (1987) and Bashir and Akhtar (2018), Kabeeruddin (1938)
	Mausshaeer (barley water) (good quality of barley is taken and soaked in water so that it swells up. Later the barley is taken out and crushed to remove its peel. From this peeled barley 60 g is taken then again boiled till the water turned reddish, barley swells up and split, thereafter the water is filtered and left it to cool).	Cold and moist	Nutritious	It is used with *misri* (crystal sugar) or syrup; it expels the morbid matter from the body and specifically from the stomach. It is easily absorbed by the body; provides balanced nutrition and quenches thirst. But it is not much suitable to the stomach and other organs of cold temperament.	
	Maushshaeer Malham When meat is added during the preparation of *Maushsheer* to increase its nutritional value then it is called as *Maushshaeer Malham*		More nutritious	It is used in general debility.	
	Kashkusshaeer (it is prepared by taking water and barley in a ratio of 20:1 and then it is boiled on light flame till the ratio becomes 8:1. During boiling froth is removed till the complete disappearance. After that the light reddish water is filtered and collected.)	Cold and moist	Nutritious	Useful for all but especially effective in those persons whose temperament is hot and dry and feel more thirst.	

(continued)

Table 15.2 (continued)

S. No.	Cereals and grains	Temperament	Properties	Methods of therapeutic uses	References
	Chapati of barley (barley bread)	Cold and dry	Nutritious	It is less nutritious than wheat *chapati* and causes flatulence.	
	Barley sattu	Cold and dry	Nutritious	It is less nutritious than chapatti; stops bilious diarrhoea. For hot temperament persons, barley *sattu* is more preferable than wheat *sattu*. Nevertheless it produces more gasses.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010), Ansari et al. (2018), Zaman et al. (2013) and Bashir and Akhtar (2018)
5	*Pennisetum glaucum* (millet)	Cold and dry	Nutritious, diuretic, emmenaogogue and abortifacient	It produces dryness and cause constipation. Its nutrition is less than rice and wheat. It has diuretic effect and produces viscous humour. Millet bread is not easily digested but if flour of peeled millet dough is prepared with wheat bran water and consumed with some oil, more valuable and forms good quality of blood as well as maintains the body in healthy state. It provides strength to the body and increases sexual power. Its *hareera* and *sattu* is effective in vomiting caused by bile and black bile. It acts as diuretic, emmenaogogue and abortifacient; effective in ascites. Its hot fomentation is found to be efficient in inflammatory pains and piles as it dissolves swelling; pain and discomfort caused by flatulence.	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010) and Ghani (2011)

6	*Sorghum vulgare* (Sorghum/Jawar)	Cold and dry	Astringent, Nutritive	It is more dry than wheat, rice and *bajra* that's why it checks the diarrhoea effectively. However, its excessive consumption may cause dryness in the body which can be minimized by taking with milk. Nonetheless it provides nutrition to the body. It is less digestive, produce dryness and flatulent but can be minimized if it is consumed with some oil prepared by sweet taste drugs, *gulqand* and *murabba adrak* (preserved in honey or sugar).	Ibn Sina (2007), Al Razi (1991), Ibn al Baitar (1999), Majoosi (2010), Ghani (2011), Ansari et al. (2018), Zaman et al. (2013) and Bashir and Akhtar (2018)
	Sorghum Hareera		Nutritious, Repellent, Resolvent	*Hareera* made up of its husk by mixing with almond oil and sugar is found to be effective in respiratory congestion as it dries the viscous morbid matters of chest. Its *zemad* (paste) has *rade* (repellent) property which prevents the absorption of morbid matters towards the organs. Its *kamad* (poultice) is very effective in squeezing type of stomach pain.	
	Sorghum Chapati		Nutritious	Effective in diarrhoea	

including phenolic compounds (Khalid et al. 2015). Various varieties of rice are used in which the red variety is likely to induce constipation nevertheless after boiling with milk it is well-adjusted both in its temperament and effect. Nutritional property of rice always remains intact no matter how old it is (Ibn Sina 2007; Al Razi 1991; Ibn al Baitar 1999; Majoosi 2010). Though rice is harmless, its excessive dryness is reduced and nutritional value can be increased after cooking or by mixing it with milk, sugar, butter or ghee. In this form it digests easily. White rice is more useful than other varieties as it brings freshness, and charm to the body (Ibn Sina 2007; Al Razi 1991; Ibn al Baitar 1999; Majoosi 2010).

15.4.4 Maize or Corn (Zea mays)

Zea mays ssp. *mays*, commonly referred to as maize or corn, belong to the grass tribe Andropogoneae of the family Gramineae (Strable and Scanlon 2009). *Zea* is an ancient Greek word which means "sustaining life" and *Mays* is a word from Taino language meaning "life giver." Due to its highest yield potential among the cereals it is known globally as "Queen of cereals". The largest producer of maize is United States of America (USA) contributing about 35% of the total world maize production. It is known as mother grain of Americans and it is the driver of the US economy. In India, the major maize growing states are Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka, and Jammu and Kashmir (Shah et al. 2016). In traditional medicine, corn is used for relieving diarrhoea, dysentery, urinary tract disorders, prostatitis, lithiasis, angina, hypertension and tumour. *Stigma maydis* (Corn silk) is one of several herbs commonly used in the management of kidney stones, bed wetting, and urinary infections. Corn silk has found therapeutic applications as an insecticide, disinfectant, antioxidant, antibiotic, and immune booster (Wang and Zhao 2019; Hasanudin et al. 2012).

As per Unani description it has three varieties white, yellow and red. It is also known as big jawar and its properties are similar to jawar. Since it is constipative and cause flatulence therefore, Unani physicians advice to take it with some correctives (*Musleh*) such as it should be combined with such drugs that help in its digestion viz. Fennel (*Foeniculum vulgare*), Ajowan (*Trachyspermum ammi*), Anise seed (*Pimpinella anisum*) etc. (Ibn Sina 2007; Al Razi 1991; Ibn al Baitar 1999; Majoosi 2010; Ghani 2011).

15.4.5 Millet

Pearl millet (*Pennisetum glaucum*), also known as Bajra, is one of the four most important cereals (rice, maize, sorghum and millets) grown in tropical semi-arid regions of the world primarily in Africa and Asia (Pal and Pandey 2017;

Tako et al. 2015). Pearl millet is a gluten free grain and is the only grain that retains its alkaline properties after being cooked which is ideal for people with wheat allergies. Pearl millet grains are very high in calories precisely the reason they do wonders for growing children and pregnant women. Gluten intolerance persons (Celica) are allergic to gliadin, aprolmine specific to wheat and some other common grains (Nambiar et al. 2011).

15.4.6 Jawar (Sorghum vulgare)

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum is used in a variety of foods. The white sorghums are processed into flour and other products, including expanded snacks, cookies and ethnic foods, and are gaining popularity in areas like Japan (Awika and Rooney 2004). The nutrients in the sorghum millets have identified prospective for reducing the risk of coronary heart disease, diabetes, tumour incidence, cancer risk, blood pressure, reduces the rate of cholesterol and fat absorption, delaying gastrointestinal emptying and provide in gastrointestinal health. Thus, the regular intake of sorghum millets and their processed products can make a payment to health endorsement and disease avoidance (Mathanghi 2012).

15.5 Nutritional Composition and Bioactive Compounds in Cereals and Grains

The phytochemicals in fruits and vegetables are different from those in the grains, which contain tocotrienols, tocopherol and oryzanol. The phenolic like ferulic acid and diferulate are predominant in grains, but are not significant in some fruit and vegetables. Thus, the regular insertion of cereals and their processed products can make a payment to health endorsement and disease avoidance (Mannan et al. 2013). Whole grains are a source of carbohydrates, multiple nutrients and dietary fibers (https://en.wikipedia.org/wiki/Whole_grain). Moreover, they contain a substantial amount of starch, a carbohydrate that provides dietary energy (https://en.wikipedia.org/wiki/Legume). Cereals include dietary fibres such as glucan and arabinoxylan, carbohydrates such as resistant starch and oligosaccharides (galacto- and fructo-oligosaccharides). Cereals can also be used as fermentable substrates for the growth of probiotic microorganisms. Several of the nutrients in cereals such as the linoleic acid, fibre, vitamin E, selenium and folate have known potential for reducing the risk factors for coronary heart disease. (Ötles and Cagindi 2006).

Wheatgrass (*Triticum aestivum*) is one of the most widely used health foods; Wheat germinated over a period of 6–10 days is generally called wheatgrass. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts. Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals in their methanolic leaves extract (Suriyavathana et al. 2016). Presences of various bioactive compounds in wheat are reported (Table 15.3), these compounds might be responsible for their therapeutic effect. HPTLC studies of alcoholic extracts chromatogram shows many coloured spots which indicate the presence of biomolecules in the drug. The findings of the study reveal a strong hope for development of more chemotherapeutic agents (Pathak and Shrivastav 2015).

The nutrients content of rice varies depending on the variety of rice soil, and the conditions they grow. Rice contributes to the major dietary energy for body. Pre-germinated brown rice has protein two times more than white rice i.e. 14.6 g/100 g (brown rice) vs 7.3 g/100 g (white rice). On the other hand, the fat content is so high, namely 24.8 g/100 g for pre-germinated brown rice and 1.5 g/100 g for white rice (FAO 2004; Rohman et al. 2014). Roasted maize kernels are also used as coffee substitute (Shah et al. 2016). Maize kernels contain various vitamins and minerals (Table 15.3) and an average fatty acids composition of 11% palmitic (16:0), 2% stearic (18:0), 24.1% oleic (18:1), 61.9% linoleic (18:2), and 0.7% linolenic (18:3) acids (Ignjatovic-Micic et al. 2015).

Pearl millet grains can be considered a possible alternative for food diversification because they have the fibers, minerals, proteins and antioxidants with similar or even higher levels than those found in traditional grains such as rice and maize (Dias-Martins et al. 2018). The chemical composition of pearl millet (dry basis) is, on average, 72.2% carbohydrate, 11.8% protein, 6.4% lipid, 7.8% dietary fibre and 1.8% minerals. However, variations of these levels are possible due to genotype, climatic conditions, soil nutrient content and type of processing (Dias-Martins et al. 2018).

Sorghum is a rich source of various phytochemicals (Table 15.3). These constituents have potential to significantly impact human health. Sorghum fractions possess high antioxidant activity in vitro relative to other cereals or fruits (Awika and Rooney 2004; Mathanghi 2012). Among cereals, sorghum has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties. Almost all classes of phenolic compounds are found in sorghum important for human nutrition (Dicko et al. 2006).

The phytochemical analysis of aqueous and methanolic *Zea mays* hair extracts summarized in Table 15.3. Terpenoid compounds however present only in the methanolic extract sample. In addition, the total phenolic content (TPC) in aqueous extract was significantly higher compared to methanolic extract. The findings suggested that phytochemicals present in *Zea mays* hair are potentially beneficial as therapeutic and antioxidative agents in pharmaceuticals, food and other related industries (Solihah et al. 2012). GCMS analysis of its aqueous extract revealed the presence of major identifiable apoptogenic phytonutrients.

Phenolic compounds are the most widely distributed secondary metabolites, universally present in the plant kingdom. Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole grains. Among health-promoting phytochemicals residing in whole grains of cereals, phenolic compounds

Table 15.3 Bioactive Compounds from Cereals and Grains and their Health Benefits

Source	Bioactive compounds	Pharmacological actions	References
Triticum aestivum	Protein, fat, carbohydrate, starch, alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides, vitamin E, thiamine, ribofalvin, niacin, folate, trace minerals	Antihyperglycemic, antimicrobial, antioxidant, hypolipidemic	Suriyavathana et al. (2016), Pathak and Shrivastav (2015), Ashok (2011), Kothari et al. (2008), Kumar et al. (2011) and Mohan et al. (2013)
Oryza sativa	Protein, carbohydrate, fibre, alkaloids, sugar, terpenoids, flavonoid and phenolic compound, glutamic and aspartic acids, thiamine, ribofalvin, niacin, pyridoxine, biotin, potassium etc.	Antioxidant, antimicrobial, anti-inflammatory	FAO (2004), Rohman et al. (2014), Khalid et al. (2015), Abbas et al. (2011), Firdous and Bharathi (2014) and Bakiyalakshmi and Boominathan (2014)
Zea mays	Flavonoids, saponin, tannins, phlobatannins, phenols, alkaloids and cardiac glycosides, maizenic acid, -carotene, ascorbic acid, gluten, o-diethyl phthalate, 2-methyl-naphthalene, thymol, cyanidin, cinnamic acid, hordenine, luteolinidin, pelargonidin, betaine, vitamin C, vitamin E, vitamin K, vitamin B1 (thiamine), vitamin B2 (niacin), vitamin B3 (riboflavin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folic acid, selenium, N-p-coumaryl tryptamine, and N-ferrulyl tryptamine and potassium	Antioxidant, diuretic, anti-depressant, anti-fatigue, anti-hyperlipidemic, anti-diabetic, Nephroprotwctive, anti-inflammatory, Neuroprotective	Solihah et al. (2012), Shah et al. (2016), Hasanudin et al. (2012), Milind and Isha (2013) and Wang and Zhao (2019)
Hordeum vulgare	Vitamins, minerals, fiber, b-glucan fiber, phenolic acids, flavonoids, lignans, tocols, phytosterols, and folates	Cardioprotective, hepatoprotective, antioxidant, antidepressant	Idehen et al. (2017), Abulnaja et al. (2015), Ames and Rhymer (2008) and Yamaura et al. (2012)
Sorghum vulgare	Tannins, phenolic acids, anthocyanins, phytosterols, policosanols and phenolic compounds	Antioxidant, blood thinning effect, amylolytic, helpful in obesity, arthritis and rheumatism and cancer	Awika and Rooney (2004), Mathanghi (2012), Dicko et al. (2006) and Ciornea et al. (2008), Singh and Naithani (2014)
Pennisetum glaucum	Fibers, minerals, proteins, lipids, carbohydrate, amino acids, fats, phenolic compounds, iron and zinc and other minerals	Antioxidant, anticancer, probiotic and prebiotics, Hypoglycemic	Dias-Martins et al. (2018), Nambiar et al. (2011), Nani et al. (2011), Pal and Pandey (2017) and Tako et al. (2015)

have gained much attention in many scientific research areas, as they have strong antioxidant properties. In vivo experiments confirmed that polyphenol rich diets may decrease the risk of chronic diseases by reducing oxidative stress. Also, Meyer et al. (1998) reported that hydroxyl cinnamates such as ferulic, caffeic and p-coumaric acid reduce low-density lipoprotein oxidation, potentially protecting the body from atherosclerosis. Ferulic acid, as the most common phenolic acid in whole cereal grains, was predominantly found in bound form in corn, wheat, oats, and rice (Zilic et al. 2011).

15.6 Pharmacological Studies on Cereals and Grains

Phytoconstituents such as ascorbic acid, tocopherols, carotenoids and phenolic compounds (polyphenols), besides other bioactive compounds are reported to have antioxidant activity. A majority of the antioxidant activity is attributed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins (Firdous and Bharathi 2014). In recent years, cereals and its ingredients are accepted as functional foods and nutraceuticals because of providing dietary fibres, proteins, energy, minerals, vitamins and antioxidants required for human health. It is widely recognized that dietary ingredients have a dual role, one of them is nutritional and another is pharmaceutical. So now it's often called nutraceuticals.

The antidiabetic and antioxidant potential of *Triticum aestivum* were evaluated in streptozotocin-induced diabetic rats. Ethanolic extracts of *Triticum aestivum* (100 mg/kgb.w.) were administered orally for 30 days. Result showed that a significant increase in the liver glycogen and a significant decrease in fasting blood glucose, glycosylated hemoglobin levels, and serum marker enzyme levels. The total cholesterol and serum triglycerides levels, low density lipoprotein, and very low density lipoprotein was also significantly reduced and the high density lipoprotein level was significantly increased upon treatment with the ethanolic wheat extract. A significant decrease in the levels of lipid peroxides, superoxide dismutase, and glutathione peroxidise and increase in the levels of vitamin E, catalase, and reduced glutathione were observed in treated diabetic rats (Mohan et al. 2013). In other study ethanolic extract of the leaves of *Triticum aestivum* were evaluated for its effect in the treatment of chronic fatigue syndrome (CFS) in an experimental mice model. After 7 days, various behavioural tests (mirror chamber and elevated plus maize test for anxiety, open field test for locomotor activity) and biochemical estimations (malondialdehyde [MDA] and catalase activity) in mice brain were performed. Result showed that forced swimming in the stressed group resulted in a significant increase in immobility period, decrease in locomotor activity and elevated anxiety level. The brain homogenate showed significantly increased MDA and decreased catalase levels. The extract-treated groups showed significantly ($P < 0.05$) improved locomotor activity, decreased anxiety level, elevated catalase levels and reduction of MDA (Borah et al. 2014). One more study was conducted on fresh grass juice of *Triticum aestivum* to investgate the hypolipidemic activity in normal rats. Freshly prepared wheatgrass juice was administered to normal rats (5 ml/kg and 10 ml/kg orally once daily) for 21 days. Fresh grass juice administration produced dose related significant reduction in total chloesterol, triglycerides, low density lipoprotein-cholesterol and very lowdensity lipoprotein-cholesterol levels in normal rats as compared to control (Kothari et al. 2008).

Aqueous leaf extract of *Oryza sativa* exhibited enormous activity against *E. coli*. Moderate amount of inhibition zones was recorded in Pseudomonas (Firdous and Bharathi 2014).

Recently, barley flour and whole-grain products have been formulated in food research laboratories to increase the diversity of barley food products available and to improve the utilization potential of this healthful grain. Another motivation for advancing barley research and product development is the potential for barley foods to improve consumer health and reduce the risk of prevalent diseases especially cardiovascular disease. The β-glucan soluble fibre levels in barley varies in different varieties of barley e.g. Kalra and Jood compared cultivars of barley differing in β-glucan content and showed that quantity and solubility of barley β-glucan were strong predictors of cholesterol-lowering ability in rats. However, Wilson et al. showed that both high-molecular-weight and low-molecular-weight b-glucan concentrates from barley, lowered cholesterol to similar levels and through similar mechanisms in hamsters. In addition, Pins et al. and Keenan et al. conducted randomized, controlled clinical studies to determine the cholesterol-lowering effects of isolated barley b-glucan, when low- (50–400 kDa) vs. high-molecular-weight (1000 kDa) b-glucan was added to food products although a dose response was evident, 9–15% reduction in LDL cholesterol, molecular weight did not significantly affect cholesterol lowering effect (Ames and Rhymer 2008).

Young green barley leaf is one of the richest sources of antioxidants and has been widely consumed for health management in Japan. Antidepressant effect was evaluated on the forced swimming test in mice, a significant anti-depressant effect was observed. The expression of mRNA for NGF detected in the hippocampus immediately after the last swimming test was higher than that in the non-swimming group. Young green barley leaf also showed a moderate decrease in the expression of mRNA for NGF, in a dose-dependent manner (Yamaura et al. 2012).

The current study focused on testing the hypolipidemic activity of two doses of barley bran on hypercholesterolemic male rats. Administration of the two doses of barley bran to the hypocholesterolemic rats ameliorated the level of lipids, liver enzymes, lactate dehydrogenase, and creatinekinase-MB. In addition, the histology of heart, liver, and kidney tissues nearly restored the normal state as in negative control group (Abulnaja and El Rabey 2015).

Naniet al (1993) have reported that pearl millet (*Penniseteum typhoideum*), has the lowest GIIt also favours increased glucose uptake into skeletal muscle and improves insulin sensitivity by increasing the viscosity of the stomach contents and impeding digestion of carbohydrate and absorption of macronutrients. According to the obtained results, it was concluded that diets containing millet when administered to diabetic rats induces notable and probable changes of the glucidic metabolism. The use of whole grain millet appears useful to correct the hyperglycaemia caused by diabetes mellitus and can thus prevent, and even decrease the intensity of this disease (Nani et al. 2011).

The presence of omega-3 fatty acids in pearl millet as compared to any other cereal grain highlights its potential in prevention and treatment of cardiovascular diseases, diabetes, arthritis and certain types of cancer. Pearl millet grains can be processed and consumed as ingredients in diversified foods. They are called "nutri-cereals" because of their high protein, fibre, mineral, and fatty acids contents, as well as their antioxidant properties. Also, they are an alternative food for celiacs and gluten sensitive individuals (Dias-Martins et al. 2018). The protein in millet consists of all varieties of essential amino acids including leucine. It is a good source of Tryptophan, an amino acid which can raise serotonin level and helps stress reduction. Also, from the result obtained in this study, pearl millet possesses ability to serve as reducing agent and therefore can serve as a of antioxidants which counter the accumulation of free radicals in the body (Odusola et al. 2013).

Zea mays hair has been claimed to have effect particularly on renal diseases including chronicnephritis, benign prostate hyperplasia, gout, chronic nephritis andcystitis. It helps to pass stone from kidney and urinary tract and prevent inflammation. Besides, *Zea mays* hair has anti-prostatitis and anti-spasmodic activities (Solihah et al. 2012).

A study evaluated membrane stabilization and detoxification potential of ethyl acetate fraction of *Zea mays Stigma maydis* in acetaminophen-induced oxidative onslaughts in the kidneys of Wistar rats. The acetaminophen-mediated significant elevations in the serum concentrations of creatinine, urea, uric acid, sodium, potassium, and tissue levels of oxidized glutathione, protein-oxidized products, lipid peroxidized products, and fragmented DNA were dose dependently assuaged in the fraction-treated animals. The fraction also markedly improved creatinine clearance rate, glutathione, and calcium concentrations aswell as activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in the nephrotoxic rats. These improvements may be attributed to the antioxidative and membrane stabilization activities of the fraction. The overall data from the present findings suggest that the fraction could prevent or extenuate acetaminophen-mediated oxidative renal damage via fortification of antioxidant defence mechanisms (Sabiu et al. 2016).

The present study targeted the possible association between cereals and legumes and risk of colorectal cancer (CRC) development. Results indicated that higher consumption of refined cereals and white bread is associated with higher CRC risk. The study also showed that higher intake of whole grains and legumes is associated with lower risk for developing CRC. The protective effect of whole grains against risk of developing CRC could be attributed to nutrients and phytochemicals content of whole grains including dietary fibres, resistant starch, oligosaccharides escaping from digestion in the small intestine and then being fermented in the gut to produce short-chain fatty acids. Furthermore, whole grains are rich in antioxidants, including trace mineralsand phenolic compounds. These phenolic compounds may exert a preventive effect in the disease process. Additionally, whole grains may mediate insulin and glucose responses (Tayyem et al. 2016).

15.7 Conclusions and Future Prospects

Cereals and grains are an essential food source that not only provide major nutrients like proteins, carbohydrates, fats etc. but also offer ample amount of vitamins and minerals. Despite the fact that they are consumed as food they have medicinal properties too. They are an excellent source of antioxidants such as phenolic acids and flavonoids. Additionally, some of them are characterized to be potentially prebiotic and can enhance the viability of probiotics with potential health benefits. Unani system of medicine emphasises the use of food to treat several ailments and a lengthy list of food medicine and medicinal food are mentioned in the Unani classics. The properties of cereals and grains mentioned in the literature are based on the empirical research of Unani physicians. Though several studies are carried out regarding its phytoconstituents and pharmacological properties, the medicinal benefit described in Unani literature are still untouched. Hence there is a dire need to reinvestigate them by using modern methodologies and technologies so that the claims of Unani physicians can be validated and the safety and efficacy of various nutraceutical preparations (*Maushsheer*, *Kashkushsheer*, *Sattu* etc.) can be established.

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Chapter 16
Effect of Germination Processing on Bioactive Compounds of Cereals and Legumes

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16.1 Introduction

The present global prevalence of lifestyle associated maladies is irrefutable (Essa and El Shemy 2015), with experimental and epidemiological indications devastatingly showing that poor, unhygienic nutrition and lifestyle of most of the people are main trends added to the etiology of long lasting and non-transferable maladies for example over weight, many type of cancers related to diet, diabetes and heart disease (Chaput et al. 2011). Due to upper mentioned maladies nutritionist and diet planners recommend healthy diet which improves health of consumers and reduces risk of diet related disorders (National Health and Medical Research Council [NHMRC] 2013). In current life style people consume food containing high number

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of calories rather than nutrition so causes over weightiness, also modified the normal signaling and homeostasis of body and creates wide range of physiological defects (Fernández-Sánchez et al. 2011). Bunch of maladies produced due to poor diet are diabetes, hypertension, several types of inflammation, dyslipidemia along with considerable risk of heart diseases (Cheung and Li 2012).

Various health conditions could be produced by using different diets, so functional and nutritional components of food are fundamental in controlling and improving maladies produced due to lifestyle (Cencic and Chingwaru 2010). Therefore, consumption of proper and balanced diet along with functional components impacts health most importantly. Now a day's industry of functional foods growing exponentially throughout the world as functional components has many benefits having various physiological effects which proved to be helpful in improving health (Abuajah et al. 2015). Grains (cereals, pulses and legumes) commonly used worldwide are staple foods globally containing many bioactive and functional components affecting health positively and protect consumers from adverse health defects produced to present lifestyle thus received major attention of researchers (Fardet 2010). They are good source of vitamins, minerals, fibre and notable quantities of bioactive compounds including phenolics, carotenoids, lignans, starch, sterols, and phytates. These compounds either have additive and synergistic effects because of having role as free radical scavengers, as cofactors with antioxidant enzymes, or as indirectly performing as antioxidants providing positive effects on human health by reducing the hazards of chronic diseases other than the basic nutrition (Topping 2007).

Among many findings, the application of controlled germination process of seeds is a valuable technique in improving the nutritional contents of these grains which are excessively used in the world. This technique proves to be practical in enhancing the nutritional value of these grains especially in the countries where diet related maladies are major issue due to lifestyle.

16.2 Grains (Cereals and Legumes)

Cereals and legumes rich source of minerals and vitamins (as a whole) are excessively used especially in Asian region. Most of the grains are crushed and converted into flour prior to use while other grains like rice and legumes are used as a whole (Oghbaei and Prakash 2016). If we speak about, these are edible portion derived from caryopsis of cereal grasses, belong to Poaceae (Gramineae) family including many other crops which are grain producing for example barley, rye, wheat, triticale, rice, oats, sorghum and maize (Davidson et al. 2012), along with these some minor grain crops are also included in this family like millet. By description whole grains contain equal amounts of nutrients and grain components (germ, bran, endosperm) and available in various forms such as crushed, cracked, rolled, flaked, cooked or extruded grains (US Food and Drug Administration [USFDA] 2011). Many cereals which do not contain true seeds are considered as whole grain, e.g. amaranth, quinoa and buckwheat, but few grain containing crops like oilseeds and legumes are not included in whole grains (Whole Grains Council n.d.). Furthermore, few grains which were sprouted or malted are also included in whole grains but condition is, they should contain parts like bran, germ, and endosperm and retain their nutritional value (American Association of Cereal Chemists [AACC] 2008).

Cereals are major part of diet of many people throughout the world. Widely growing cops are wheat, maize, rice, sorghum and barley. All these crops belong to a same family called Poaceae, and contain monocotyledon in their grain. Cereals are processed and converted into variety of products and gives energy, dietary fiber, minerals, vitamins and proteins (Sarwar et al. 2013). Besides these cereals are also rich source of many bioactive and functional compounds e.g. tocopherols (antioxidant), phytosterols, phenolic acids, lignans and folates (Van Hung 2016). Consumption of whole grain cereals or their products resulted in many positive effects on health which includes reduction in risk of certain cancers, reduction in level of blood cholesterol and risk of heart related maladies (Sarwar et al. 2013). Several research studies have been conducted using cereals alone or their products as an ingredient for their functional foods (Das et al. 2016).

In developing agricultural countries legume crops also play key role due to their nutritional value for both the producers and consumers. Another form of seeds which are enclosed in pods belongs to legume family defined as “bean” are consumable and nutritious. Legumes also called pulses are grown in various countries. They include wide range of varieties like alfalfa, clover, lupins, green beans, peas, peanuts, dry beans, broad beans, dry peas, kidney beans, fava beans, black beans, soy beans, chickpeas, and lentils etc. In many regions of the world legumes used along with cereals proved to be complementary diet as these are rich source of proteins and minerals, therefore perform a key role in human diet (Bouchenak and Lamri-Senhadji 2013). Dicotyledons contain as many as 700 genera and about 20,000 species among these legumes belongs to family Leguminosae (Petchiammal and Hopper 2014). Among flowering plants on the basis of population Leguminosae is a third most important family (del Socorro López-Cortez et al. 2016).

Plant of legumes recognized by its seeds which are edible and born in pods (Megat Rusydi et al. 2011). Among legumes researchers give their major attention to dry seed besides these dry edible seeds, legumes are also used in the form of salad as these are green in color for example fresh pods, leaves and seedlings (Mitchell et al. 2009). These foods contain many essential nutrients which proved valuable to human health as they contain antioxidant, and are recognized as safe (GRAS) to use for human. Free radicals which are usually produced in biomolecules like DNA, lipids, and other proteins, their oxidative effect is masked by protein of these legumes (Rochfort and Panozzo 2007).

16.3 Biological Activities of Grains

As the grains are used worldwide and are staple foods of many countries therefore their use has the potential of impacting health outcomes. Major portion of grains is carbohydrates which are useful source of energy, it also contains other valuable dietary components e.g. phytochemicals (Okarter and Liu 2010), therefore mixing of these grains into foods either whole grain or in the form of other products gives vital nutritional differences. If we compare nutritional profile of whole grains and milled grains, major difference noticed in dietary fiber, minerals, vitamins and other bioactive compounds e.g. polyphenol which are antioxidants (Jonnalagadda et al. 2011). Various varieties of whole grains contain different nutritional composition and therefore contains different bioactive compounds and activities, so produced various biological effects in body (Donkor et al. 2012). Number of bioactive compounds present in grains along with other components are characterized with different physiological responses (Björck et al. 2012; Fardet 2010). In past many researchers had reviewed the functional characteristics of whole grains, but still they are not clarified completely (Björck et al. 2012). From literature, it is concluded that dietary fiber (important part of whole grains) provides number of health benefits, but data from research shows that all whole grains are not reliable source of dietary fibers e.g. brown rice contain 4.6 g/100 g while dark rye flour contain 23.8 g/100 g (US Food and Drug Administration [USFDA] 2012).

Many explanations regarding mechanism of whole grains activity were discussed e.g. structure of starch, solubility properties and viscosity produced by fiber, along with size of particles (Jonnalagadda et al. 2011), structure of food and gelatinization (Björck et al. 2012). Soluble fiber present in whole grains may help to reduce level of cholesterol in blood by production of bile while production of cholesterol may also be inhibited by shot chain fatty acids which produced after fermentation of grain polysaccharide. Risk of diabetes could be reduced by many minerals (Mg, Se) and antioxidant (Vitamin E) present in whole grains (Jonnalagadda et al. 2011). Oxidative stress and inflammation may also be reduced by polyphenolic antioxidants (Mateo Anson et al. 2010).

Importantly, it was observed that superior health effects were exerted through consumption of whole grains in comparison to milled grains in diet, this effect proved through epidemiological studies (Masters et al. 2010). Basal metabolic rate, LDL and total cholesterol improved by consuming whole grains (Newby et al. 2007). Numerous studies had been conducted related dietary intake of whole grains which associated with reduced risk of various disease but recommended consumption of whole grains is not met in many contraries including Canada, USA and Australia. In developed country like USA recommendation to consume whole grains were 500% more than present consumption while just 20% increment observed in consumption, which were not up-to the mar (Whole Grains Council 2009).

Similar conditions also observed in Australia where less than 1/3rd of whole grain were consumed, which are far less than recommendation (Go Grains Health and Nutrition [GGHN] 2010), In Canadian community, consumption of whole grains was also less than recommendation e.g. data collected in 2004 through a Canadian Health Community showed that between 21% and 66% of Canadian citizens aged 19 years or older were not taking their minimum intake of whole grains (Garriguet 2007). Many reasons discussed regarding reduction in consumption of whole grains, among of these lack of understanding, consumption of refined food and availability of various products have been suggested as obstacles for less whole-grain utilization (Go Grains Health and Nutrition [GGHN] 2010). Besides these barriers many anti-nutritional factors which inherent the grains may also impair the availability of many minerals e.g. iron and zinc such as phytic acid which may inhibit absorption of these mineral (Gupta et al. 2015). A novel grain processing method was recognized by "The European HEALTHIGRAIN" which will improve the palatability and nutritional profile of whole grains and ultimately positively affect the health of consumer (Delcour et al. 2012). Epidemiological and interventional studies mainly focused to non-germinated (non-sprouted) whole-grain and so, creating the gap in understanding many wider effects of whole grains on health.

Additionally, legumes are also rich source of phytochemicals (phytosterols, natural anti-oxidants), polyphenols and other bioactive carbohydrates. Therefore, legumes have increasingly used in diet to treat and prevention of number of dieses in developed countries (Amarowicz and Pegg 2008). Through numerous studies it was proved that correlation exist between consumption of legumes and reduction in risk of several disease e.g. cancer, heart related disease, overweightness and diabetes (Boudjou et al. 2013). As legumes are reliable source of phenolic compounds therefore, antioxidant ability (Heimler et al. 2005) and the anti-cancer (Cardador-Martínez et al. 2002), related to apoptosis (Aparicio-Fernández et al. 2008), and antiproliferative effects are inserted by legumes (Segev et al. 2010).

Many anti-cancer compounds e.g. flavonoids, phenolic acids, lignans, and tannins, which are richly present in legumes (seed coat). Such ant-oxidant (phenolic contents) found in legumes proves to be helpful against many chronic diseases (Aberoumand and Deokule 2008). Legumes are usually as a seed but many products could also be produced from legumes such as dhal, snack foods, flour etc. which could be used for further food preparation (Villegas et al. 2008) or also as germinated grains. It is universally accepted that simple and less costly conventional processing methods are effective techniques to bring useful changes in composition of legume seeds. Among these conventional techniques soaking, cooking, fermentation and germination may be used to enhance the quality of legumes and their nutritional value due to the removal of few anti-nutritional factors (Singh et al. 2014).

16.4 Germination Processing

Germination is a natural processing technique used for biological activation of grains to improve their nutritional and functional properties (Hefni and Witthöft 2011). Sometimes seeds germination in controlled environment is achieved to get desire benefits in which seeds are grown thus sprouts formed during germination process. The environmental conditions required for germination are optimal humidity level, accessibility of oxygen (aerobic respiration), acceptable temperature for proper time duration to stimulate metabolic processes (Sangronis and Machado 2007). These environmental factors could be controlled like moisture, temperature and humidity in controlled environment in the laboratory or alternatively seed could also have germinated in field, process called preharvest sprouting (PHS). In both condition, basic purpose is sprouting which resulted in higher amounts of essential nutrients like soluble dietary fiber, vitamins, minerals, antioxidants and phytochemicals which proved beneficial for consumers (Van Hung et al. 2011). It's necessary to carry out germination for several days to increase nutritional value of grains (Rakcejeva et al. 2014).

Additionally, sprouts are also rich source of health improving vital nutrients like glucosinolates, phenolics and selenium-containing components in various legumes for example in the Brassica plants and isoflavones in the soybean plant. It is very important to note that the sprouts which produced during germination are consumed as such so gives high amount of nutrients. Addition to nutrients sprouts are also rich source of various phytochemicals, various minerals and vitamins, enzymes and essential amino acids which exert heath promising effects on humans (Gan et al. 2017). Recently researcher gave more attention to biological value of the nutritional sprouts (Marton et al. 2010). Many anti-nutritional compounds such as trypsin inhibitor, phytic acid, pentosan, tannin etc. reduced in considerable amount, and on the other hand it also observed many compounds which proved to be health improving compounds are increased which showed anti-cancer activity, example of these compounds is glucosinolates. Therefore, sprouting can increase the amount of nutrients which exert positive effect on human health and reduces the risk of many serious diseases (Sangronis and Machado 2007).

Exact nutritional composition of sprouts is not clear as nutritional profile varies with variety of legumes but fundamentally germination modified the nutritional and physicochemical characteristics of seeds to support the growth of baby plant (Noda et al. 2004). As compared to non-germinated (non-sprouted) grains, germinated exert beneficial effect on human health as they contain huge amount of beneficial nutrients and antinutritional factors are also reduced during germination process. For germination grains are soaked in water for the short time duration which facilitate the germination process resultantly improve the technological and nutritional properties of grains. Alongside the modification in nutritional value germination also changes the appearance, taste, and flavor of the grains (Kaukovirta-Norja et al. 2004). In germination process kernels are usually soaked in water and after that allowed to germinate in controlled environment. Therefore, sprouting conditions and water contents exert significant impact on metabolic process of grains. And this metabolic process occurs in embryo results in the formation and/or release of metabolic compounds which carry health maintaining effects (Hübner and Arendt 2013).

16.5 Mechanism of Germination

Germination processing can be conducted in different simple steps like sterilization, steeping and sprouting. Germination can be activated in controlled environment having optimum range of temperature, light and moisture factors. Before soaking the seeds in water, sterilization is carried out to reduce the microbial load. Many sterilization solutions available but solution of sodium hypochlorite (NaClO) with various concentration proved to be promising and is commonly used to reduce to microbial load before seed germination (0.07% NaClO solution) (Limón et al. 2014). By using above mentioned solution usually sterilization is carried out at room temperature for 15–30-min duration in the ratio of 1:5 or 1:6 with 85 (g) seed weight/solution volume. Beside sodium hypochlorite pure ethanol or ethanol 70% solution and 0.2% formaldehyde solution also successfully used in literature for 3-min time duration (Nour et al. 2015; Pająk et al. 2014). Results of few research shows effect of sterilization process on seed germination. While in few research sterilization, process is omitted (Guo et al. 2012; Guajardo-Flores et al. 2013), researcher claim that sterilization process can cause potential hazardous effects and create seed food safety risks. So, sterilization of seeds is not compulsory before seed germination, it depends upon many factors including seed condition, water changing frequency during germination process and germination purpose.

Before carrying germination, seed is usually soaked in water to re-hydrate the seed and during this process temperature, time of soaking, ratio of seed weight and water volume should be considered. Soaking the seed at room temperature from few to 24 h and seed weight/volume ratio of 1:1.5 to 1:20 is a frequent practice which carried out before seed germination. The variation in seed soaking depends upon inherent characteristics of seeds for example water absorbing capacity of seeds, seed coat thickness and seed size. After carrying out soaking process seeds could be placed in incubators for proper germination (Gan et al. 2017). The metabolic activity of dry grains boosts rapidly as it is hydrated during soaking. For proper seed germination process several processes should be note such as the light, temperature, humidity, watering and time. Successful germination usually performed in dark and temperature for germination is kept 20 to 30 °C. Proper care is required during germination process, for example watering to should be done every day to provide seed optimum humidity for growth, water should be replaced periodically (twice a day), it will help to eliminate metabolites of sprouted seed and stop the growth of microorganisms (Kandil et al. 2015). To start germination in the grains presence of water is compulsory which initiates extensive biochemical and physiological process which provides support to growing baby plant and this process ceased after elongation of embryonic axis. Emergence of radical round the embryo of the grain is the visible sign that germination had completed (Bewley and Black 1994). Water penetration enhances seed rehydration which stimulates gibberellin synthesis in embryo which leads to gene expression of hydrolytic enzyme (Nelson et al. 2013).

As grains always remain in dormant in unfavorable condition therefore, for proper germination grains are exposed to appropriate environmental condition e.g. proper temperature and moisture which will stimulate the hormones present in grains to initiate the germination process. Optimum moisture contents and temperature will stimulate the debranching and hydrolytic enzymes and some hormones which will liberate nutrients from germ (embryo), endosperm and scutellum from within the seed (Gan et al. 2017). Period for sprouting usually varied seed to seed for example 3–5 days are enough for successful germination of edible beans. Germination process is simple, less costly, environmental friendly and recommended as safe to sprout a seed within less duration of time (Gan et al. 2017).

During germination process, complex biochemical changes occur in seeds like hormones (gibberellin) release from embryo and reached up-to aleuronic layer of seed and stimulate the release of different enzymes (amylases, proteases) into the endosperm, and also lower the activity of enzyme inhibitors, all these activities will act on compounds present in germ and endosperm therefore, convert seed from dormant to active metabolism (Iordan et al. 2013). These enzymes will act on stored carbohydrates and proteins and convert them to smaller molecules which will be used by the baby plant for growth. It is interesting to know that most of these stored compounds are not soluble into water therefore growing embryo unable to use them until they converted to smaller components by enzymes which are soluble (Miransari and Smith 2014).

As the germination process starts in the grains numerous phytochemical and physiological changes took place in which complex nutrient flux occur which includes degradation, remobilization and accumulation. Stored complex compounds e.g. starch, lipids and proteins are catabolized during germination which resultantly produced smaller molecules such as nitrogen and carbon which are used for the proper growth and photosynthesis by the plant (Theodoulou and Eastmond 2012). During germination process lot of nutrients and other bioactive compounds increased (Donkor et al. 2012), and reduced as these compounds consumed by the growing plant (Yang et al. 2001). It was given by Hung et al. (2012) that sprouted waxy wheat had improved and beneficial nutritive profile as compare to non-sprouted, as it contains high amount of dietary fiber, free amino acids and phenolics. In another study, it was reported that after 102 h germination at 20–25 °C grains contain 2 times high amount of α-tocopherol and 2–3 times more minerals (Ozturk et al. 2012). In same study 3.6-time higher amount of folate was found (Koehler et al. 2007). Another research conducted by Yang and co-workers (2001), reported that amount of ascorbic acid and tocopherol and β-carotene were not easy to detect in un-germinated wheat grains, while in the same grains these compounds were present in higher amounts and their amount continuously increasing after lengthening the time of germination and reached up-to optimum value after 7 days of germination. Values of vitamin C were 550 μg/g, and of α-tocopherol were 10.92 μg/g while for β-carotene were 3.1 μg/g. In the same study amounts of ferulic and vanillic acids were also noticeably enhanced, reached optimum level after 7 days of germination which became 932.4 μg/g and 12.9 μg/g, respectively.

Many variables such as variations in grain types, germination conditions along with laboratory technologies may confused the findings. Due to these variable results achieved after germination of same type of whole grain could be changed (decrease or increase) or unchanged. Such as amount of sugar were resulted both increase or decrease when compare with control group (un-germinated) while different results were showed by using different type of rice varieties. (Moongngarm and Saetung 2010). Related results were also found in another study in which antioxidant ability of sprouted rice resulted both increase and decrease, antioxidant activity was measured by using scavenging activity of the rice variety (Imam et al. 2012). Due to above mentioned reasons care should be taken during comparison of the results from other studies. It proved from previous discussion that optimum conditions might be varied with grain type, parameter to be studied and analytical techniques being used for analysis. The degree of the variation detected may change with other parameters like temperature, time, moisture, available oxygen for germination (Koehler et al. 2007), type of whole grain and environmental conditions (Mak et al. 2009).

If talk about life-cycle of the cereals grains it comprised upon two phases which were separated from each other by dormancy i.e. germination and development. During germination seeds play a vital role as a reproductive unit and this unit guarantees survival of all species of plants. There are three distinct parts of the wheat grains namely endosperm, bran layer (sometime called peripheral layer) which comprised of aleurone, nucleus tegument, testa and pericarp, and important part germ which is comprised of embryos and scutellum (Tasleem-Tahir et al. 2011). As the germination process starts complex compound present in grain like carbohydrates, protein and lipids are converted to simple and soluble molecules by the action of various enzymes which are present in the kernel. These enzymes usually stimulate upon germination. Mostly such enzymes present in upper aleurone layer, bran and in germ (Poutanen 1997). For example, amylase enzyme required to break-down complex starch usually present in the pericarp. Another important enzyme required to break-down complex protein (proteases) are present in endosperm, germ and aleurone layer. Lipoxygenase enzyme which break-down fats is dominantly present in embryo, while polyphenol oxidase and peroxidase are dominantly present in bran of seeds (Rani et al. 2011). These enzymes convert complex compounds into simple fractions which initiate the complex physiological and bio-chemical changes in the seeds.

16.6 Bioactive Compounds

A vast number of organic compounds are produced by the plants and these organic compounds didn't take part directly in growth and development of the plants. Conventionally, such compounds are called secondary metabolites and are found in various amounts in taxonomic group of plants. In contrast, secondary metabolites plant also produced primary metabolites such as acyl acids, phytosterols, organic acids and amino acids which are produced by all types of plants and participate essentially in development and growth of the plant (Hussain et al. 2012). Secondary metabolites which are limitedly produced by the plants have complex structure and biosynthetic pathways and conventionally these compounds didn't get much intentions of the researchers as these compounds are taken biologically insignificant (Parsaeimehr et al. 2011).

In contrast to biochemists, pharmaceutical researchers gave much intention to secondary metabolites (organic compounds) and since 1850s they intensively investigated their biochemical properties. Interestingly, investigation on these bioactive compounds was not just on academic level but on industrial level several compounds were developed from these bioactive components, few examples are dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and drugs. These compounds have huge biochemical properties due to which researchers focused to develop innovative drug, herbicides, insecticides and antibiotics (Grindberg et al. 2011).

Based on their synthetic pathways, these compounds are divided into three major categories (nitrogen containing compounds, terpenes and phenolics) (Krzyzanowska et al. 2010). Research had been conducted on their mechanism of synthesis including firstly the mevalonic pathway (to synthesize terpenes), secondly shikimic acid pathway or the mevalonic pathway (to synthesize phenolic compounds), thirdly tricarboxilic acid pathway (to synthesize nitrogen containing secondary metabolites). In pharmaceutical industry, vast number of products are produced by using these secondary metabolites (Parsaeimehr et al. 2011).

16.7 Phenolic Compounds

In numerous studies, (Table 16.3) polyphenolic compounds recognized as major antioxidant components of the whole grains (Zieli'nski and Kozłowska 2000). Radical compounds produced from oxidation are stabilized by the phenolic compounds as phenolic compounds are antioxidant in nature. Whole grains contain large amount of antioxidant compounds, few examples of such compounds are vitamins, sterols, and phenolics as well as phytic acid. Above mentioned compounds mostly found in bran or germ of the whole grain (Fardet et al. 2008). All these mentioned bioactive compounds are participated in degree of antioxidant properties and germination process also affect such compounds. Various in vitro studies showed the antioxidant effect of these compounds however, in vivo antioxidant effect is not completely recognized yet (Fardet et al. 2008). It is interesting to know that few bioactive compounds might act as synergists/antagonists, having antioxidant characteristics in-vitro, might not observed of such compounds in-vivo. Polyphenols primarily occur in both soluble or bound forms in plants but edible grains mostly have high ratio of bound phenolics (Agati et al. 2012).

Polyphenolic contents (secondary metabolites) produced by plants contains variety of structurally and functionally diverse compounds which are produced through the shikimate-phenylpropanoids flavonoids pathways (Krzyzanowska et al. 2010). Majority of the phenolic compounds show estrogenic activities, and defined as the compounds having one or more aromatic ring along with hydroxyl substituents, including many functional derivatives like esters, methyl ethers, glycosides, etc. (Parsaeimehr et al. 2011). Polyphenolic compounds could be divided into two categories, firstly soluble phenols e.g. phenolic acids, flavonoids and quinones, secondly insoluble phenols e.g. condensed tanins, lignins and cell wall bounded hydroxycinammic acids (Krzyzanowska et al. 2010).

Many types of compounds are included in polyphenolic compounds like furanocoumarins, lignin, flavonoids, isoflavonoids, and tannins. Another class of phenols flavonoids comprised of large and varied units of polyphenolic compounds found in plants. Among flavonoids vital group of compounds are flavonols comprised of quercetin, kaempferol, and isorhamnetin, other important group is flavones for example apigenin, luteolin, and chrysoeriol (Hounsome et al. 2008). Another class called phenolic acids contains gallic and caffeic acids, vanillic acid, cinnamic acid and coumaric acid (Krzyzanowska et al. 2010). Most commonly phenolic acids and flavonoids are found in sprouted seeds which have recognized as anti-oxidant and could be used as functional compounds (Fu et al. 2011). As germinated (sprouted) are rich source of poylphenolic compounds and other bioactive compounds therefore, these seeds could be the best alternative of fruits and vegetables in our healthy diets.

16.8 Germinated Grains and Polyphenols

Through several studies in cereal grains it was shown that germination process could enhance the amount of solvent-extractable phenolic compounds (Tables 16.1–16.3). Several authors suggested that increment of water soluble polyphenolic compounds during sprouting process can be attributed to the *de novo* synthesis and transformation (Kim et al. 2013; Tang et al. 2014). Negative impact of seed germination also observed, as many studies showed the reduction in polyphenolic content in sprouted seeds. And this contradictory result associated with increment in moisture content during germination process, during which soluble phenols may be wasted (Guo et al. 2012). These opposing results stated are mainly accredited to difference in soaking, temperatures, germination time and methods of drying the germinated grains. Increased phenolic contents and antioxidant activities has been stated through different *in vitro* studies in cereal grains, like wheat (Van Hung et al. 2011), sorghum (Donkor et al. 2012), buckwheat (Alvarez-Jubete et al. 2010), and rice (Imam et al. 2012).

Enhanced polyphenol oxidase activity (PPO) might be the reason of increased polyphenol contents in germinated grains as compared with non-germinated grain (Demeke et al. 2001). The increase in polyphenols during seed geminating could be due to solubilization of condensed tannins during water soaking and its movement to the outer layer during germination. During maize germination phytate contents

Table 16.1 Phenolic compounds in different germinated cereals sprouts

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Maize	Phenolic contents	UV spectrophotometer	Increased	Mihafu et al. (2017)
Barley	Total flavonoid content Total phenolic content Individual polyphenols (gallic acid, vanillic acid, catechin, epicatechin, chlorogenic acid, ferulic acid sinapic acid, myricetin, quercetin, kaempferol) Antioxidant activity	UV spectrophotometer HPLC DPPH & ABTS,	Increased	Aborus et al. (2017)
Soybean	Total flavonoid content Total phenolic content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Xue et al. (2016)
Buckwheat	Individual phenolic compound (qrientin, isoorientin, vitexin, isovitexin, rutin)	HPLC	Increased	Nam et al. (2015)
Wheat	Total phenolic content	UV spectrophotometer	Increased	Van Hung et al. (2015)
Soybean	Total phenolic content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Koo et al. (2015)
Soybean	Antioxidant activity	UV spectrophotometer	Increased	Victoria et al. (2015)
Wheat	Total phenolic content Individual phenolic compound (ferulic acid, isoferulic acid) Antioxidant activity	UV spectrophotometer HPLC FRAP, DPPH, ABTS	Increased	Zilic et al. (2014)
Finger millet	Polyphenols content	UV spectrophotometer HPLC	Increased	Sudha and Usha (2014)
Barley, Oat, Wheat	Polyphenol content Antioxidant activity	UV spectrophotometer FRAP	Increased	Panfil et al. (2014)
Soybean	Total phenolics Antioxidant activity Isoflavone content (daidzein, glycitein, genistein, total aglycones, daidzin, glycitin, genistin)	UV spectrophotometer DPPH HPLC	Increased	Huang et al. (2014)

(continued)

Table 16.1 (continued)

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Rye, Sorghum, Brown rice, Wheat, Oat, Barley, Buckwheat	Total polyphenol content Individual polyphenol content (gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, *p*-coumaric acid, ferulic acid, luteolin) Antioxidant activity	UV spectrophotometer HPLC DPPH	Increased	Donkor et al. (2012)
Rice	Total polyphenol content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Imam et al. (2012)
Soybean	Total phenolic content Antioxidant activity	UV spectrophotometer FRAP	Decreased (fresh weight basis)	Shohag et al. (2012)
Waxy wheat	Total phenolic compounds Antioxidant activity	UV spectrophotometer DPPH	Increased	Hung et al. (2012)
Wheat	Total polyphenol content Phenolic compounds profile (hydroxybezoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic) Antioxidant activity	UV spectrophotometer UPLC DPPH	Increased	Van Hung et al. (2011)
Rice	Total polyphenol content	UV spectrophotometer	Non-significant change	Moongngarm and Saetung (2010)
Amaranth, Buckwheat, Quinoa, Wheat	Total polyphenol content Individual polyphenol content (protocatechuic acid, protocatechuic acid, vanillic acid derivative, vanillic acid, caffeic acid derivative, syringic acid derivative, caffeic acid, caffeic acid derivative, 3-coumaric acid derivative, ethyl gallate) Antioxidant activity	UV spectrophotometer HPLC DPPH, FRAP	Increased	Alvarez-Jubete et al. (2010)
Rice	Ferulic acid	HPLC	Increased	Banchuen et al. (2009)

Table 16.2 Phenolic compounds in different germinated legume sprouts

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Adzuki bean	Total phenolic content Total flavonoid content Phenolic acids content Antioxidant activity	UV Spectrophotometer DPPH, ABTS	Increased	Złotek et al. (2015)
Mung bean	Total phenolic content Antioxidant activity	UV Spectrophotometer FRAP	Increased Decreased	Tiwari et al. (2017)
Faba bean, White bean, Chickpea, Lentil, Fenugreek seeds	Total phenols Total flavonoids Individual phenolic compounds (ferulic, pyrogallol, protocatechuic, catechin, syringic, epicatechin, vanillic, gallic, caffeic, chlorogenic, ellagic and coumarin) Total antioxidant	UV Spectrophotometer HPLC DPPH	Increased	Salem et al. (2014)
Pigeon pea	Total phenolic content Antioxidant activity	UV Spectrophotometer DPPH	Increased	Uchegbu and Ishiwu (2016)
Mung bean	Total Phenolics Antioxidant activity	UV Spectrophotometer DPPH	Increased	Kim et al. (2012)
Mung bean	Total Phenolics Total flavonoid Individual phenolic compounds (quercetin, myricetin, quercetin-3-O-glucoside) Antioxidant activity	UV Spectrophotometer HPLC-UV Hydro-PSC	Increased	Guo et al. (2012)
Green mung, Arhar, Masur, Chickpea	Total phenolic content Antioxidant activity	Colorimetric method DPPH	Decreased Increased	Singh et al. (2014)
Lentil	Total phenolic compounds Antioxidant activity	UV Spectrophotometer Oven test method	Increased	Gharachorloo et al. (2012)
White bean, Common vetch, Lentil, Chickpea	Total phenolic contents Antioxidant activity	UV Spectrophotometer Oven test method	Increased	Gharachorloo et al. (2013)
Black gram, Desi chickpea, Cowpea, Yellow mustard	Total antioxidant Total phenol content Total flavonoid content	DPPH UV Spectrophotometer	Increased	Khyade and Jagtap (2016)

(continued)

Table 16.2 (continued)

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Green mung bean, Black mung bean	Total phenol content Individual phenolic compounds (gallic acid, *p*-coumaric acid, catechin, rutin, vitexin and isovitexin) Antioxidant activity	UV Spectrophotometer HPLC ABTS, FRAP	Increased	Gan et al. (2017)

reduced which could be credited to the increased activity of endogenous enzyme phytase, which hydrolyzes phytic acid (Pawar et al. 2006). The level of the phytate breakdown depends upon several factors like grain variety, germination stage, pH, moisture level, temperature, solubility of phytate and the presence of certain inhibitors (Egli et al. 2002). In a study conducted by Liukkonen et al. (2003) rye grains were germinated for 6 days at different temperatures like 5, 10 and 25 °C all treatments exhibited an increment in methanol-extractable phenolic compounds but highest increase was observed in samples germinated at 25 °C. The reason suggested for this increase was that synthesis or activation of a variety of hydrolytic enzymes started in grains through germination which caused different alterations in structure or synthesis of new compounds having high bioactivity and nutritional value (Wang et al. 2014).

In other study Tian et al. (2004) found that during germination for 24 h in brown rice main soluble phenolic compounds including 6-*O*-feruloylsucrose and 6-*O*-sinapoylsucrose were decreased, while the free compounds including ferulic acid and sinapinic acid increased significantly. In soybean and mung bean after germination total phenolics content in seeds and sprouts expressed on a fresh weight basis (Shohag et al. 2012) decreased compared with other seed contents due to dilution effect of phenolics after imbibition and growth, and due to increased water absorption. While in case of dry weight basis the total phenolics content increased with germination time, because moisture content is eliminated (Cevallos-Casals and Cisneros-Zevallos 2010).

Total antioxidant activity has been generally associated with increased antioxidant activity in a variety of grains (Imam et al. 2012). Avenanthramides are phenolic compounds exclusively found in oats. They have been reported to contribute to the fresh taste of oat products. An increase in these compounds by about 20% upon germination was reported (Skoglund et al. 2008). Tannins, phenolic compounds with high molecular weight, have traditionally been nutritionally negative, as they can form insoluble complexes with proteins and can complex minerals. On a positive note, they contribute to the antioxidant activity and the breakdown products also have antioxidant properties. During germination, tannins are partially broken down, as reported for millet (Hemalatha et al. 2007), and sorghum (Dicko et al. 2005).

Table 16.3 Commonly present phenolic acids in cereal grains

Phenolic acids	Predominant	Major	Low amount	References
Cinnamic acid	–	Millet	Wheat, sorghum, oat	Dykes and Rooney (2006, 2007) and Shahidi and Naczk (2004)
Caffeic acid	–	Oat, sorghum, rice, rye, barley, wheat	Maize, rye, millet	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
p-Coumaric acid	–	Maize, wheat, barley, rye, sorghum, millet, rice, oat	–	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Ferulic acid	Maize, wheat, barley, rye, millet, sorghum, rice	Oat	–	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Gallic acid	–	Rice	Sorghum, millet, oat, wheat	Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
p-Hydroxy benzoic acid	Barley	Oat, rice, millet	Wheat, maize, rye, sorghum	Beta et al. (2012), Cai et al. (2011), Dykes and Rooney (2007), and Laokuldilok et al. (2010)
Protocatechuic acid	–	Rice, sorghum	Millet, rye, barley, oat, maize	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Sinapic acid	–	Rye, rice	Wheat, maize, barley, sorghum, millet, oat	Beta et al. (2012), Cai et al. (2011), and Laokuldilok et al. (2010)
Salicylic acid	–	–	Wheat, barley, sorghum	Dykes and Rooney (2007) and Kim et al. (2006)
Syringic acid	–	Sorghum, wheat, maize	Oat, millet, barley	Dykes and Rooney (2007), Kim et al. (2006), and Zhang and Hamaker (2012)
Vanillic acid	–	Barley, oat, millet, wheat	Sorghum, rye, maize	Beta et al. (2012), Cai et al. (2011), and Zhang and Hamaker (2012)

Among whole seeds plant, legumes have very wide range of antioxidant activity, as it relies upon variety and origin of plant. While if we review the impact of seed germination and technological processing also effect the inherent ant-oxidant activity of the seeds of legumes for example phenolics, tocopherols, vitamin C etc. Currently researchers greatly interested in function and efficiency of anti-oxidants present in food components which increase the attentions to test the anti-oxidant activity of foods. Therefore, some researched had worked on the germination effect upon nutritional profile of legumes **(**Gharachorloo et al. 2012). Lentil sprout flour could be the extremely good source of natural anti-oxidants as germination modify phenolic compounds therefore, their anti-oxidant activity also improved. Research data showed that germination process improves the quantity and quality of polyphenolic compounds in legumes (López-Amorós et al. 2006).

Sprouting is a natural process improving the nutritional value of legumes due to increasing phenolic contents. According to one study (Tiwari et al. 2017) of mung beans it may be possible that germination caused enzymatic degradation of carbohydrates which in results increased polyphenols production (Perron and Brumaghim 2009). Results of this study showed decreases the antioxidant activity because boiling of sprouted mung bean may cause denaturation of proteins and enzymes having antioxidative properties. According to several other studies researchers shown that during germination high temperature causes decrease in many vitamins, protein contents and secondary metabolites in food grains (Soris et al. 2010). In another study conducted by Salem et al. (2014) showed an increase in total flavonoids, total phenolics and total antioxidant activity. Increase in phenols may be due to solubilization of condensed tannin during soaking process and relocation of phenols to the outer layer during germination as appeared through browning of the germinated seeds (Sokrab et al. 2012).

But further study requires describing the composition of the seed extract for identification and determination the level of biologically active compounds. Further data required to shows the effect of food processing technologies and various physiological process (digestion) on the availability and stability of these functional and bio-active compounds for using lentils for food supplement (Malik and Kapoor 2015). A large no of research had been conducted in past which shows a direct relation with consumption of foods and reduction in risk of many diseases like cardiovascular and cancer (Fernandez-Orozco et al. 2009). As many health benefits are related with anti-oxidants compounds of the foods, therefore pulses are intensively studied for their anti-oxidant activity. Furthermore, data also present on modification of their anti-oxidant components while processing which also create interest in carrying research. Many compounds are present on pulses which shows anti-oxidant activity, major compounds are ascorbic acid and tocopherol, phenolic compounds and reduced glutathione. From many years sprouted seeds had been used in healthy diet as they provide phytochemicals which have ability to reduce the incidence of various diseases (Fernandez-Orozco et al. 2006).

16.9 Future Perspective

All over the world cereal grains and legumes remain an important staple diet making considerable involvement to intake of different nutrients including carbohydrates, proteins, vitamins, minerals, and fiber as well as different bioactive compounds (Gani et al. 2012). Different health benefits provided by cereals grains and legumes are due to these bioactive compounds present in them. These compounds having covalent bounding with indigestible polysaccharides are key source of antioxidants in legumes and cereal grains. Due to interference created by these covalent bonds, most of phenolic compounds are not properly bioavailable to body because they are not available for enzymatic digestion in human gastrointestinal tract. However, their bioavailability could be improved by increasing their availability to enzymes in gastrointestinal tract mainly through decreasing particle size, interruption of structural matrices, and their release from matrices by using appropriate processing techniques (Wang et al. 2014).

Different processing techniques their parameters and grain types affect the bioavailability of phenolic compounds from cereals and legumes. The nutritional profile of grains can be improved through germination leading to products with better nutritional and sensory properties having improved bioavailability in body as compared to raw form grains. Major objective of germination is to activate hydrolytic enzymes from their inactive form in raw seeds (Ayernor and Ocloo 2007). Moreover, as germination has been claimed of improving the nutritional quality of grains through softening their structure and reducing antinutritional factors so have potential for the industrial applications in development of new ingredients, reengineer processes, and products (Tian et al. 2010).

More research work is required for isolation and characterization of these bioactive compounds after germination from sprouts contributing to beneficial health effect. Research is also desired in determination of metabolism, bioavailability and health influence of these compounds in humans because grains contain varied range of compounds having antioxidant potential.

16.10 Conclusion

Seed germination an important economical processing technique which improves nutritive value and profile of bioactive compounds in grains by removing antinutritional factors and enhancing their digestibility. In addition to changing the nutritional level and biochemical activities, germination process through enzymatic actions also produces bioactive compounds and increases antioxidant activity having health-promoting activities. Germinated biologically stimulated grains should be widely recognized and used as functional foods due to their nutritional and health related benefits. The biological stimulation in grains through germination can enhance their functional properties which could be further used to formulate new healthy functional food products.

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Chapter 17
Mushroom: Nutraceutical, Mineral, Proximate Constituents and Bioactive Component

Sakshi Painuli, Prabhakar Semwal, and Chukwuebuka Egbuna

17.1 Introduction

The kingdom Fungi is a diverse group of decomposers which majorly includes moulds, yeasts and mushrooms. Mushrooms are found worldwide and are also known to be the earliest form of fungi to mankind (Okhuoya et al. 2010). Generally, they are the fruiting bodies of macroscopic filamentous fungi which are usually known for high nutritional as well as medicinal properties. There are several evidences in history which shows that mushrooms have been consumed by mankind since a long back in the form of food or medicine. The ancient civilizations of Greek, Romans, Egyptians, Japanese, Chinese and Mexicans prized mushrooms for their medicinal properties and consumed them as dietary supplement or medicinal food (Agrawal and Dhanasekaran 2019; Chang and Zhao 2002; Guzmán 2015; Hobbs 2002). According to Greeks, mushrooms are the source of strength for soldiers in battle, whereas Romans believed them as "God's food" and Chinese

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consider them as a health food or a medicine of life. Many researchers have studied and documented that mushrooms are the reservoir of variety of nutraceuticals, nutriceuticals and bioactive compounds. During the last few decades, consumption and cultivation of mushrooms increased continuously as nutritional resource. Internationally, China is the biggest producer of mushrooms, whereas *Agaricus bisporus* is highly cultivated mushroom species followed by *Pleurotus* spp., and *Flammulina velutipes* (Valverde et al. 2015). Mushrooms are important inclusions for human diet as they contain significant amount of proximate constituents, prominent amount of minerals, proteins, vitamins and low fat contents along with several medicinally important active components (Kalač 2013; Isabel CFR Ferreira and Heleno 2017; Beelman et al. 2019). Different species of mushrooms are recognized to possess different medicinal properties like anti-tumor, neuroprotective, anti-oxidant, anti-hypoglycemic, anti-cancer, anti-bacterial, immunomodulatory, anti-inflammatory, anti-viral, anti-atherosclerotic properties, etc. (Wani et al. 2010; Thatoi and Singdevsachan 2014; Kim et al. 2008; Khatun et al. 2012; Gaglarirmak 2011; Elkhateeb et al. 2019; Cohen et al. 2014). The chapter presents an overview of the research on the nutraceutical, mineral, proximate constituents and bioactive component from beneficial mushrooms.

17.2 Proximate Analysis and Mineral Composition

The proximate composition including moisture content, crude fat content, ash content, carbohydrate content, total sugar content and energy value are given in Table 17.1. Many reports have been documented the proximate composition of edible and wild mushrooms. The water content of mushroom varies greatly, as their fruiting body get influenced by the type of species, growth and climatic condition, collection strategy and storage measures. The moisture content of mushrooms have been recorded from a range of 2.71 to 93.25 g/100 g dry weight. The lowest and highest moisture content has been recorded in *Astraeus hygrometricus* and *Pholita microsporeo,* respectively (Meng et al. 2019; Pavithra et al. 2018). Few species such as *Agaricus albertii*, *Termitomyces clypeatus*, *Russula delica*, *Hygrophorus chrysodon* and *Suillus variegates* have also showed high moisture content. The ash content generally estimates the mineral content and the value usually varies between 2.80 and 33.10 g/100 g dry weight for different mushrooms. The lowest and highest ash content has been assessed in *Ganoderma lucidum* and *Lycoperdon umbrinum* (Pereira et al. 2012; Stajic et al. 2013). While, the content of crude fat ranges between 0.18 and 17.52 g/100 g dry weight in *Bovista aestivalis* and *Amanita princeps* (Pereira et al. 2012; Srikram and Supapvanich 2017).

Carbohydrates are the major composition of mushrooms; they are basically present in the form of glycoproteins or polysaccharides. The highest and lowest carbohydrate content has been detected in *Boletus edulis* (81.00 g/100 g dry weight) and *Lepista inversa* (10.35 g/100 g dry weight), while some heterogeneity

Table 17.1 Nutraceutical and proximate profiling of few selected species of mushrooms from different regions

Species name	Moisture	Crude fat	Crude protein	Ash	Carbohydrate	Energy	Total sugar	Country	References
Agaricus albertii	90.73	1.38	19.83	22.13	56.66	318.36	05.98	Portugal	Reis et al. (2014)
Agaricus bisporus	91.64	1.67	15.43	11.36	71.53	345.10	51.08	Portugal	Reis et al. (2012)
Agaricus bisporus	–	1.56	29.14	–	51.05	–	–	India	Ahlawat et al. (2016)
Agaricus blazei	–	–	31.6	06.20	48.00	325.30	39.50	Portugal	Taofiq et al. (2019)
Agaricus comtulus	87.94	0.46	21.29	28.14	50.11	289.74	18.99	Portugal	Pereira et al. (2012)
Agaricus excellens	87.72	1.37	14.47	29.64	54.52	288.29	01.51	Portugal	Reis et al. (2014)
Agaricus subrufescens	–	02.20–15.70	37.20–50.40	–	18.00–23.90	–	–	China	Liu et al. (2019)
Amanita calyptroderma	87.43	1.46–11.61	3.58–28.49	1.34–11.82	3.56–22.16	–	–	Thailand	Srikram and Supapvanich (2017)
Amanita curtipes	80.00	8.60	06.40	17.20	67.80	347.00	15.10	Portugal	Fernandes et al. (2015)
Amanita princeps	89.67	1.81–17.52	3.16–30.59	0.47–4.55	2.18–17.04	–	–	Thailand	Srikram and Supapvanich (2017)
Amanita rubescens	–	–	21.8	7.0	–	–	–	India	Lalotra et al. (2018)
Astraeus hygrometricus	2.71–2.93	–	16.80–17.30	18.43–15.53	46.17–48.42	–	–	India	Pavithra et al. (2018)
Astraeus odoratus	84.15	1.16–7.32	4.18–26.37	0.98–10.17	3.91–20.68	–	–	Thailand	Srikram and Supapvanich (2017)
Boletus edulis	–	2.23	10.65	05.26	81.86	390.09	14.38	Portugal	Heleno et al. (2015c)
Boletus edulis	–	–	31.3	6.4	–	–	–	India	Lalotra et al. (2018)
Boletus erythropus	88.36	0.75	20.92	25.90	52.44	300.15	34.46	Portugal	Grangeia et al. (2011)
Boletus porosporus	65.57	0.96	15.74	04.20	79.11	388.01	41.26	Portugal	Leal et al. (2013)
Boletus regius	79.15	1.59	05.22	04.40	88.79	390.36	20.95	Portugal	Leal et al. (2013)
Bovista aestivalis	23.23	0.18	15.59	31.86	52.37	273.44	00.38	Portugal	Pereira et al. (2012)

(continued)

Table 17.1 (continued)

Species name	Moisture	Crude fat	Crude protein	Ash	Carbohydrate	Energy	Total sugar	Country	References
Catathelasma ventricosum	89.62	1.52	29.13	12.59	56.75	362.42	–	China	Liu et al. (2012b)
Chlorophyllum rhacodes	88.28	3.29	19.32	12.10	65.29	368.03	44.00	Portugal	Pereira et al. (2012)
Clavariadelphus pistillaris	84.22	0.59	16.27	20.77	62.37	319.88	25.36	Portugal	Pereira et al. (2012)
Clitocybe subconnexa	–	1.02	07.42	05.98	27.35	381.18	30.71	Portugal	Heleno et al. (2015a)
Coprinus comatus	–	1.80	11.84	10.07	76.29	368.72	07.25	Netherlands	Stojković et al. (2013)
Cortinarius praestans	89.16	2.58	14.56	18.89	63.98	337.34	60.88	Portugal	Pereira et al. (2012)
Craterellus cornucopioides	–	4.87	47.21	10.08	–	413.46	14.08	Croatia	Beluhan and Ranogajec (2011)
Dictyophora indusiata	–	2.6–2.7	01.90–19.50		40.20–41.60	–	–	China	Liu et al. (2019)
Entoloma clypeatum	–	6.21	27.98	09.21	–	374.80	21.50	Croatia	Beluhan and Ranogajec (2011)
Flammulina velutipes	–	6.45	27.95	07.39	–	343.69	30.10	Croatia	Beluhan and Ranogajec (2011)
Ganoderma amboinense	–	0.8–1.0	12.70–14.60	–	45.00–61.00	–	–	China	Liu et al. (2019)
Ganoderma lucidum	–	4.43	11.34	02.80	81.48	410.93	00.75	Netherlands	Stojković et al. (2014)
Geopora arenicola	–	–	23.8	9.9	–	–	–	India	Lalotra et al. (2018)
Gyromitra esculenta	85.68	0.73	14.74	32.10	52.43	275.23	06.13	Portugal	Leal et al. (2013)
Heimiella retispora	88.56	1.63–14.25	2.73–23.86	0.39–3.41	2.33–20.37	–	–	Thailand	Srikram and Supapvanich (2017)
Helvella lacunosa	82.37	2.40	04.40	21.70	71.50	325.21	04.36	Portugal	Leal et al. (2013)
Hericium coralloides	–	2.38	07.25	09.31	81.06	374.67	10.79	Portugal	Heleno et al. (2015b)

Hericium erinaceum	–	1.75	15.40	3.49	79.39	394.79	23.63	Portugal	Heleno et al. (2015b)
Hygrophorus chrysodon	92.09	3.48	15.11	26.91	54.51	309.74	07.27	Portugal	Pereira et al. (2012)
Laccaria amethystea	86.13	2.78	29.84	10.31	57.07	377.70	–	China	Liu et al. (2012b)
Lactarius citriolens	–	5.37	10.89	06.99	76.76	398.89	08.76	Portugal	Vieira et al. (2014)
Lactarius turpis	–	2.06	13.06	07.21	77.68	381.47	19.54	Portugal	Vieira et al. (2014)
Lentinula edodes	–	1.22	18.85	–	63.60	–	–	India	Ahlawat et al. (2016)
Lentinus edodes	9.00	0.67	19.50	4.82	45.42	–	–	Korea	Olawuyi and Lee (2019)
Lentinus edodes	–	1.14	16.00	06.24	76.62	380.74	15.61	Portugal	Heleno et al. (2015a)
Lycoperdon umbrinum	71.98	0.37	14.53	33.14	51.96	269.29	01.46	Portugal	Pereira et al. (2012)
Macrolepiota procera	–	2.23	24.22	05.37	–	389.46	24.40	Croatia	Beluhan and Ranogajec (2011)
Morchella deliciosa	–	–	25.6	7.0	–	–	–	India	Lalotra et al. (2018)
Morchella esculenta	90.79	2.59	11.52	11.34	74.55	367.56	15.66	Portugal	Heleno et al. (2013)
Pholita microsporeo	87.58–93.25	0.71–1.15	19.22–21.22	–	–	–	26.13–33.53	China	Meng et al. (2019)
Pleurotus eous	–	1.05	19.59	–	64.34	–	–	India	Ahlawat et al. (2016)
Pleurotus eryngii	82.59	4.36	02.09	14.95	78.60	362.00	15.63	Portugal	Reis et al. (2014)
Pleurotus geesteranu		1.71–2.26	32.70–39.80	–	29.00–42.80	–	–	China	Liu et al. (2019)
Pleurotus giganteus	–	3.10	19.20	–	64.70	364.00	–	Malaysia	Phan et al. (2019)
Pleurotus ostreatus	78.28	–	28.40	16.68	52.74	–	–	Nigeria	Ikon et al. (2019)
Pleurotus ostreatus	–	2.06–2.19	21.10–36.40	–	34.70–50.70	–	–	China	Liu et al. (2019)
Pleurotus sajorcaju	–	1.98–2.7	23.20–35.50	–	40.1–49.4	–	–	China	Liu et al. (2019)
Ramaria aurea	79.99	1.24	10.33	12.75	75.68	355.18	11.85	Portugal	Leal et al. (2013)

(continued)

Table 17.1 (continued)

Species name	Moisture	Crude fat	Crude protein	Ash	Carbohydrate	Energy	Total sugar	Country	References
Russula alboareolata	86.35	0.63–4.61	4.08–29.90	1.32–10.99	3.27–22.62	–	–	Thailand	Srikram and Supapvanich (2017)
Russula cyanoxantha	89.45	0.83–7.87	5.19–49.20	0.27–2.56	1.01–9.56	–	–	Thailand	Srikram and Supapvanich (2017)
Russula delica	92.00	3.4	13.80	08.80	74.00	363.00	10.30	Portugal	Fernandes et al. (2014)
Russula emetic	87.57	0.49–3.94	4.13–33.24	1.03–8.29	3.37–27.09	–	–	Thailand	Srikram and Supapvanich (2017)
Russula virescens	86.51	1.69–12.54	3.98–29.50	0.87–5.40	2.73–27.67	–	–	Thailand	Srikram and Supapvanich (2017)
Russula virescens	92.49	1.85	21.85	11.04	62.27	365.09	11.10	Portugal	Leal et al. (2013)
Sparassis crispa	–	–	16.2	4.9	–	–	–	India	Lalotra et al. (2018)
Suillus variegates	90.77	3.31	17.57	15.36	63.76	355.12	04.85	Portugal	Pereira et al. (2012)
Termitomyces clypeatus	90.13	0.78–7.90	2.60–26.34	0.29–2.94	2.73–27.67	–	–	Thailand	Srikram and Supapvanich (2017)
Volvariella volvacea	–	0.97	38.10	–	42.30	–	–	India	Ahlawat et al. (2016)
Volvopluteus gloiocephalus	–	4.62	19.66	14.19	13.97	366.34	03.37	Portugal	Heleno et al. (2015a)
Xerocomus badius	–	4.22	08.08	07.32	80.38	391.83	11.77	Portugal	Heleno et al. (2015a)

has also observed between species (Leal et al. 2013). The energy value for mushrooms ranges between 269 and 413.46 kcal/100 g dry weight in *Lycoperdon umbrinum* and *Craterellus cornucopioides* (Beluhan and Ranogajec 2011; Pereira et al. 2012). The two major forms of sugar detected in mushrooms are mannitol and trehalose. Basically, mannitol contributes in growth and firmness of fruiting body and can vary from species to species, it is abundantly present in wild mushrooms (Kalač 2009). In wild mushrooms, mannitol ranges between 11.03 and 43.34 g/100 g dry weight, the lowest and highest values has been observed in *Lyophyllum decastes* and *Clavariadelphus truncates*, whereas in cultivated species the highest amount of mannitol has detected in *Agaricus bisporus* (64.15 g/100 g dry weight) and the lowest amount has detected in *Lentinula edodes* (49.51 g/100 g dry weight), respectively (Pereira et al. 2012; Reis et al. 2012). Trehalose, found profusely from a range of 14.21 to 25.57 g/100 g dry weight in *Pleurotus eryngii* and *Chlorophyllum rhacodes*, respectively, however in cultivated mushrooms the value of trehalose has detected to be 72.82, 42.82 and 60.51 g/100 g dry weight, the lowest and highest has been recorded in *Pleurotus eryngii*, *Coprinus comatus* and *Cortinarius praestans*, respectively (Pereira et al. 2012; Reis et al. 2012; Vaz et al. 2011). Other than mannitol and trehalose, low content of other sugars like sucrose, fructose, mannose, and arabinose has also been detected in mushrooms.

Mushrooms (wild or cultivated), are capable of accumulating micro and macro minerals in their fruiting bodies to perform several functions. The trace mineral contents for different species of mushrooms are given in Table 17.2. Few minerals like, Iron (Fe), Phosphorus (P) and Potassium (K) are abundantly present in mushroom fruiting bodies (Wang et al. 2014). The other minerals detected in mushrooms includes Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn) and Copper (Cu). The mineral content in the fruiting body depends on the type of species or on the substrate on which they are cultivated. The highest and lowest potassium content has been detected in *Macrocybe gigantea* and *Volvopluteus gloiocephalus* from a range of 1300 to 46,926 μg/g dry weight (Heleno et al. 2015a). While, the highest and lowest phosphorus content has been estimated in *Lentinus cladopus* and *Stropharia rugosoannulata* from 1005 to 7290 μg/g dry weight, respectively (Liu et al. 2012b; Mallikarjuna et al. 2012). For other elements like Mg, Cu, Mn, Fe and Zn, a huge variation in values have been reported, ranging from 88 to 2289, 1.53 to 88.8, 0.03 to 103.9, 0.02 to 6762 and 0.94 to118.84 μg/g dry weight, respectively. Along with beneficial minerals some toxic minerals like, Chromium (Cr), Lead (Pb), Cadmium (Cd) and Arsenic (As) have also been detected in fruiting bodies. These toxic elements are absent or very less in the cultivated species of mushrooms (Mallikarjuna et al. 2012).

Table 17.2 Trace elements in few selected mushrooms from different regions

Name of species	Mg	Cu	Mn	Fe	Zn	Pb	Co	Cr	Country	References
Agaricus bisporus	–	1.54	–	5.14	2.55	0.07	–	0.07	Spain	Rubio et al. (2018)
Agaricus bisporus	–	–	7.97	85.86	79.64	–	–	–	India	Ahlawat et al. (2016)
Agrocybe cylindracea	630–851	–	–	–	–	–	–	20.5–21.1	Poland	Siwulski et al. (2019)
Amanita caesarea	833.1	19.32	47.99	356.90	65.65	0.09	0.75	1.23	Greece	Ouzouni et al. (2009)
Amanita rubescens	–	39.2	33.6	105	52.3	–	–	–	India	Lalotra et al. (2018)
Armillaria mellea	1063.1	17.38	55.59	499.00	54.12	0.49	0.16	4.20	Greece	Ouzouni et al. (2009)
Armillaria tabescens	1150.7	17.47	11.18	60.40	64.45	0.79	0.14	4.37	Greece	Ouzouni et al. (2009)
Boletus aereus	0.14–0.18	0.75–0.96	0.01–0.02	0.003–0.10	0.94–1.19	–	–	–	Italy	Alaimo et al. (2018)
Boletus aureus	755.1	41.47	18.31	112.80	89.45	0.09	0.18	0.86	Greece	Ouzouni et al. (2009)
Boletus edulis	–	34.4	54.4	812	96.3	–	–	–	India	Lalotra et al. (2018)
Boletus griseus	200.0	52.00	63.00	47.00	94.00	–	1.70	0.84	China	Liu et al. (2012a)
Boletus griseus	–	29.1–35.3	16.2–16.6	484–523	75.9–121.5	–	1.2–1.8	–	China	Wang et al. (2017)
Boletus impolitus	–	9.8–16.2	16.2–41.5	672–1831	38.7–78.1	–	5.2–12.7	–	China	Wang et al. (2017)
Boletus luridus	–	26.6–34.2	17.2–18.5	288–371	118–164.5	–	3.5–8.3	–	China	Wang et al. (2017)
Boletus reticulatus	–	16.5–33.8	29.8–34.7	516–714	41.7–108.5	–	3.9–4.3	–	China	Wang et al. (2017)
Boletus speciosus	110.0	28.00	02.00	78.00	50.00	–	1.00	0.45	China	Liu et al. (2012a)

Boletus speciosus	–	32.5–70.4	12.5–13.3	568–581	115.2–187.4	–	2.8–3.7	–	China	Wang et al. (2017)
Boletus umbriniporus	–	36.3–62.6	29.2–36.4	591–816	71.1–172.9	–	4.9–7.5	–	China	Wang et al. (2017)
Cantharellus cibarius	–	16.7–56.1	–	–	69.3–94.0	0.21–0.89	–	–	Slovakia	Árvay et al. (2019)
Catathelasma ventricosum	1538.0	38.00	09.00	673.00	88.00	–	–	–	China	Liu et al. (2012b)
Clitocybe subconnexa	148.86	05.22	0.10	06.53	06.37	–	–	–	Portugal	Heleno et al. (2015a)
Clitocybe maxima	520.0	52.00	33.00	308.00	127.00	1.44	–	–	China	Liu et al. (2012b)
Clitocybe maxima	1846–2289	–	–	–	–	–	–	30.0–34.1	Poland	Siwulski et al. (2019)
Clitopilus prunulus	0.38	0.50	0.03	0.02	1.27	–	–	–	Italy	Alaimo et al. (2018)
Coprinus atramentarius	–	57.12	64.20	1183.60	288.40	–	–	–	Turkey	Bengu (2019)
Craterellus cornucopioides	978.0	43.00	27.00	413.00	61.00	–	–	–	China	Liu et al. (2012b)
Flammulina velutipes	1058–1143	–	–	–	–	–	–	20.8–21.9	Poland	Siwulski et al. (2019)
Ganoderma lucidum	427–632	–	–	–	–	–	–	14.9–17.4	Poland	Siwulski et al. (2019)
Geopora arenicola	–	88.8	50.5	267	36.5	–	–	–	India	Lalotra et al. (2018)
Hericium coralloides	134.00	00.72	0.31	77.96	04.76	–	–	–	Portugal	Heleno et al. (2015b)
Hericium erinaceus	85.57	00.22	0.09	06.77	02.11	–	–	–	Portugal	Heleno et al. (2015b)
Imleria badia	–	21.4–43.8	–	–	62.7–140.0	0.07–0.33	–	–	Slovakia	Árvay et al. (2019)
Laccaria amethystea	1482.0	36.00	35.00	211.00	59.00	0.74	–	–	China	Liu et al. (2012b)
Lactarius deliciosus	–	1.64	–	10.9	2.32	0.08	–	0.16	Spain	Rubio et al. (2018)

(continued)

Table 17.2 (continued)

Name of species	Mg	Cu	Mn	Fe	Zn	Pb	Co	Cr	Country	References
Lactarius hygrophoroides	140.0	28.00	3.70	28.00	16.00	–	2.10	1.50	China	Liu et al. (2012a)
Laetiporus sulphureus	–	5.00	19.36	162.92	28.36	–	–	–	Turkey	Bengu (2019)
Leccinum rugosiceps	–	15.1–35.7	19.3–19.7	323–449	62.1–91.6	–	0.7–2.1	–	China	Wang et al. (2017)
Lentinula edodes	–	1.53	–	10.5	2.23	0.09	–	0.15	Spain	Rubio et al. (2018)
Lentinula edodes	856–942	–	–	–	–	–	–	15.6–18.4	Poland	Siwulski et al. (2019)
Lentinula edodes	–	–	17.48	37.55	89.63	–	–	–	India	Ahlawat et al. (2016)
Leucopaxillus giganteus	84.0	50.00	60.00	510.00	85.00	–	0.72	6.30	China	Liu et al. (2012a)
Macrocybe gigantean	550.0	13.00	5.90	79.00	160.00	–	0.29	0.65	China	Liu et al. (2012a)
Melanoleuca arcuate	230.0	22.00	1.40	22.00	38.00	–	1.60	2.50	China	Liu et al. (2012a)
Morchella deliciosa	130.0	55.00	70.00	42.00	58.00	–	0.51	5.90	China	Liu et al. (2012a)
Morchella deliciosa	–	33.4	53.3	213	117	–	–	–	India	Lalotra et al. (2018)
Mycena haematopus	270.0	23.00	24.00	180.00	54.00	–	0.63	1.50	China	Liu et al. (2012a)
Pholiota nameko	–	1.73	–	10.9	1.93	0.08	–	0.10	Spain	Rubio et al. (2018)
Pleurotus eous	–	–	6.47	183.07	162.18	–	–	–	India	Ahlawat et al. (2016)
Pleurotus eryngii	699–1228	–	–	–	–	–	–	19.0–21.0	Poland	Siwulski et al. (2019)
Pleurotus ostreatus	–	1.99	–	11.0	2.91	0.10	–	0.17	Spain	Rubio et al. (2018)
Pulveroboletus ravenelii	210.0	58.00	58.00	370.00	34.00	–	0.65	5.60	China	Liu et al. (2012a)
Sparassis crispa	–	25.5	41.0	555	137	–	–	–	India	Lalotra et al. (2018)
Stropharia rugosoannulata	1135.0	29.00	59.00	195.00	102.00	0.07	–	–	China	Liu et al. (2012b)
Suillus luteus	421–1210	–	–	–	34.7–141	–	–	–	Germany	Zocher et al. (2018)

Suillus luteus	–	27.8–36.2	–	–	92.3–98.8	0.16–0.34	–	–	Slovakia	Árvay et al. (2019)
Suillus luteus	–	13.36	22.84	283.24	118.84	–	–	–	Turkey	Bengu (2019)
Tricholoma matsutake	370.0	20.00	3.00	34.00	62.00	–	2.3	2.60	China	Liu et al. (2012a)
Volvariella volvacea	–	–	–	72.51	94.28	–	–	–	India	Ahlawat et al. (2016)
Volvopluteus gloiocephalus	200.19	05.01	0.13	69.91	10.89	–	–	–	Portugal	Heleno et al. (2015a)
Xerocomus spadiceus	–	19.8–28.0	29.0–103.9	1315–6762	47.5–77.8	–	7.2–25.9	–	China	Wang et al. (2017)

17.3 Nutritional and Nutraceutical Potential of Mushrooms

Nutraceuticals and nutriceuticals are considered as food or a part of diet that have therapeutic and health benefits, which helps in fighting against several diseases. They may vary from herbal products to dietary supplements, isolated nutrients, genetically engineered/designer food and processed food products like beverages, cereals and soups. There are several examples of nutritive nutraceuticals/functional food ingredients like proteins, peptides, keto acids, amino acids, polyunsaturated fatty acids (PUFA), vitamins, minerals and antioxidants (Barros et al. 2008). Mushrooms are extremely nutritious with low fat content and high protein, essential amino acid, mineral and vitamin content (Agrahar-Murugkar and Subbulakshmi 2005). Studies showed that around 35 different species of edible mushrooms are cultivated commercially, however almost 200 species of wild mushrooms are used for their medicinal properties (Beulah et al. 2013). Recently, researchers gain interests in exploring mushrooms as a functional food, as a reservoir for development of potent therapeutic products and as a rich nutraceuticals, responsible for their antioxidant, anti-microbial and anti-tumor properties (Barros et al. 2007; Çağlarırmak 2007; Elmastas et al. 2007; Gupta et al. 2018; Ribeiro et al. 2007; Salihović et al. 2019). Several researchers, have documented the therapeutic potential of nutraceutical compounds isolated from mushrooms in cure and prevention of fatal diseases like cancer, hypertension, diabetes, heart diseases and cerebral stroke (Pardeshi and Pardeshi 2009; Wasser and Weis 1999). The investigation of therapeutic and nutraceutical potential of mushrooms gives an idea that they can be future food, which provides high quality protein and minerals (Barreira et al. 2014; Cheung 2013; El Enshasy and Hatti-Kaul 2013; Heleno et al. 2015a; Khatua et al. 2013; Kumar 2015; Ruthes et al. 2016; Singh et al. 2016; Tel-Cayan et al. 2017).

17.3.1 Proteins and Peptides

Proteins are one of the major macronutrients found in a quality food. In the last few decades, researchers have studied the importance of protein, essential amino acids, peptides and their significance for nutrition and health. Particularly, the biological functions of several dietary or bioactive proteins & peptides have been studied in more detail (Walther and Sieber 2011). Proteins and peptides are substantial bioactive nutraceuticals in mushrooms which possess numerous health benefits, like improving the digestion and absorption of nutrients, enhancing immune activity and regulating enzymatic activity (Valverde et al. 2015). The protein and peptides commonly found in mushrooms are ribosome inactivating proteins (RIPs), lectins, laccases, fungal immunomodulatory proteins (FIPs), and ribonucleases (Ko et al. 1995; Munoz et al. 1997; Xu et al. 2011). Among these proteins, lectins are glycoproteins or non-immune proteins have the property of cell agglutination and binds with the carbohydrates of cell surface. It was reported that the lectins isolated from *Clitocybe nebularis*, *Pholiota adiposa*, *Narcissus tazetta*, *Xylaria hypoxylon*, *Russula lepida*, *Russula delica* and *Hericium erinaceum* possess anti-proliferative, immunomodulatory, anti-viral, anti-microbial and anti-tumor activities (Li et al. 2010; Liu et al. 2006; Ooi et al. 2010; Singh et al. 2015; Zhang et al. 2010).

Mushrooms FIPs has been extracted from *Ganoderma lucidium*, *Ganoderma microsporum*, *Flammulina velutipes*, *Volvariella volvacea*, *Antrodia camphorate*, etc., and reported with immunomodulatory activity (Xu et al. 2011). They also prevent the invasion and metastasis of tumour cells and therefore can be used as adjuvants for treating tumour (Lin et al. 2010). Bioactive protein "RIPs" belongs to enzyme class and has been extracted from several mushroom species including *Pleurotus tuberregium*, *Flammulina velutipes*, *Lyophyllum shimeiji*, *Calvatia caelata*, and *Hypsizigus marmoreus* (Lam and Ng 2001; Lin et al. 2010; Ng et al. 2003; Wang and Ng 2001a, b, 2006). They are capable to inactivate the ribosomes by removing one or more adenosine from rRNA and also they help in inhibiting the HIV-1 reverse transcriptase activity and fungal proliferation (Puri et al. 2012). Like RIPs, laccases also belong to the enzyme category, and are considered as a promising candidate in the field of industry and biotechnology. They are majorly isolated from *Pleurotus eryngii*, *Pleurotus ostreatus*, *Tricholoma mongolicum* and *Clitocybe maxima*, and reported for anti-viral and anti-proliferative activity (M EL-Fakharany et al. 2010; Wang and Ng 2006; Zhang et al. 2010). They are known as Green tool/green catalyst in the field of biotechnology and have immense applications in the field of medicine, food, cosmetics, etc. (Agrawal et al. 2018). Ribonucleases, another bioactive protein isolated from mushroom has been documented for their antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Alves et al. 2013).

According to Rathore and co-workers (Rathore et al. 2017), the highest content of crude protein has observed in *Auricularia auricular* (360 g/kg dry weight), followed by *Pleurotus ostreatus* (284 g/kg dry weight) and *Lentinus edodes* (228 g/kg dry weight). The protein content from beneficial mushrooms in the past few years are shown in Table 17.1. The higher content of protein have been determined in *Agaricus subrufescens* (50.40 g/100 g dry weight), followed by *Russula cyanoxantha* (49.20 g/100 g dry weight), *Craterellus cornucopioides* (47.21 g/100 g dry weight), *Pleurotus geesteranu* (39.80 g/100 g dry weight), *Pleurotus ostreatus* (28.40 g/100 g dry weight), and *Laccaria amethystea* (29.84 g/100 g dry weight) (Beluhan and Ranogajec 2011; Ikon et al. 2019; Liu et al. 2019, 2012b; Srikram and Supapvanich 2017), whereas the lowest protein content have been reported in *Pleurotus eryngii* (2.09 g/100 g dry weight), followed by *Helvella lacunose* (4.40 g/100 g dry weight), *Boletus regius* (5.22 g/100 g dry weight), *Amanita curtipes* (6.40 g/100 g dry weight) and *Clitocybe subconnexa* (7.42 g/100 g dry weight), respectively (Fernandes et al. 2015; Heleno et al. 2015b; Leal et al. 2013; Reis et al. 2014). The research findings could confirm the anti-tumor, anti-viral and anti-inflammatory activities of proteins isolated from mushrooms (Chatterjee et al. 2017; Lin et al. 2010).

17.3.2 Amino Acids and Vitamins

The essential amino acids are vital for humans as they are not synthesized by the body, therefore they are obtained from the food. Mushrooms are ideal source of essential amino acids (Kıvrak et al. 2014). They contain all essential amino acid along with few non-essential amino acid (Manzi et al. 1999). Free amino acid are leading components of functionally active compounds, contributing for the unique flavors of mushroom (Kalač 2009; Kıvrak et al. 2014). Research findings showed the presence of 18 free amino acids in mushrooms which includes lysine, leucine, isoleucine, tryptophan, threonine, valine, methionine, histidine and phenylalanine from the class of essential amino acids and glycine, tyrosine, arginine, serine, aspartate, alanine, cysteine, glutamate and proline from non-essential amino acid group. The content of major amino acids detected in beneficial mushrooms are shown in Table 17.3. *Boletus edulis*, *Clavulina rugosa*, *Lepista nuda*, *Cantharellus tubaeformis*, *Leucopaxillus giganteus*, *Hydnum repandum*, *Russula rosea* and *Tricholoma saponaceum* showed highest (18 known amino acids) diversity of amino acids, whereas *Inonotus obliquus* showed the least (2 known amino acids) diversity of amino acids (Ayaz et al. 2011). Usually, the number of amino acids in mushroom varies between 14 and 18. Mushrooms, also known as a rich source of vitamins especially riboflavin (B2), niacin (B3), folates (B9) and traces of vitamin A, B1, B5, B12, C, D, and E in mushrooms (Anderson and Fellers 1942; Bernaś et al. 2006; Cardwell et al. 2018; Jaworska et al. 2014).

17.4 Bioactive Components

Polysaccharides are one of the most common but potent compounds extracted from mushrooms. All the polysaccharides present in mushrooms possess b-linked glucose backbone but vary from species to species on the basis of pattern and degree of branching. Most common monosaccharides found in mushrooms are glucose, fructose, xylose, galactose, mannose, trehalose, arabinose, mannitol and rhamnose (Valverde et al. 2015). The few types of polysaccharides detected in mushrooms are homopolysaccharides, heteropolysaccharides (heteroploysaccharides grifloan and lentinan), b-glucans type polysaccharides and Glucan-protein complex, respectively (Lakhanpal 2014). Polysaccharides isolated from mushrooms are well studied for their health benefits and also utilized to develop functional foods.

Lentinan, erothionine, ganoderan, pleuran, calocyban, schizophyllan and agaritine, proteoglycans are well studied polysaccharides, which are derived from *Lentinus edodes*, *Ganoderma lucidium*, *Pleurotus* species, *Calocybe indica*, *Schizophyllum commune* and *Agaricus blazei*, respectively (Badalyan 2014; Kim et al. 2005; Villares et al. 2012). These polysaccharides has been reported with antioxidant, anti-tumor, immunomodulatory, anti-viral, anti-inflammatory, anti-fatigue and anti-carcinogenic activities (Jeong et al. 2012; Kim et al. 2005; Liu et al. 2015;

Table 17.3 Amino acids in different species of mushrooms

Species name	Thr	Val	Met	Ile	Leu	Phe	Lys	His	References
Agaricus abruptibulbus	5.91	4.18	1.62	9.46	–	1.92	–	8.26	Sudheep and Sridhar (2014)
Agaricus bisporus	–	45.74	00.00	–	74.93	82.20	24.44	–	Salihović et al. (2019)
Agaricus bisporus	–	30.95	23.80	–	70.30	77.10	31.90	–	Salihović et al. (2019)
Agaricus subrufescens	11	11.9	33.5	9.8	16.8	10.9	16.6	5.5	Liu et al. (2019)
Amanita caesarea	–	16.00	00.00	–	44.60	48.92	26.00	–	Salihović et al. (2019)
Amanita hemibapha	199.9	–	114.7	184.1	373.0	392.5	241.2	234.8	Sun et al. (2017)
Antrodia camphorate	1.2	–	–	1.1	1.6	0.9	0.8	0.4	Liu et al. (2019)
Astraeus hygrometricus	4.88	5.75	1.67	4.61	1.67	2.91	7.7	2.7	Pavithra et al. (2018)
Boletinus pinetorus	125.3	–	28.2	70.1	145.0	126.6	13.2	75.8	Sun et al. (2017)
Boletus aestivalis	–	16.60	00.00	–	10.60	11.66	85.20	–	Salihović et al. (2019)
Boletus bicolor	119.1	–	19.1	45.8	25.4	40.8	61.8	102.3	Sun et al. (2017)
Boletus craspedius	28.0	–	–	–	–	–	16.5	65.3	Sun et al. (2017)
Boletus griseus	97.0	–	6.2	36.7	47.3	17.2	76.7	103.1	Sun et al. (2017)
Boletus ornatipes	116.0	–	18.2	44.4	86.3	97.3	74.4	125.9	Sun et al. (2017)
Boletus sinicus	140.5	–	–	25.0	30.8	–	171.7	81.6	Sun et al. (2017)
Boletus speciosus	132.3	–	24.7	58.3	106.8	113.9	27.0	100.6	Sun et al. (2017)
Cantharellus cibarius	–	25.30	17.67	–	65.74	72.10	65.80	–	Salihović et al. (2019)
Catathelasma ventricosum	6.07	18.52	10.82	0.22	1.39	2.44	13.42	11.49	Liu et al. (2012b)
Clitocybe maxima	7.24	2.74	–	–	0.44	6.91	0.79	27.34	Liu et al. (2012b)

(continued)

Table 17.3 (continued)

Species name	Thr	Val	Met	Ile	Leu	Phe	Lys	His	References
Craterellus cornucopioides	6.37	0.41	12.74	0.08	17.51	–	8.09	22.07	Liu et al. (2012b)
Dictyophora indusiata	0.9	2.7	0.7	0.7	1.2	0.9	1	0.4	Liu et al. (2019)
Ganoderma amboinense	3.6–4.8	3.5–4.7	33.2–36.3	2.7–3.4	4.2–5.3	2.7–3.8	3.5–3.9	1.3–1.7	Liu et al. (2019)
Hericium erinaceus	3.2	2.9	0.8	2	3.8	2.3	4.1	1.6	Liu et al. (2019)
Laccaria amethystea	12.82	7.99	2.59	–	16.83	7.31	11.97	0.72	Liu et al. (2012b)
Laccaria amethystea	0.49	13.12	10.34	2.20	2.57	–	10.32	–	Liu et al. (2012b)
Lactarius deliciosus	–	5.99	00.00	–	12.79	14.04	7.74	–	Salihović et al. (2019)
Lactarius deliciosus	131.76	122.17	25.41	47.82	109.53	133.61	208.46	278.57	Xu et al. (2019)
Lactarius piperatus	–	1.80	00.00	–	4.40	4.87	2.92	–	Salihović et al. (2019)
Lentinus edodes	10.3	9.6	29.7	7.1	11.7	8.5	10.2	3.8	Liu et al. (2019)
Lycoperdon pyriforme	–	21.72	00.00	–	65.00	71.40	50.60	–	Salihović et al. (2019)
Macrolepiota procera	–	8.42	17.67	–	35.30	38.70	70.60	–	Salihović et al. (2019)
Pholita microsporeo	0.13–0.27	0.7–0.81	1.29–1.76	0.53–0.59	1.17–1.25	0.68–0.83	0.78–0.85	0.31–0.37	Meng et al. (2019)
Pleurotus geesteranu	1.7–1.9	2.4–2.7	0.8–0.8	1.5–1.6	2.1–2.6	1.3–1.3	2.0–2.5	0.8–0.9	Liu et al. (2019)
Pleurotus ostreatus	0.8–1.4	1.6–2.4	0.5–0.8	0.6–1.2	1–1.9	0.6–1.0	0.9–1.8	0.3–0.7	Liu et al. (2019)
Pleurotus ostreatus	–	281.20	00.00	–	137.72	318.70	196.80	–	Salihović et al. (2019)
Pleurotus sajorcaju	0.8–1.5	1.6–2.3	0.4–0.9	0.7–1.3	1.1–2	0.7–1.0	0.9–1.9	0.4–0.7	Liu et al. (2019)
Pleurotus sapidus	0.4	1.8	0.2	0.8	1.4	0.8	0.3	0.4	Liu et al. (2019)
Stropharia rugosoannulata	7.44	0.35	31.94	–	–	–	7.11	2.17	Liu et al. (2012b)
Stropharia rugosoannulata	–	22.96	6.69	0.63	7.61	–	–	0.97	Liu et al. (2012b)

(continued)

Table 17.3 (continued)

Species name	Thr	Val	Met	Ile	Leu	Phe	Lys	His	References
Stropharia rugosoannulata	0.8	1.6	11.6	0.6	1.3	0.6	0.9	0.4	Liu et al. (2019)
Suillus placidus	79.6	–	–	46.3	83.4	65.5	60.5	89.3	Sun et al. (2017)
Termitomyces globulus	6.25	5.31	0.68	10.87	–	2.73	–	5.21	Sudheep and Sridhar (2014)
Termitomyces microcarpus	653.6	–	180.4	689.6	1197.3	785.8	512.1	357.5	Sun et al. (2017)
Tricholoma terreum	125.3	–	24.0	98.0	83.3	87.0	58.8	44.6	Sun et al. (2017)
Tricholomopsis lividipileata	86.9	–	38.6	74.3	118.3	79.1	99.4	34.1	Sun et al. (2017)
Xerocomus	187.2	–	6.0	76.8	160.5	83.7	92.1	109.9	Sun et al. (2017)

Ma et al. 2014; Meng et al. 2016; Rathore et al. 2017). Few polysaccharides have also been discussed in the proximate analysis and mineral content section.

During last few decades researchers are more concerned about oxidative damage because the risk of oxidative species, including free radicals is increasing constantly, resulting from unhealthy habits and pollution exposure. A free radical is known as any unstable molecule which are very reactive and cause oxidative stress to body, resulting in growth of several fatal diseases like cancer, neurodegenerative disease, cardiovascular disease, etc., (Carocho and Ferreira 2013; Gutteridge and Halliwell 2000). An anti-oxidant can stabilize these free radicals and help in preventing the diseases caused by oxidative stress (Carocho and Ferreira 2013; Ferreira et al. 2009, 2015; Painuli et al. 2018, Painuli and Kumar 2016, Semwal and Painuli 2019; Stajic et al. 2013). Although, human body can prevent oxidative stress by endogenous antioxidant defense mechanism, we can also obtain anti-oxidants by inclusion of healthy food in our diet (Ferreira et al. 2009).

In this view, mushrooms are considered to be a rich source of anti-oxidants and have been extensively studied for their antioxidant properties. The antioxidants reported in mushrooms are phenolic acids, tocopherols, terpenes, carotenoids, steroids and ascorbic acid (Ferreira et al. 2009, 2015; Stajic et al. 2013). Alkaloid is one of the important class of phenolic compounds. They possess immense therapeutic properties and have been used from a long back for curing diseases. In past few years mushrooms are reported with significant content of alkaloids, which are further studied for their bioactivity. *Astraeus odoratus*, *Flammulina velutipes*, *Ganoderma sinense*, *Hericium erinaceum*, *Macrolepiotaneo mastoidea*, *Pseudobaeospora pyrifera* are examples of such mushrooms which possess alkaloids with anti-tubercular activity, cytotoxic activity, anti-cholinesterase and anti-mycobacterial activity (Arpha et al. 2012; Kim et al. 2012; Liu et al. 2010; Rhee et al. 2001; Song et al. 2009).

Terpenoids are hydrocarbon formed by the combination of different isoprene units. They are reported for various medicinal and therapeutic activities like anti-malarial, anti-cancer, anti-viral, anti-microbial, anti-inflammatory and anti-cholinesterase (Arpha et al. 2012; Kim et al. 2013; Lee et al. 2011; Liu 1995; Mann et al. 1994; Mothana et al. 2003; Shibata et al. 1998; Wang et al. 2012, 2013a, b). The major terpenoid isolated from mushroom is lanostane, a triterpenes well studied for its anti-cancer activities (Arpha et al. 2012; Kim et al. 2013; Wang et al. 2012, 2013a, b). Ganoderic, lucidenic, ganodermic, ganolucidic, ganoderols, applanoxidic acids and lucidones are terpenoids isolated from *Ganoderma lucidium* which are reported for anticancer treatments (Li et al. 2005). Terpenoids isolated from mushrooms have also been observed to their anti-mycobacterial and anti-candidal activities (Öztürk et al. 2015).

Steroid is an organic compound, resulting from the characteristic arrangement of four cycloalkane rings that are linked with each other. They play an important role in biological systems and are responsible for cell functioning. Steroids have been isolated from different species of mushrooms including *Tomophagus cattienensis*, *Fomes fomentarius*, *Ganoderma lucidum*, *Hypsizigus marmoreus* and *Sarcodon joedes*, respectively (Hien et al. 2013; Liu et al. 2013; Seo et al. 2009; Zang et al. 2013). Steroids isolated from the mushrooms have anti-microbial, cytotoxic, anti-tubercular, anti-complement and anti-viral activities (Itoh et al. 2008; Liu et al. 2013; Niedermeyer et al. 2005; Seo et al. 2009).

Phenolic compounds are the largest group of phytochemicals which are well known for their interesting pharmacological activities. Flavonoids, coumarins, anthocyanin, catechins and phenolic acid, etc., are few examples of phenolic compound. The rich source of these compounds are vegetables, fruits, herbs, seeds, juices, etc. Several mushrooms including *Albatrellus caeruleoporus*, *Boletus pseudocalopus*, *Cortinarius purpurascens*, *Ganoderma colossum*, *Ganoderma pfeifferi*, *Ganoderma fornicatum*, and *Tricholoma oriruben* have been reported with biologically active phenolic compounds (Awadh Ali et al. 2003; Bai et al. 2013; El Dine et al. 2009; Kawagishi et al. 2004; Mothana et al. 2000; Niu et al. 2006; Quang et al. 2006; Song et al. 2009; Venkateswarlu et al. 2002; Yousfi et al. 2009). Current report on antioxidant activity of wild-growing (*Agaricus campestris*, *Boletus edulis*, *Cantharellus cibarius*, *Macrolepiota procera*, *Pleurotus ostreatus*, *Russula alutacea*, and *Russula vesca*) and cultivated (*Agaricus bisporus*, *Pleurotus ostreatus*) mushrooms suggests them as a rich source of phenolics and flavonoids (Buruleanu et al. 2018). The pharmacological activities reported in phenolic compound of mushrooms are anti-cancer, anti-diabetic, anti-inflammatory, anti-microbial activities, etc., (Öztürk et al. 2015). Different biological activities of different compounds isolated from mushrooms are presented in Table 17.4.

Table 17.4 Biological activities of few beneficial mushrooms from different regions

Species name	Bioactivities	References
Agaricus bisporus	Antioxidant, Antimicrobial	Özcan and Ertan (2018)
Agaricus bisporus	Infectious diseases	Soković et al. (2014)
Agaricus bisporus	Diabetic mellitus	Calvo et al. (2016)
Agaricus blazei	Anti-cancer	Taofiq et al. (2019)
Agaricus brasiliensis	Infectious diseases	de Sousa Cardozo et al. (2014)
Agaricus brasiliensis	Cytoprotective	Fang et al. (2016)
Agaricus brasiliensis	Anti-HSV-1, HSV-2	de Sousa Cardozo et al. (2014)
Agrocybe aegerita	Anti-influenza	Ma et al. (2017)
Agrocybe cylindracea	Antioxidant	Rathee et al. (2012)
Amanita rubescens	Antioxidant	Lalotra et al. (2018)
Auricularia auriculajudae	Infectious diseases	Cai et al. (2015)
Auricularia auriculajudae	Hypertension	Ardigò (2017)
Auriporia aurea	Anti-influenza (H1N1)	Krupodorova et al. (2014)
Boletus edulis	Antioxidant, Antimicrobial	Özcan and Ertan (2018)
Boletus edulis	Antioxidant	Lalotra et al. (2018)
Boletus edulis	Antimicrobial	Salihović et al. (2019)
Boletus spp.	Antioxidant	Yuswan et al. (2015)
Cantharellus cibarius	Antioxidant, Antimicrobial	Özcan and Ertan (2018)
Cantharellus cibarius	Antimicrobial	Salihović et al. (2019)
Cordyceps militaris	Bladder, Leukemia Cancers	Patel and Goyal (2012)
Cordyceps sinensis	Bladder, Colon, Leukemia, Liver Cancer	Wang et al. (2016)
Cortinarius caperatus	Antioxidant	Zacchigna (2017)
Craterellus cornucopioides	Antioxidant, Antimicrobial	Özcan and Ertan (2018)
Craterellus cornucopioides	Antioxidant	Palacios et al. (2011)
Craterellus cornucopioides	Antioxidant, Antimicrobial, Cytotoxicity	Kosanić et al. (2019)
Entoloma lividoalbum	Cytoprotective	Lima et al. (2016)
Flammulina velutipes	Anti-influenza	Krupodorova et al. (2014)
Fomes fomentarius	Anti-influenza	Krupodorova et al. (2014)
Fomitopsis pinicola	Antifungal, antitumor	Scaglione (2017)
Ganoderma adspersum	Antimicrobial, Antioxidant	Shomali et al. (2019)
Ganoderma lucidum	Anti-influenza	Krupodorova et al. (2014)
Ganoderma lucidum	Antioxidant, improve hormone production	Scaglione (2017)
Ganoderma lucidum	Virologic and clinical efficacy	Scaglione (2017)
Ganoderma pfeifferi	Anti-HSV-1	Lindequist et al. (2015)

(continued)

Table 17.4 (continued)

Species name	Bioactivities	References
Ganoderma pfeifferi	Anti-influenza	Lindequist et al. (2015)
Geopora arenicola	Antioxidant	Lalotra et al. (2018)
Grifola frondosa	Cytoprotective	Berven et al. (2015)
Grifola frondosa	Bladder, Prostate, Stomach Cancer	Patel and Goyal (2012)
Grifola frondosa	Anti-enterovirus	Zhao et al. (2016)
Hericium erinaceus	Cytoprotective	Cheng et al. (2016)
Hohenbuehelia serotina	Anti-HIV-1	Zhang et al. (2014)
Hydnum repandum	Antioxidant, Antimicrobial	Özcan and Ertan (2018)
Hypsizygus marmoreus	Antioxidant, Anti-inflammatory	Jang et al. (2013)
Inonotus hispidus	Antimicrobial, Antioxidant	Shomali et al. (2019)
Inonotus obliquus	Antioxidant	Rathee et al. (2012)
Inonotus obliquus	Anti-HIV-1	Shibnev et al. (2015)
Inonotus obliquus	Anti-HSV-1	Polkovnikova et al. (2014)
Inonotus obliquus	Anti-influenza (H3N2, H5N6)	Tian et al. (2017)
Lactarius deliciosus	Antioxidant	Xu et al. (2019)
Lentinula edodes	Antioxidant	Chowdhury et al. (2015)
Lentinula edodes	Antioxidant	Olawuyi and Lee (2019)
Lentinula polychrous	Antioxidant	Attarat and Phermthai (2015)
Lentinula squarrosulus	Antioxidant	Attarat and Phermthai (2015)
Lentinus edodes	Anti-influenza	Krupodorova et al. (2014)
Lyophyllum shimeji	Anti-influenza	Krupodorova et al. (2014)
Macrolepiota dolichaula	Cytoprotective	Samanta et al. (2015)
Morchella deliciosa	Antioxidant	Lalotra et al. (2018)
Phellinus baumii	Anti-influenza (H1N1, H5N1,H3N2)	Li et al. (2011)
Pleurotus abalonus	Anti-HIV-1	Li et al. (2011)
Pleurotus eryngii	Breast Cancer	Xue et al. (2015)
Pleurotus eryngii	Cervix, Colon Cancer	Milovanović et al. (2014)
Pleurotus eryngii	Anti-influenza	Krupodorova et al. (2014)
Pleurotus Florida	Antioxidant	Ganeshpurkar et al. (2015)
Pleurotus giganteus	Antioxidant	Phan et al. (2019)
Pleurotus nebrodensis	Cytoprotective	Hai-Yan et al. (2015)
Pleurotus ostreatus	Anti-microbial	Ikon et al. (2019)
Pleurotus tuber-regium	Diabetic mellitus	Wu et al. (2014)
Rozites caperata	Anti-HSV-1	Yan et al. (2015)
Russula chloroides	Antimicrobial, Antioxidant	Shomali et al. (2019)
Sarcodon imbricatus	Antimicrobial, Antioxidant	Shomali et al. (2019)
Schizophyllum commune	Anti-influenza	Krupodorova et al. (2014)
Sparassis crispa	Antioxidant	Lalotra et al. (2018)
Trametes versicolor	Anti-mutagenic, Antioxidant	Milovanovic et al. (2015)
Trametes versicolor	Anti-influenza	Krupodorova et al. (2014)
Tylopilus ballouii	Cytoprotective	Lima et al. (2016)

17.5 Conclusion

Presently, the global production of cultivated mushrooms has exceeded ten million tons and is continuously increasing, with China being the largest producer. More than 2000 mushroom species are safe and eatable. Further, mushrooms can help in boosting the economic growth in a society. Worldwide, both cultivated and wild species of mushrooms are consumed as they possess distinctive and unique taste, texture and smell along with low calorific values and high fibre content. They synthesize active compounds with broad spectrum of biological activities. *In vitro* studies, clinical trials and animal studies validated the traditional knowledge and indicated the immense potential of mushroom-derived compounds and products, for the suppression and treatment of various diseases. The present chapter covers the information on proximate content, proteins, vitamins, minerals, bioactive compounds and potential benefits of medicinal mushrooms. Mushrooms can be consumed as tonics, medicines, nutritional products, etc. and can be used as cosmetics and natural bio-control agents. In view of promising outcomes, additional efforts are required to explore the ability of mushrooms as functional food along with the therapeutic potential. The major tasks comprise the understanding of clinical trials, development of mushroom derived products with standardized protocols, and their sustainable production under appropriate environment.

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Chapter 18
Aloe Species as Valuable Sources of Functional Bioactives

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18.1 Introduction

Herbal medicines occupy distinct position right from the primitive period to present day. In every ethnic group, there exists a traditional health care system, which is culturally patterned. In rural communities, health care seems to be the first and fore-

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most line of defence. The WHO has already recognized the contribution of traditional health care in tribal communities. These medicines have fewer side effects and are easily accessible to mankind in the nature. It has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been formulated out of it. Therapeutically, interesting and important drugs have been developed from plant sources which are being used in traditional system of medicines. The use of plants as a therapeutic material due to their chemical substances of medicinal value is very common all over the world from ancient period of time.

The Genus Aloe belongs to the family Xanthorrhoeaceae which is also previously known as Asphodelaceae, Aloaceae or Liliaceae (Chen et al. 2012). The genus contains over 500 species of Aloe which vary greatly in their heights and sizes ranging from very small shrub-like plants to very large trees (Grace 2011). Out of these 500, about 160 are indigenous to South Africa and some species are found in Jordan, Madagascar, Arabian Peninsula and Indian Ocean Islands contributing to regional bio-cultural diversity (Cottam and Curtis 1956; Cock 2015). The genus is thought to have originated from the highlands of Southeast Africa and almost 82% of the rec-

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ognized species are found to be present in High Africa (Holland 1978). There is a presence of rosette of leaves at the base except stem in many members of the genus Aloe. Its name is derived from the Arabic word “Alloeh”, which basically means “shining bitter substance”. In many cultures for long time its name is used as a remedy. The well-investigated Aloe species includes *Aloe vera*, *Aloe barbadensis*, *Aloe arborescens* and *Aloe ferox*. There is a cultivation of different evergreen species of Aloe as ornamentals due to its spiny sharp-pointed sword-shaped leaves terminating at the trunk and clusters of colourful red and yellow flowers. The plants of the genus Aloe have been used worldwide for their medical and cosmetic benefits and their effectiveness has been accepted internationally. Despite their common use, internationally scientific studies and experimental data on the pharmacological uses and the toxicology of these Africa species is seriously lacking (Srikanth et al. 2014).

Aloe vera is considered as the most potent and popular plant of the genus (Eshun and Qian 2004). *Aloe vera* has been used in folk medicine for over 2000 years, and has remained an important component in the traditional medicine of many contemporary cultures, such as in China, India, West Indies and Japan (Foster et al. 2011). *Aloe vera* has been used for many centuries for its curative and therapeutic properties. *Aloe vera* is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The aloe leaf can be divided into two major parts, namely the outer green rind, including the vascular bundles, and the inner colourless parenchyma containing the aloe gel. Main chemical constituents of *Aloe vera* include (Table 18.1): amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins, and phytosterols (Surjushe et al. 2008).

Apart from *Aloe vera*, *Aloe ferox* also known as Cape aloe or bitter aloe is the second most studied member and has been shown to exhibit wound healing, laxative properties, antioxidant, anti-inflammatory, antimicrobial, anticancer, antimalarial and anthelmintic activities (Chen et al. 2012). The plant is also used as an ornamental plant as it shows flowering of red, yellow, orange and a rare white color. The plant is native to the Cape coastal region of South Africa and hence the name Cape aloe. The botanical name ferox is attributed to the presence of thorns and sharp spines of reddish color giving the plant its ferocious appearance (Chen et al. 2012).

Aloe vera exhibits many pharmacological activities due to the phytochemical such antioxidant, antimicrobial, immune boosting, antitumor, hypolipidemic, wound healing, and antidiabetic (Cosmetic Ingredient Review Expert Panel 2007). It is also reported that *Aloe vera* helps in reducing serum cholesterol and triglycerides and increasing level of high density lipoprotein cholesterol. Many traditional uses are also reported such as in burn injury, eczema, cosmetics, inflammation, and fever, which continue to be studied, although further research still has to be done. Thus, it is quite promising as a multipurpose medicinal agent so further experiments are needed to elucidate and to find out the mechanism of the bioactive chemicals using modern instruments, such as high-performance liquid chromatography, high-performance thin layer chromatography, and clinical trials has to be done to generate novel drugs. The US Food and Drug Administration have already approved the developmental study of *Aloe vera* in the treatment of cancer and AIDS. In future, controlled studies are required to prove the effectiveness of *Aloe vera* under various conditions.

Table 18.1 Active components of *Aloe vera*

Class	Active components
Anthraquinones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose
Chromones	8-C-glucosyl-(2′-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)- aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-O-methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoaloesin A
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, superoxide dismutase
Inorganic compounds	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Miscellaneous including organic compounds and lipids	Arachidonic acid, γ-linolenic acid, steroids (campestrol, cholesterol, βsitosterol), triglicerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid
Non-essential and essential amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine
Proteins	Lectins, lectin-like substance
Saccharides	Mannose, glucose, L-rhamnose, aldopentose
Vitamins	B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol

Ni and Tizard (2004), Dagne et al. (2000), Femenia et al. (1999), and Choi and Chung (2003)

For centuries, Aloe plants have been widely used as therapeutic and topical agents due its medicinal and pharmacological properties, where it participate in the treatment of different types of diseases through molecular and biochemical pathway modulation (Surjushe et al. 2008; Rahmani et al. 2015). Research studies demonstrate that there is a presence of wide variety of phytochemicals and nutrients in the different Aloe species (Table 18.1) which includes simple and complex polysaccharides (notably glucomannans), fatty acids, vitamins, minerals, enzymes and many interesting secondary metabolites like phenolic compounds, flavonoids, phytosterols, glycoproteins, coumarins, alkaloids, pyrones, anthrones, naphthalenes, indoles, anthraquinones (aloe-emodin, aloins, aloectic and barbaloin), alkanes, aldehydes, ketones and dicarboxylic acids with potential toxicological and biological activities, however presence of the active components elude definition. (Nejatzadeh-Barandozi 2013; Boudreau et al. 2013a). It has been reported that due to presence of these secondary metabolites Aloe plants have been shown to possess numerous biological properties such as antimicrobial, antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, anticancer, antidiabetic, anti-rheumatoid, anti-arthritic, detoxification, laxative, treating constipation, promoting digestion, wound or burn healing and enhancing immune system (Boudreau et al. 2013a). Conversely, ingestion of some toxic variety of Aloe plants produces adverse symptoms like kidney failure, hypersensitive reactions, diarrhoea, pseudomelanosis coli, phototoxicity and hypokalemia (Guo and Mei 2016). The different parts of Aloe plants like fleshy leaves, latex and gel have been used commercially in food, cosmetics and pharmaceutical industries. This review article summarizes the phytochemistry, pharmacotherapy and toxicological profile of the Aloe genus plants.

18.2 Botany

Aloe genus (see classification below) is a succulent, monoecious, perennial species with shallow roots. Aloe species are widely spread throughout the warmer regions mainly in arid areas like Africa, India, etc. Although they can also be grown in subtropical winter rainfall and summer rainfall regions. There are some key factors which limit the genus distribution such as temperature, rainfall, fire tolerance and soil moisture. A wide diversity of habitats is occupied by Aloe species ranging from altitudes of 2700 m to sea level. Some Aloe species are particularly restricted to some geographical area although distribution of Aloe species is effected by specificity and seed pollinator morphology (Jordan 1996). In a broad range of soils Aloe species can be cultivated and mainly loamy mixture is preferred with temperature and pH ranging from 4 to 21 °C and 7.0 to 8.6. Conversely, some cold tolerant species can grow below 4 °C. Generally, grass Aloe species like *Aloe plicatilis*, *Aloe commixta* and *Aloe haemanthifolia* desires to grow in acidic soils (Giddy 1973). Most of the Aloe species under optimal environmental conditions can reach heights of up to 61–99 cm and flowering period is between the months of May to June. Entire Aloe species are evergreen with majority of species are with separate rosettes of fleshy, sword-shaped and thick leaves terminating at the trunk or branches in case of branched species. In individual species the leaves differ in colour, size and prickles distributed on the faces and margins of leaves. Usually flowers are of different shades (red, orange or yellow) with narrow to tubular shaped usually clustered at the apex of long stemmed spikes. Subsequently, up to 5 cm long oval fruits are formed after fertilization. The most commonly used part of Aloe plants is leaf which can be divide into three major parts as shown in Fig. 18.1. Outer green rind consisting of structural components containing glycosides and anthraquinones (Reynolds 2004), below the rind is outer pulp region with sap or latex and vascular bundles. Principally most phenolic compounds (Flavonoids, anthraquinones, anthrones, preanthraquinones, pyrones, chromones and coumarins) are present in the latex region (Gutterman and Chauser-Volfson 2000).

Kingdom:	Plantae- Plants
Subkingdom:	Tracheobionta- Vascular plants
Superdivision:	Spermatophyta- Seed plants
Division:	Magnoliophyta- Flowering plants
Class:	Liliopsida- Monocotyledons,
Subclass:	Liliidae
Order:	Liliales
Family:	Aloaceae (Aloe family)
Genus:	*Aloe L.- Aloe*

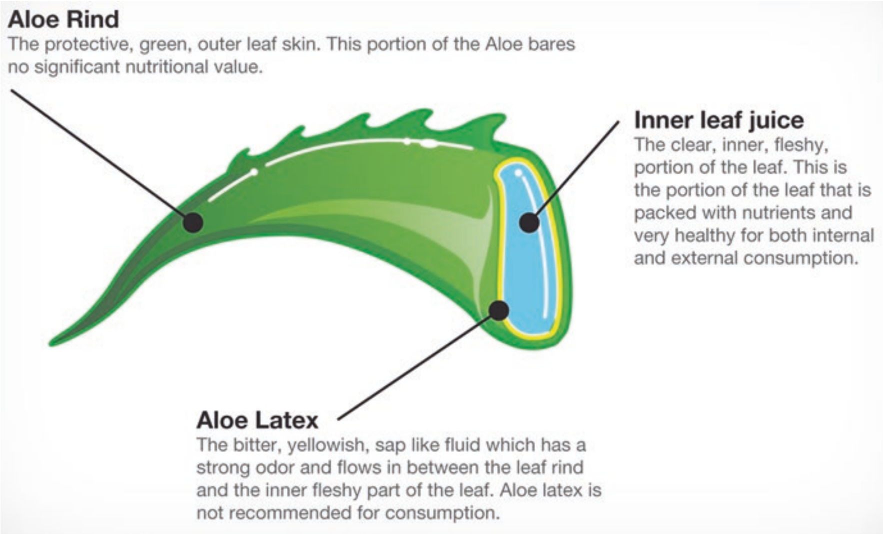

Fig. 18.1 A schematic illustration of Aloe leaf morphology showing a leaf cross section

The parenchyma cells are present in the inner leaf pulp of Aloe gel. In all aloe species the pulp consists of high acemannan polysaccharide and water content (approximately 99% for *Aloe vera*). There is a presence of numerous proteins, minerals, vitamins, enzymes, phytochemicals or secondary metabolites in the inner leaf pulp which includes flavonoids, alkaloids, coumarins, chromones, anthrones and anthraquinones (Reynolds 2004; Dagne et al. 2000; Boudreau and Beland 2006).

18.3 Phytochemistry

Aloe vera is a succulent plant meaning that the plant can bear droughts of water and can survive in areas of less water availability, due to possessing large water storage inside it. Though the studies are lacking, the plant's pulp is said to be associated with the storage of water (Ni et al. 2004). Succulents also use a different photosynthetic metabolism called as crassulacean acid metabolism (CAM) that involves malic acid (Denius and Homann 1972; Kluge et al. 1979).

In diverse Aloe genus plants, a variety of biologically active substances are present which shows interesting pharmacological activities. The potential bioactive compounds are present in different parts of Aloe plant such as leaves, outer pulp, inner leaf pulp (gel) or latex. The phytoconstituents present in Aloe genus includes enzymes, amino acids, saccharides, lignin, salicylic acids, alkaloids, flavonoids, sterols, saponins, coumarins, pyrans, pyrones, anthrones, benzene, anthroquinones, naphthalene, chromones, and furan derivatives (Cock 2015). In leaf gels of unspecified Aloe species various vitamins are present namely vitamin E (α-tocopherol), vitamin C (ascorbic acid), vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6

(pyridoxal phosphate) and vitamin B_{12} (cyanocobalamin) along with presence of various inorganic minerals (copper, calcium, phosphorus, magnesium, potassium, zinc, iron, copper, manganese, molybdenum and sodium). According to researches, the diverse biological activities of different Aloe species is due to synergistic action between numerous compounds instead of single chemical compound. The various phytoconstituents present in different parts of Aloe species are presented in Table 18.2.

Many beneficial activities of the plant have been reported and attributed especially to the plant's polysaccharides. The activities may include, anti-bacterial, anti-viral, anti-inflammatory property, laxative, immunomodulation, and protection against radiations.

The various polysaccharides isolated from the plant include galactan, mannan, arabinanarabinorhamnogalactan, glucuronic acid-containing polysaccharide, and pectic substance. Mannan and pectic substance have been found to be the primary polysaccharides with some studies suggesting mannan to be the main polysaccharide while still others suggesting pectic substance to be the main one (Ovodova et al. 1975; Mandal and Das 1980). These controversies have been attributed to the plant's different geographical occurrences (Mandal and Das 1980; Grindlay and Reynolds 1986).

The leaves of the *Aloe vera* plant have shown strong antioxidant activities. One study showed that the plant is more antioxidant than some of the standards like butylated hydroxytoluene (BHT) and α-tocopherol. The study was conducted using plants of different age such as 2–4 year old plants. It was determined that the 3 years old plant contained a significantly higher number of flavonoids and polysaccharides than the 2 and 4 years old plants. Also the extract of the 3 year old plant showed a higher antioxidant potential than the extracts of 2 years old plants in the first 10 min of the experiment. When the different plant's extracts were ranked in terms of their antioxidant activity along with butylated hydroxytoluene (BHT) and α-tocopherol, it was found that in first 10 min α-tocopherol had the highest antioxidant potential while the 3 years old plant was at the second highest rank. However when their antioxidant potential was observed for 110 min it was observed that radical scavenging potential of α-tocopherol had decreased significantly ranking the 3 years old plant's extract at the highest point in terms of showing antioxidant potential. The study also concluded that the 2 years old plant showed the lowest antioxidant activity (Hu et al. 2003).

The plant contains very high amounts of the phenolic compounds such as anthrones, anthraquinones, chromones, saponins, coumarins, and polysaccharides in the leaf gel. The phenolic compounds have been attributed to showing antioxidant activities of the plant (Rice-Evans et al. 1997).

18.3.1 Anthraquinones

Several anthraquinones occur in roots and leaves of the species of aloe genus. Aloe emodin is found widely in the species of aloe and is found mostly in leaves of the plant. The roots of aloe contain two types of anthraquinones such as aloe saponarin

Table 18.2 Phytoconstituents present in various parts of Aloe species

Phytoconstituents	Description	Source	Plant part used	Molecular Formula	References
Alkaloids	O,N dimethyltyramine,	*Aloe* spp.	Leaves	$C_{10}H_{15}NO.HCl$	Nash (1992)
	N-methyltyramine	*Aloe* spp.	Leaves	$C_9H_{13}NO$	Nash (1992)
	γ-coniceine	*A. gillilandii*	Leaves	$C8H_{15}N$	Cock (2015)
	coniine	*A. viguleri*	Leaves	$C_8H_{17}N$	Cock (2015)
Flavonoids	Dihydro-isorhamnetin	*Aloe* spp.	Leaves	$C_{16}H_{14}O_7$	Cock (2015)
	Naringenin	*Aloe* spp.	Leaves	$C_{15}H_{12}O_5$	Cock (2015)
	Isovitexin	*Aloe* spp.	Leaves	$C_{21}H_{20}O_{10}$	Cock (2015)
	Apigenin	*Aloe* spp.	Leaves	$C_{15}H_{10}O_5$	Cock (2015)
Sterols	Lupeol	*A. barbadensis*	Leaves	$C_{30}H_{50}O$	Cock (2015)
	Cholesterol	*A. barbadensis*	Leaves	$C_{27}H_{46}O$	Cock (2015)
	β-sitosterol	*A. arborescens*	Leaves	$C_{29}H_{50}O$	Cock (2015)
	Campesterol	*A. barbadensis*	Leaves	$C_{28}H_{48}O$	Cock (2015)
Coumarins	Dihydroisocoumarin	*A. hildebrandtii*	Leaves	$C_{15}H_{12}O_4$	Wintola and Afolayan (2011)
	Feralolide	*A. ferox, Cape Aloe*	Leaves	$C_{23}H_{26}O_{13}$	Veitch et al. (1994)
Pyrans and pyrones	Bisbenzopyran	*A. barbadensis*	Root	$C_{22}H_{26}O_8$	Saleem et al. (1997)
	Aloenin (Aloecarbonoside)	*Aloe nyeriensis*	Leaves	$C_{19}H_{22}O_{10}$	Conner et al. (1987)
	Aloenin B	*Kenya aloe*	Leaves	$C_{34}H_{38}O_{17}$	Speranza et al. (1986)
	Aloenin acetal	*A. arborescens*	Leaves	NS	Woo et al. (1994)
	Aloenin aglycone	*A. nyeriensis*	Leaves	$C_{13}H_{12}O_5$	Conner et al. (1987)
	Aloe-2″-p-O-coumaroyl ester	*A. nyeriensis*	Leaves	NS	Conner et al. (1987)

	10-O-β-D-glucopyranosyl aloenin	*A. arborescens*	Leaves	NS	Duri et al. (2004)
Benzene	Pluridone	*A. pluridens*	Leaves	$C_{12}H_{12}O_3S$	Confalone et al. (1983)
	Protocatechuic acid	*A. berhana*	Leaves	$C_7H_6O_4$	Dagne and Alemu (1991)
	Fluridone	*A. pluridens Haw*	Leaves	$C_{19}H_{14}F_3NO$	Cock (2015)
	Methyl-p-coumarate	*A. ferox*	Leaves	$C_{12}H_{12}O_4$	Graf and Alexa (1982)
Naphthalene	Plicataloside	*A. plicatilis*	Leaves	$C_{23}H_{30}O_{13}$	Wessels et al. (1996)
	Feroxidin,	*A. ferox*	Leaves	$C_{11}H_{14}O_3$	Speranza et al. (1990)
	Feroxidin A	Cape aloe	Leaves	$C_{11}H_{14}O_3$	Rainsford et al. (2015)
	Feroxidin B	Cape aloe	Leaves	$C_{11}H_{14}O_3$	Rainsford et al. (2015)
	Aglycone isoeleutherol	Aloe spp.	Roots and leaves	$C_{14}H_{12}O_4$	Salehi et al. (2018)
	Isoeleutherol-5-O-glucoside	*A. saponaria*	Roots and leaves	NS	Salehi et al. (2018)
Furan derivatives	3-methylnaphto[2,3-c]furan-4,9-dione	*A. ferox*	Leaves	NS	Cock (2015)
	3-methylnaphto[2,3-c]furan-4(9H)-one,	*A. ferox*	Leaves	NS	Cock (2015)
	5-OH-3-methylnaphto[2,3-c]furan-4(1H)-one	*A. ferox*	Leaves	NS	Cock (2015)
Chromones	Aloeresin A	*Aloe* spp.	Leaves	$C_{28}H_{28}O_{11}$	Dell'Agli et al. (2014)
	Aloesin (Aloeresin B)	*Aloe* spp.	Leaves	$C_{19}H_{22}O_9$	Manitto et al. (1990)
	Aloeresin C	Cape aloe	Leaves	$C_{38}H_{38}O_{16}$	Cock (2015)

(continued)

Table 18.2 (continued)

Phytoconstituents	Description	Source	Plant part used	Molecular Formula	References
	Aloeresin D	*Aloe* spp.	Leaves	$C_{29}H_{32}O_{11}$	Cock (2015)
	Aloeresin E	*A. peglerae*	Leaves	$C_{34}H_{38}O_{15}$	Van Heerden et al. (1996)
	Aloeresin F	*A. peglerae*	Leaves	$C_{28}H_{28}O_{10}$	Van Heerden et al. (1996)
	Aloesol	*Aloe* spp.	Leaves	$C_{13}H_{14}O_4$	Cock (2015)
	Iso-aloesin	*A. vera var. chinensis*	Leaves	$C_{19}H_{22}O_9$	Cock (2015)
	Iso-aloeresin A	Cape aloe	Leaves	$C_{28}H_{28}O_{11}$	Speranza et al. (1988)
	Iso-aloeresin D	*A. vera*	Leaves	$C_{29}H_{32}O_{11}$	Okamura et al. (1996)
	7-O-methylaloesin	*A. rupestris*	Leaves	$C_{20}H_{24}O_9$	Bisrat (2000)
	7-O-methylaloesinol	*A. capensis*	Leaves	NS	Park et al. (1997)
	7-O-methylaloeresin A	*A. marlothii*	Leaves	NS	Bisrat (2000)
	8-[C-β-D-[2-O-(E)-cinnamoyl] glucopyranosyl]-2-[(R)-2-hydroxypropyl]-7-methoxy-5-methylchromone	*A. barbadensis*	Leaves	NS	Hutter (1996)
	8-C-glycosyl-7-O-methylaloediol	*A. vera*	Leaves	NS	Okamura et al. (1997)
	8-C-glycosyl-7-O-methyl-(S)-aloesol	*A. vera*	Leaves	NS	Okamura et al. (1996)
	2-acetonyl-7-hydroxy-8-(2-furanonyl)-7-hydroxy-5-methylchromone	Cape aloe	Leaves	NS	Speranza (1997)
	7-hydroxy-2,5-dimethylchromone	Cape aloe	Leaves	$C_{11}H_{10}O_3$	Speranza et al. (1993)
	8-*C*-glucosyl-(2'-*O*-cinnamoyl)-7-*O*-methyl-aloediol	*A. vera*	Leaves	NS	Okamura et al. (1998)

	8,2-acetonyl-8-(2′,6′-di-*O*,*O*-coumaroyl)-glucopyranosyl-7-hydroxy-5-methylchromone	*A. speciosa*	Leaves	NS	Holzapfel (1997)
	2-acetonyl-8-(2′, cinnamoyl)-glucopyranosyl-7-hydroxy-5-methylchromone	*A. broomii*	Leaves	NS	Cock (2015)
	6’-*O*-coumaroylaloesin	*A. castanea*	Leaves	$C_{28}H_{28}O_{11}$	Van Heerden et al. (2000)
	2′-p-*O*- methylcoumaroylaloesin	*A. excelsa*	Leaves	NS	Cock (2015)
Anthrones	Barbaloin	*A. vera*	Leaves	$C_{21}H_{22}O_9$	Patel et al. (2012a)
	Nataloin	*A. ellenbeckii*	Leaves	NS	Grace et al. (2008)
	Homonataloin	*A. marlothii* Berger, *A. jacksonii*	Leaves	$C_{22}H_{24}O_9$	Conner et al. (1990)
	Homonataloside	*A. lutescens*	Leaves	NS	Van Heerden et al. (2000)
	Aloe barbendol	*A. barbadensis*	Roots	$C_{15}H_{14}O_4$	Saleem et al. (1997)
	Aloinoside A/B	*Aloe* spp.	Leaves	$C_{27}H_{32}O_{13}$	Cock (2015)
	Aloe-emodin anthrone	*Aloe* spp.	Leaves	$C_{15}H_{12}O_4$	Sigler and Rauwald (1994)
	Aloe emodin-10-C-rhamnoside	*A. rabaiensis*	Leaves	NS	Cock (2015)
	Chrysophanolanthrone	*Aloe* spp.	Leaves	$C_{15}H_{12}O_3$	Sigler and Rauwald (1994)
	8-*O*-methyl-7-hydroxyaloin A/B	*A. barbadensis*	Leaves	$C_{22}H_{24}O_{10}$	Rauwald (1990)
	6′-*O*-*p*-coumaroyl-7-hydroxyaloin	*A. barbadensis*	Leaves	NS	Rauwald (1990)
	6′-*O*-cinnamoyl-8-*O*-methyl-7-hydroxyaloin	*A. barbadensis*	Leaves	NS	Rauwald (1990)
	6′-*O*-cinnamoyl-5-hydroxyaloin A	*A. broomii*	Leaves	NS	Koroch (2009)
	7-hydroxyaloin-4′,6′-*O*-diacetate 6′-*O*-diacetate	*A. succotrina*	Leaves	NS	Sigler and Rauwald (1994)
	Deacetyllittoraloin	*A. littoralis*	Leaves	$C_{26}H_{30}O_{12}$	Koroch (2009)

(continued)

Table 18.2 (continued)

Phytoconstituents	Description	Source	Plant part used	Molecular Formula	References
	Microstigmin A	*A. microstigma*	Leaves	$C_{30}H_{28}O_{13}$	Koroch (2009)
	Littoraloside	*A. littoralis*	Leaves	$C_{32}H_{40}O_{17}$	Dagne et al. (1997)
	Littoraloin	*A. littoralis*	Leaves	$C_{28}H_{32}O_{13}$	Koroch (2009)
	Microdontin	*A. microdonta*	Leaves	$C_{30}H_{28}O_{11}$	Koroch (2009)
Anthraquinones	Aloesaponarin	*A. saponaria*	Roots	$C_{15}H_{10}O_4$	Cock (2015)
	Aloechrysone	*A. berhana*	Roots	$C_{15}H_{16}O_4$	Koroch (2009)
	Aloesaponol	*A. saponaria*	Roots	$C_{15}H_{14}O_4$	Cock (2015)
	Desoxyerythrolaccin	*A. saponaria*	Roots	$C_{15}H_{10}O_5$	Cock (2015)
	Chrysophanol	*A. saponaria*	Roots	$C_{15}H_{10}O_4$	Cock (2015)
	Prechrysophanol	*A. graminicola*	Roots	$C_{15}H_{14}O_4$	Yenesew et al. (1993)
	Helminthosporin	*A. saponaria*	Roots	$C_{15}H_{10}O_5$	Cock (2015)
	1,5-dihydroxy-3-hydroxy Methylanthraquinone	*A. excelsa*	Leaves	NS	Koroch (2009)
	7-hydroxyaloe emodin	*A. succotrina*	Leaves	NS	Sigler and Rauwald (1994)
	Nataloe emodin	*A. nyeriensis*	Leaves	$C_{15}H_{10}O_5$	Koroch (2009)
	Nataloe emodin-8-methyl ester	*A. specrosa*	Leaves	NS	Cock (2015)
	Isoxanthorin	*A. saponaria*	Roots	$C_6H_5N_5O_2$	Cock (2015)
	Laccaic acid-d-methyl ester	*A. saponaria*	Roots	NS	Cock (2015)
	Aloe emodin-11-*O*-rhamnoside	*A. rabaiensis*	Leaves	NS	Koroch (2009)
	Aloesaponol-*O*-methyl-4-*O*-glucoside	*A. barbadensis*	Leaves	NS	Yagi et al. (1998)
	Aloesaponol-6-*O*-glucoside	*A. saponaria*	Roots	NS	Cock (2015)
	Aloesaponol-8-*O*-glucoside	*A. saponaria*	Roots	NS	Cock (2015)
	Nataloe emodin-2-*O*-glucoside	*A. nyeriensis*	Leaves	NS	Cock (2015)
	Asphodelin A	*A. saponaria*	Roots	$C_{15}H_{10}O_6$	Cock (2015)

	Bianthracene	*A. saponaria*	Roots	$C_{28}H_{18}$	Cock (2015)
	Elgonicardine	*A. elgonica*	Leaves		Conner et al. (1990)
	5-hydroxyaloin A	*A. khamiensis Pillans*	Leaves	$C_{21}H_{22}O_{10}$	Rauwald and Beil (1993)
	7-hydroxyaloin A/B	*A. succotrina* Lam	Leaves	$C_{21}H_{22}O_{10}$	Sigler and Rauwald (1994)
	10-hydroxyaloin A	*A. arborescens; A. barbadensis*	Leaves	$C_{21}H_{22}O_{10}$	Park et al. (1998)
	5-hydroxyaloin A 6′-*O*-acetate	*A. marlothii* and *A. rupestris*	Leaves	$C_{23}H_{24}O_{11}$	Bisrat et al. (2000)
	7-hydroxyaloin-6′-*O*-monoacetate	*Aloe* spp.	Leaves	NS	Cock (2015)
	10-hydroxyaloin-6-*O*-acetate	*Aloe* spp.	Leaves	NS	Cock (2015)

NS Not Specified

I-type and chrysophanol-type while chrysophanol-type is also found in the leaces of aloe (Dagne et al. 2000). Anthraquinones such as aloe emodin and aloin exhibit strong anti oxidant activities probably due to their ability to scavenge free radicals and also inhibit peroxidation of lipids.

In the study, the antioxidant activity of anthraquinones, anthrones, aloe emodin, rhein amodin have been studied using a linoleic acid system. Thiocyanate method was used to determine the degree of peroxidation inhibition of the linoleic acid system at 37 °C. Anthrones and alzirin were identified to show the strongest antioxidant potential with no significant difference between them. Their scavenging power was found to be even stronger than α-tocopherol and BHT while anthraquinone had antioxidant potential stronger than rhein emodin but lesser than α-tocopherol. It was also determined that anthrone and alzinrin's antioxidant activity is due to their strong reducing powers and their reducing potential increases with their concentrations. The other anthraquinones did not show any reducing activity in the experiment. Similarly in the same study the scavenging activity of anthrones and anthraquinones was determined on the hydroxyl radicals with the result that emodin exhibits strong scavenging potential at a concentration of 0.25 mg/ml while chrysophanol, anthraquinone, rhein and alizarin at the same dose increase the formation of such radicals. Anthrone and aloe emodin also exhibit scavenging potential but their capacity is lesser than that of emodin at the same concentration (Yen et al. 2000).

In another study, aloin and aloe emodin were studied for their pro-oxidant and antioxidant activities at different doses. Radicals scavenging properties were determined using Chemiluminescence assay. Epigallocatechin-3-gallate (EGCG) was used as a comparative antioxidant agent. The experiment established that both aloin and aloe emodin showed antioxidant potential which increased by increasing their dose however it was less than that of EGCG at all concentrations and aloe emodin showed scavenging activity lesser than that of both aloin and EGCG at all concentrations. It was also found that aloin had a greater reducing tendency than aloe emodin at all concentrations attributing it's antioxidant potential to its reducing capacity. Also at high doses of 1.25–2.5 mM aloin prevented DNA breakage due to OH radicals by 5–30% over the control value but increases DNA damage at lower concentrations of 300–8 μM. Both aloe emodin and EGCG at high concentrations show pro-oxidant effects. The experiments established that the different effects of both aloin and aloe emodin on DNA may be attributed to the difference in their structures and concentration dependencies (Tian and Hua 2005). Studies have established that several antioxidant agents can become pro-oxidants at different concentrations and therefore their right doses should be established before their clinical use (Lee and Park 2003).

18.3.2 Bianthraquinoids

Genus does not contain many dimers however elgonica-dimers A and B (Elgonicardine) are found in *Aloe elgonica* while *Aloe saponaria* contains Asphodelin, Bianthracene II, III and IV in the plant's rhizomes and roots.

18.3.3 Anthrones

Anthrones has been reduced from anthraquinones and are a class of compounds mainly responsible for producing laxation and purgation effects. Alloin A and B are collectively called as barbaloin or alloin are C-glycosyl anthrones and are the first anthrones to be discovered in aloe. Anthrones have been found not only in *Aloe vera* but in amost a hundred species of genus Aloe in high concentrations of 10–20% (Dagne et al. 2000). Aloe ferox has almost a 30 percentage of such anthrones (Van Wyk et al. 1995).

Alloin however is not only confined to the genus Aloe but several other plant species belonging to different genus also contain alloin. Nataloin and homonataloin have been detected to be present in a species of aloe called *Aloe marlothii* Berger. The anthrone 10-Hydroxyaloin B is found in abundance in *Aloe llittoralis*. Anthrones are mostly present in the leaves and have not yet been discovered to present in the roots of the species (Dagne et al. 2000).

Anthrons such as alloin have been reported to act as strong antioxidants and protective of the DNA breakage at high concentrations (Yen et al. 2000; Tian and Hua 2005), but at lower concentrations, they act as prooxidants.

18.3.4 Chromones

It is one of the most abundant phenolic compound present in the aloe species including aloesin and aloeresin. These have been found and identified in most of the aloe species. Aloesin has been identified to occur in at least 30% of the examined species of the genus aloe (Dagne et al. 2000). Chromines such as aloesin and 7-O-methylaloesin are the major components found in Aloe rupestris Bak (Dagne et al. 2000).

18.3.5 Coumarins, Pyrans and Pyrones

Aloe ferox has been reported to contain coumarins a such as dihydroisocoumarin glycoside and feralolide (Speranza et al. 1993). Apart from his species *A. hildebrandtii* has also been reported to contain the said two coumarines (Veitch et al. 1994). Coumarin such as aloenin is a very bitter tasting component found in the leaf exudant of aloe and has been derived from phenylpyrone. These constituents of the aloe plants have been reported to suppress hunger (Costa et al. 2016). A double blind placebo controlled study, in an attempt to treat lymphedema of arms and legs with 5,6-Benzo- α -pyrone was carried out with the findings that the said pyrone stimulates macrophagaes to cause degradation of the albumins present outside the cells ultimately resulting in a rapid reabsorption of the fluid and thus lowering of the edematous swellings at a dose of 400 mg for a duration of 6 months. After the first month of therapy, the side effects were reported to have diminished as well (Casley-Smith et al. 1993).

18.3.6 Flavonoids

The studies on the presence of flavonoids had been seriously lacking as the focus was mainly on the presence of anthraquinones, anthrones and coumarines in aloe species. Out of three hundreds of the species flavonoids have been reported to occur in only thirty one species of the genus exhibiting their rare occurance. The major flavonoids found in these species are identified to include apigenin, naringenin, dihydroisorhamnetin, and isovitexin. Flavonoids have a strong antioxidant potential as they have been observed in inhibiting the lipid peroxidation in several trials. Due to the antioxidant properties of flavonoids they have been reported to prevent coronary heart diseases (Martikainen et al. 2007).

18.3.7 Alkaloids

O, N-dimethyltryamine and N-methyltryamine have been reported as the most common alkaloids found in aloe species. Gama-coniceine has been reported to occur in 6 species including *Aloe gillilandii* while coniine is found to occur only in one aloe species namely *Aloe viguieri* (Dagne et al. 2000). Alkaloids have been reported to exhibit toxic properties and aloe's toxicology may be attributed to their presence as exudates of the plant have been reported to be used as arrow poisons. It has been identified in a study that the alkaloids cause muscle paralysis. The mechanism underlying is observed to be their ability to block the nicotinic receptors of the post-synaptic neurons (Reynolds 2005).

18.3.8 Sterols

A number of important sterols have been found in the leaves of the aloe species including campesterol, cholesterol, lupeol, β-sitosterol and their glucosides (Dagne et al. 2000). These sterols present in plants have been reported to exert healing properties thus justifying the uses of aloe for healing purposes. The sterols are identified to increase production of the cells of endothelial walls of arteries as they increase the production of proteins important in keeping the integrity of vessels intact (Moon et al. 1999). The sterols found in the plant may also play a role attributing to the plant's anti-inflammatory properties as the sterols suppress the pain associated with inflammation and may provide analgesic properties (Sahu et al. 2013).

18.3.9 Other Compounds

Other compounds reported to be present in aloe include benzene, furan and naphthalene derivatives. Protocatechuic acid a benzene derivative found in aloe have been identified to exhibit antioxidant activities as it gets rid of free radicals as reported in a study (Lodovici et al. 2001). The aloe species also contain a number of important minerals such as magnesium, calcium, zinc, copper, and iron etc. The plants also contain sugars such as glucose, arabinose, mannose, galactose, and xylose apart from containing important dietary vitamins like vitamins B1, B2, B6, B12 and also vitamin C. The plant is also a source of amino acids and folic acid. An important polysaccharide acemannan is also present in *Aloe vera* gel and is thought to be the major reason behind the various benefits of the plant (Dagne et al. 2000).

18.4 Acceptable Daily Intake (ADI)

In India, Caribbean, China, and Japan Aloe plants are considered as traditional medicine for more than 2000 years. Researchers reported that various parts of Aloe plant especially gel and latex possesses wide range of pharmaceutical activities due to presence of numerous beneficial bioactive compounds. It has been reported that 8.5–13.8% of people of Hispanic populations in the southern USA frequently use *Aloe vera* in alternative and complementary medicine. According to surveys, it is also used regularly by 7.6%, 10.8% and 10.3% of adults in Jamaica, Australia, and Italy respectively (Ngo et al. 2010). A single-strength leaf gel of fluid ounces (59–237 mL) of *Aloe vera* was recommended for total daily consumption by the International Aloe Science Council (IASC 2013). Pure *Aloe vera* gel can be used generously as topical cream for skin. *Psoriasis vulgaris* and genital herpes can be treated by using hydrophilic cream, three times per day for five consecutive days per week made with 0.5% (by weight) of a 50% ethanol extract of *Aloe vera* (Ulbricht et al. 2007).

The gel/latex of Aloe plant can be used as medicinal laxative. The European Medicines Agency (EMA 2006) suggested that consumption of a correct individual dose of 30 mg of hydroxyanthracene glycosides per day produce soft-formed stool. The recommended dose for children and adults above 10 years of age is 10–30 mg of hydroxyanthraquinones/day, 40–110 mg of the dried latex or 100 mg as a single dose in the evening (WHO 1999). In adults oral dose of 4.5:1 *Aloe vera* gel concentrate of 25–100 mL per day was suggested for therapeutic applications (Morgan et al. 2005).

Recent research evidences on *Aloe vera* by the Natural Standard Research Collaboration reported that *Aloe vera* extract, gel or latex is safe if used as topical application for the treatment of mild to moderate skin conditions like inflammation, burn or wound (Ulbricht et al. 2008), its oral use has shown potential benefits like hypoglycaemic property, regulation of blood pressure, treatment of ulcerative colitis, stabilization of metastatic cancer and laxative effect (Ulbricht et al. 2008). Conversely, long term consumption of the latex or gel is unsafe as it can lead to allergic reactions, electrolyte imbalance, dehydration and diarrhoea. Individuals should follow the recommended dosing mentioned on package labelling before using the Aloe products. There is a need to understand in detail optimal dosage and of Aloe plant's preparations in the treatment of definite disorders.

18.5 Pharmacotherapy

Worldwide, traditional healers from a wide variety of cultural and ethnic group use members of genus Aloe for a broad range of medicinal purposes. In different parts of the world mostly in Asia (India and Nepal) and Africa maximum Aloe plants are used. The different parts of Aloe plants are used traditionally in the treatment of various ailments.

18.5.1 Antimicrobial Activity

Recent researches reported that different Aloe species possess antibacterial, antifungal and antiviral activities. The leaf extract of four different Aloe species *A. barbadensis*, *A. rupestris, A. juvenna* and *A. maculata* var. pulchra showed varied levels of antimicrobial activity against pathogenic bacteria *Bacillus cereus* (Sonam and Tiwari 2015). Water, petroleum ether and dichloromethane extracts of leaves, young bark, roots and upper stem of South African species *A. barberae* exhibit antimicrobial activity against Gram-negative (*Klebsiella pneumonia, Escherichia coli)* and Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria (Ndhlala et al. 2009). In vitro studies have shown that acetone extract of *A. vera* shows strong activity against *E. coli*, *S. aureus, Pseudomonas aeruginosa and Streptococcus pyogenes* compared to ethanol and aqueous extracts. Antibacterial activity of leaf extract from *Calotropis procera, Pongamia pinnata, Aloe vera, Lantona camara, Datura stromonium* were studied by Johnson et al. (2011). In a separate studies, Adetunji et al. (2011) evaluated the effect of aqueous extracts and ethanolic extract obtained from *Aloe vera* leave extracts against some pathogenic microorganism of clinical origin. The result obtained shows that the ethanolic extract exhibited a higher antibacterial activity against all the tested isolates. The high antimicrobial activity might be linked to the presence of phytochemical constituents available in the *Aloe vera* plant such as phenolics, tannins, alkaloids and saponin and cardiac glycosides. The antimycobacterial potential of *Aloe vera* were *Aloe vera* by Chandran et al. (2017) against *Mycobacterium smegmatis*. The result shows that

1000 mg/mL exhibited the highest antimycobacterial of 31 mm against the tested pathogen while the purified fraction exhibited a zone of inhibition of 40 mm. The antimicrobial activity exhibited by the purified compound were linked to the availability of acemannan or aloverose.

Kedarnath et al. (2012) evaluated the biologically active compound present in *Aloe vera and* the effect of these extract obtained from the leave was against some clinical isolates. The clinical isolates includes *Neurospora crassa, Aspergillus niger, and Aspergillus fumigates.* The antifungal activity was observed from ethanol and petroleum ether having 22 mm.

Aloe vera gel has been identified to show antibacterial activities against *Pseudomonas aeruginosa*. The polysaccharide acemannan was found to hinder its attachment to the epithelial cells of the human lungs (Azghani et al. 1995). Apart from *Streptoccocus pyogenes* and *Streptococcus faecalis* have also been found to be inhibited by *Aloe vera* gel (Heggers et al. 1995). *Aloe vera* gel also has properties to stimulate the immune system against foreign bodies. The plant has also exhibited antifungal activities against *Candida albicans* as reported in a study (Heggers et al. 1995). *Aloe vera* administration in rats showed that the plant has wound healing properties as it rids of the bacteria that may enhance the inflammation associated with wounds (Heggers et al. 1995).

Numerous research studies demonstrate that *A. vera* shows antiviral activity by preventing virus entry, adsorption, and attachment into host cells. According to Zandi et al. (2007) *A. vera* gel shows antiviral activity against herpes simplex virus (HSV) type 2 strains. Derivatives of anthraquinone (chrysophanol, aloe-emodin and emodin) present in Aloe exhibits antiviral activity by inhibiting virus-induced cytopathic effect and influenza A virus replication (Li et al. 2014).

18.5.2 Anti-dental Caries Activities

Studies has shown that Aloe vera possesses ant-dental caries activities. For instance, Fani and Kohante (2012) evaluated the effect of *Aloe vera* gel against periodontopathic (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*), opportunistic periodontopathogen (*Bacteroides fragilis*) and cariogenic (*Streptococcus mutans*) infected by patients suffering from dental caries and periodontal diseases. The authors tested the *Aloe vera* gel against 20 isolates which were bacteria using microdilution methods and disk diffusion. The highest antimicrobial activity was exhibited against *S. mutans* at the minimum inhibitory concentration of 12.5 µg/ml while *B. fragilis, A. actinomycetemcomitans*, and *P. gingivalis* show less sensitivity with minimum inhibitory concentration of 25–50 µg/ml. Their study shows that *Aloe vera* gel could be utilized for the prevention of periodontal diseases and dental caries.

18.5.3 Anti-urinary Tract Infection

Urinary tract infection (UTI) is a serious medical condition capable of affecting any part of the urinary system including the kidneys, ureters, bladder and urethra. Bukhari et al. (2017), tested *Aloe dera* gel against some UTI causing microorganisms, *Pseudomonas aeruginosa, Staphylococcus aureus,* and *E. coli.* The result observed shows that *Aloe vera* gel exhibited antimicrobial activity against all the selected uropathogens. The level of inhibition observed against the tested selected uropathogens were *E. coli* (76.9%), *Staphylococcus aureus* (75%) and *Pseudomonas aeruginosa* (40%). Their study shows that *Aloe vera gel* could be used for the management of UTI.

Begum et al. (2016) evaluated the antimicrobial efficacy of *Aloe vera* against *Streptococcus pneumonia, Staphylococcus saprophyticus*, *Klebsiella pneumonia, Staphylococcus aureus*, *Staphylococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*. The level of antimicrobial activity was assessed using zones of inhibition during antimicrobial susceptibility testing. The result obtained shows that *Aloe vera* gel exhibited an inhibitory effect against all the tested isolates most especially the ethanolic extract.

18.5.4 Antioxidant Activity

Overproduction of free radicals or reactive oxygen species (ROS) results in oxidative stress, which subsequently damages the DNA, protein and lipids of the body resulting in the development of numerous diseases. Antioxidants are those substances which at low concentration inhibits the oxidizable substrate oxidation. In vitro antioxidant potential was reported by the methanol extracts of leaf epidermis and flower of *A. vera* (López et al. 2013). The radical scavenging activity of most Aloe species is due to presence of chromone glycosides. 70% ethanol extracts of *A. vera* flower inhibit the free radical induced DNA damage and linolenic acid oxidation. High performance liquid chromatography (HPLC) was used to identify the 11 phenolic constituents present in the extract; vanillic acid content was found to be high in the extract which corresponds to high antioxidant activity. The extracts raise the antioxidant enzymes (glutathione peroxidise, superoxide dismutase and catalase) in the liver tissue of hydrogen peroxide-treated BALB/c mice. Presence of total phenolic content in the extracts promotes radical-scavenging activities. Thus, flowers of *A. bardadensis* are a valuable source of natural antioxidant (Debnath et al. 2018).

Free radical scavenging activity of Aloe gel was showed on nitric oxide radicals, 2,2-diphenyl-1-picrylhydrazyl(DPPH)and2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) + (Saini and Saini 2011). Antioxidant capacity of *A. ferox* was determined using ferric reducing antioxidant power (FRAP) analyses and oxygen radical absorbance capacity (ORAC). Results showed that it prevents or alleviates the symptoms of oxidative stress-related diseases due to presence of phytochemicals like aloeresins and 7-hydroxychromones in *A. ferox* shows strong antioxidant activity by suppressing the generation of reactive oxygen species and free radicals (Jones et al. 2002; Jia and Farrow 2005). In literature in vitro antioxidant activity of various Aloe species (*A. melanacantha*, *A. arborescens*, *A. harlana*, *A. ferox, A. saponaria, A. greatheadii* var. *davyana* and *A. marlothii*) leaf extracts have been reported (Asamenew et al. 2011; Yoo et al. 2008; Cardarelli et al. 2017).

18.5.5 Anti-ulcer Activity

Worldwide, one of the most common problem which impairs the quality of life is a chronic disease known as peptic ulcer which is linked to increased mortality and morbidity. Imbalance between aggressive factors (*Helicobacter pylori*, bile salts, chemicals, free radicals, acid, pepsin and pancreatic enzymes) and defensive factors (mucosa, adherent mucin, prostaglandins and bicarbonate) leads to ulcer formation.

In both humans and animals, *A. vera* gel has the ability to minimize the gastric ulcers (Suvitayavat et al. 2004). To promote digestion and in the treatment of peptic ulcer, extract of *A. vera* leaf have been generally recommended due to its efficient antibacterial activity and cytoprotective action. *A. vera* gel acts as a promising natural antibiotics which inhibit the growth of resistant *H. pylori* strains (Radha and Laxmipriya 2015).

A. vera gel extract shows gastro-protective nature as it acts against the aggressive factors and protects the gastric mucusa from ulceration by increasing the levels of glycoproteins and resist the attack of proteolytic enzymes. Presence of flavonoids in *A. vera* gel ameliorate the glycoprotein abnormalities and stabilize the antioxidant status of the gastric mucosa (Subramanian et al. 2007).

In pylorus ligated and lumen perfuse rats, hydrochloric acid induced-gastric mucosa damage and gastric acid secretion was studied after application of varying doses of ethanol extract of *A. vera* (Liliaceae) results demonstrate that extract possess gastroprotective activity and gastric acid inhibitory properties at lower concentration (Yusuf et al. 2004). Another study established that a mixed treatment with sucralfate and *A. vera* reduced ulcer sizes, gastric inflammation, elongate gastric glands and enhance epithelial cell proliferation (Eamlamnam et al. 2006).

18.5.6 Anticancer Activity

In the human population one of the most common and lethal malignancies is hepatocellular carcinoma with approximately 550,000 new cases. Aloe species are considered as a source of medicine in rural areas. Shalabi et al. (2015) evaluated the in vitro anticancer effect of different doses of *A. vera* and *C. comosum* extracts against hepatocellular carcinoma (HepG2) cells. Results signify that both the extracts induce genotoxic and cytotoxic effect on human hepatocellular carcinoma (HepG2) cells in a time and dose dependent manners through induction of apoptotic pathway by increasing P53 and decreasing Bcl-2 genes expressions.

Aloin[10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H)-anthracenone, l] or barbaloin is an anthraquinone glycoside, a natural phytochemical present in *A. vera* and many other plants of Aloe genus are shown to exhibit cytotoxic and chemoprotective effects against 1,2-dimethylhydrazine-induced colon preneoplastic lesions in Wistar rats (Hamiza et al. 2014). The antiproliferative nature of anthracycline aloin isolated from *A. vera* was tested against human uterine carcinoma HeLaS3 cells. Results indicate that aloin shows anti-metastatic potential by causing cell cycle arrest in the S phase and markedly increasing HeLaS3 cell apoptosis (Niciforovic et al. 2007). The polysaccharide mannan isolated from *A. saponaria* was evaluated for its anti-proliferative effects using normal human cells (PBMC), murine cells (SpMC) and many tumor cell lines. It was found that proliferation of both normal and tumor or cancer cells were inhibited by mannan and it affected the expression of CD3+ SpMC, signifying inhibition of mostly T-lymphocyte proliferative response (Sampedro et al. 2004). Aloin obtained from Aloe plant was tested for cytotoxicity against two human breast cancer cell lines with (SKBR-3) erbB-2-topolla coamplification and without (MCF-7). It was reported that SKBR-3 was less sensitive to aloin than MCF-7 cell line, as established by the clonogenic and MTT assays (Esmat et al. 2006).

An anthracenedione derivative aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione) is also derived from *A. vera* leaves are shown to possess antiproliferative effects in few cancer cell types like neuroectodermal, lung, glioma and squamous cancer cells by inhibiting gene expression and N-acetyl transferase activity (Masaldan and Iyer 2014). The bioactive component obtained from *A. ferox* is used as potential anticancer agent. It has been reported that Aloe-emodin does not affect normal cells and active against neuroectodermal tumors. It promotes cell death through uptake of specific drug by neuroectodermal tumors (Pecere et al. 2000).

In leaves of Aloe plants, sugar-binding proteins present are commonly known as lectins which have many immunological activities. In vivo studies report that plant lectin act as protein carrier which activate T cells and increase antitumor immunity (Yoshimoto et al. 1987).

Anthraquinones and anthrones at specific safe doses get rid of free radicals such as hydroxyl radicals and prevent DNA damage. In a study, aloin and aloe emodin were studied for their prooxidant and antioxidant activities at different doses. Radical scavenging properties were determined using Chemiluminescence assay. Epigallocatechin-3-gallate (EGCG) was used as a comparative antioxidant agent. The experiment established that both aloin and aloe emodin showed antioxidant potential which increased by increasing their dose however it was less than that of EGCG at all concentrations and aloe emodin showed scavenging activity lesser than that of both aloin and EGCG at all concentrations. It was also found that aloin had a greater reducing tendency than aloe emodin at all concentrations attributing its antioxidant potential to its reducing capacity. Also at high doses of 1.25–2.5 mM aloin prevented DNA breakage due to OH radicles by 5–30% over the control value but increases DNA damage at lower concentrations of 300–8 μM. Both aloe emodin and EGCG at high concentrations show prooxidant effects. The experiments established that the different effects of both aloin and aloe emodin on DNA may be attributed to the difference in their structures and concentration dependencies (Tian and Hua 2005). Studies have established that several antioxidant agents can become prooxidants at different concentrations and therefore their right doses should be established before their clinical use (Lee and Park 2003).

18.5.7 Anti-inflammatory Activity

During inflammatory process, highly active pro-inflammatory mediators (prostaglandins) are produced from arachidonic acid in the presence of cyclooxygenase (COX) enzymes (prostaglandin-H_2-synthases) which act as catalysts (Steinmeyer 2000). Inhibiting COX enzymes, particularly the COX-2 enzyme inhibit production of prostaglandin to resolve inflammation. Fifty-one different Aloe species were reported to show different activity levels against COX-1 enzymes (Amoo et al. 2014). Administration of Aloe has been resulted in proliferative and phagocytic activity by reducing prostaglandin E2 production and inhibiting COX pathways (Park et al. 2009). Methanol extract of *A. ferox* inhibit the COX-1 effects as reported by Lindsey et al. (2002). In the early phase of acute inflammatory response tumor necrosis factor (TNF)-α genes and albumin transcription levels are involved. Elimination of albumin gene transcription was observed in rats treated with aloe-emodin. After administration of aloe- emodin, decreased level of TNF-α was detected in livers. Rats treated with aloe-emodin showed a reduced inflammatory infiltration of the Kupffer cells and lymphocytes (Arosio et al. 2000). The presence of chromones and anthraquinones in the inner Aloe gel acquire strong anti-inflammatory effects in murine macrophages (Park et al. 2009).

Studies report that *A. vera* gel had strong NLRP3 (NACHT, LRR, and PYD domains-containing protein 3) inflammasome expression, immunomodulatory activity and downregulating lipopolysaccharide-induced inflammatory cytokine production in human macrophage (Budai et al. 2013). In severe traumatic–hemorrhagic rats pre-treatment of Aloe polysaccharide can attenuate reperfusion injury and cerebral ischemia by inhibiting lipid peroxidation, systemic inflammatory response and leukocyte aggregation (Liu et al. 2012).

Bradykinin is an inflammatory substance that is associated to cause pain during inflammation. Bradykinase is an enzyme that causes the breakdown of bradykinin has been discovered and isolated from aloe that cause breakdown of bradykinin thus attributing to the plant's anti-inflammatory properties. Furthermore, sterols present in the plants are natural analgesics and help suppress pain (Sahu et al. 2013).

18.5.8 Immunomodulatory Activity

Aloe has been used for its immunomodulatory properties as the plant has been studied to enhance immune responses and strengthening the immune system. In a study, rats were administered with cancerous agents before administering with aloe. It was observed that there was an increase in production and release of interleukin 1 and tumor necrosis factor which caused the necrosis of the cancerous cells. Acemannan a polysaccharide was found to cause such immune mediated response (Sahu et al. 2013).

18.5.9 Antidiabetic Activity of Phytochemicals from Genus Aloe

Medicinal plants or natural products are source of bioactive compounds which minimise adverse effects and offer promising efficient drugs with low cost. The plants that belong to genus *Aloe* contains numerous phytochemicals that can be extracted via aqueous, alcohol (Nejatzadeh-Barandozi 2013) and chloroform as solvent (Raphael 2012). Tannin, saponin, flavonoids, and terpenoids are the most significant phytochemicals that are present almost in all the plants of genus *Aloe*, irrespective of their species (Arunkumar and Muthuselvam 2009). Other than these phytochemicals, these plants contain lectins, fatty acids, chromones, anthraquinones, cholesterol, mono and polysaccharides, sterols, choline, aloetinic acid, sapogenins, choline salicylate and complex mucopolysaccharides. In addition, they also possess amino acids such as serine, aspartic acid, arginine, glutamic acid and arparagine, as well as vitamins, namely folic acid, β-carotene, B1, B2, B6, C, α-tocopherol and mannose 6-phosphate as predominant sugar component (Joseph and Justin Raj 2010). Several literatures reported that the phytochemicals extracted from genus *Aloe* are highly beneficial as an antidiabetic agent to reduce serum glucose, cholesterol level and enhance wound healing process in diabetic patients. However, their antidiabetic effect is based on the plant part where the phytochemicals are extracted, which directly correlates with the quantity of extracted phytochemicals and solvent used in the extraction process (Singh et al. 2010).

Research findings indicate that leaf latex extract of *A. megalacantha* exhibit significant antihyperglycemic activities in STZ-induced diabetic mice. This plant ameliorates diabetes and its related complications. *A. megalacantha* is showed to improve parameters like body weight, hypoglycemic activity, oral glucose tolerance, antioxidant potential and lipid profile (Hammeso et al. 2019). In vitro and in vivo studies established that the water soluble fraction of *Aloe* species possesses some components that can modulate glucose transporter-4 mRNA expression along with glucose-lowering activities (Kumar et al. 2011).

High priority research includes plant derived various naturally active ingredients used in the treatment of diabetes viz. *A. vera* is considered as an antidiabetic agent.

Research studies shown that polysaccharide present in the plant protects β-cells of islets of langerhans from oxidative damage by alloxan monohydrate and also enhance the insulin levels showing hypoglycaemic effect (Das et al. 2011). The major plant derived phytosterols like 24-methylene cycloartanol, lophenol, cycloartanol, 24-methyl-lophenol, and 24-methyl-lophenol were reported to show beneficial effects in obesity and diabetes (Misawa et al. 2012). In another study a phytoconstituent (aloe-emodin-8-*O*-glycoside) isolated from *A. vera* gel were reported to enhance transport of glucose through proximal and distal marker modulation concerned with better uptake of glucose and its transformation into glycogen (Anand et al. 2010). Aloe species shows positive effect in the treatment of diabetes over short intervention periods. Through pilot study, improvements were observed in serum insulin, end-point glucose, TC:HDL-C and HDL-C using leaf gel extracts of *A. greatheadii* var. davyana, in a STZ-induced diabetic rodent model. Another Aloe species *A. ferox* intervention showed similar positive effects but to a lesser extent (Loots et al. 2011).

Jain et al. (2010) reported that *A. vera* gel shows significant cardioprotective and antidiabetic effects, as it significantly enhance antioxidant status and reduce oxidative stress in streptozotocin-induced diabetic rats.

18.5.9.1 Leaf Extract

The leaf of genus *Aloe* is different from the normal leaves, which contains a latex and gel. In most of the literatures, the latex is referred to as leaves and gel as a separate entity from the plant (Bozzi et al. 2007). In an experiment, *Aloe vera* juice was prepared with the aloe gel, sorbitol as sweeting agent and certain preservatives are given to 50- and 22-women diabetic subjects for 42 days. The result of the study revealed that the *Aloe vera* gel has antidiabetic activity by reducing total cholesterol, post-prandial and fasting blood sugar, total lipids, triglycerides and increase high density lipoproteins (Yongchaiyudha et al. 1996). Similarly, alcoholic extract of *Aloe vera* leaf gel was orally administered to diabetic rats to evaluate their antidiabetic effect. The result confirmed that the phytochemicals present in alcoholic extract decrease blood glucose level, glycosylated hemoglobin and increases the hemoglobin level. Further, the extract brings down the level of lipid peroxidation and hydroperoxides in diabetes rat tissues, and increases superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase and glutathione-S-transferase in diabetic kidney and liver of rats (Noor et al. 2008). Also, the leaf pulp and gel extract of *A. vera* was subjected to non-diabetic, type 1 and type 2 diabetic rats to evaluate their antidiabetic activity. The result emphasized that the extracts do not reduce blood sugar level in non-diabetic subjects, whereas the pulp extract reduces glucose levels in type 1 and 2 diabetes rats, compared to glibenclamide diabetic drug. However, the gel extract enhances hyperglycemic activity in type 2 diabetic rats, which showed that the pulp extracts of *A. vera* leaves are highly beneficial in the treatment of non-insulin dependent diabetes patients (Okyar et al. 2001).

In recent times, the pulp of the *A. vera*, that are free from the peel, were lyophilized and ethanolic extract was obtained via the Soxhlet method. The result emphasized that the extract significantly decreases blood glucose level and improves body weight, which is attributed to the phytochemical combinations present in the *A. vera* pulp extract (Sacan et al. 2017). Likewise, the *A. vera* leaf powder including dried latex and gel were macerated at room temperature with ethanol for 72 h to obtain the phytochemical extract. The result showed that the presence of phenol, saponins and anthraquinones in the extract were beneficial in enhancing the wound vascularity to remove dead tissue and increase wound healing ability (Negahdari et al. 2017). Moreover, the oral administration of *A. vera* leaf powder extracts proved to be useful in improving the insulin secretion and the function of pancreatic β-cell in streptozotocin-induced diabetic rates (Noor et al. 2017). Furthermore, the leaf extracts of *A. arborescens* has been reported to reduce the blood sugar, where the phenol compounds present in the extract possess antioxidant activity in the pancreas and blood to protect islets of Langerhans from the methyl radical from streptozotocin (Beppu et al. 2006). Additionally, the combined leaf (latex and pulp) extract of plants that belong to genus *Aloe* such as *A. chaboudii*, *A. inyangensis*, *A. zebrina*, *A. barbadensis*, *A. pruinosa* and *A. arborescens* × *A. barbadensis* hybrid is widely reported to contain phytochemicals that are useful as enhanced antidiabetic agents (Grčić et al. 2016). Recently, the phytochemical constitution, hypoglycemic potential, antioxidant activity and toxicity of leaf extract of *A. lateritia*, *A. secundiflora* and *A. buettneri* is investigated. The results emphasized that the extracts are non-toxic to alloxan-induced diabetic mice and the phytochemicals such as phenols, flavonoids, tannins, steroids, saponins, alkaloids, anthraquinones and carbohydrates present in the extracts possess hypoglycemic activity (Mbithi et al. 2018; Guessan and Kouakou 2017).

18.5.9.2 Root Extract

Similar to leaves, root extract of the plants that belong to genus *Aloe* also possesses phytochemicals that can act as potential antidiabetic agent. The root extract of *Aloe ferox* contains large quantities of phenols and saponins, along with small portions of flavonoids, flavonols, tannins and alkaloids (Arowosegbe et al. 2012). The presence of these phytochemicals is already proved to be beneficial in exhibiting antidiabetic activity via several literatures. The root extract of *A. vera* and *A. barbadensis* are proven to be helpful in stimulating the synthesis and release of insulin, exhibits insulin secretagogues activity via secretion of pseudoprototinosaponin AIII and prototinosaponins AIII and initiates glucose uptake through enhanced hepatic gluconeogenesis or glycogenolysis (Patel et al. 2012b; Bnouham et al. 2006). In addition, the ethanol extract of *A. barbadensis* root has been proven to increase testosterone and cholesterol concentration depending on the dosage increase, which elevates their aphrodisiac property and eventually contributes to their antidiabetic property (Erhabor and Idu 2017). Likewise, the acetone extract of *A. pulcherrima* roots were reported to contain three unique phytocompounds such as aloesaponarin I, II and chrysophanol with antioxidant properties. Thus, these extracts can be beneficial in reducing blood sugar and other diabetes related complications (Abdissa et al. 2017). Moreover, the root extract of *A. vera* is reported to contain two unique anthraquinones namely aloesaponarin-I and II, and six of their derivatives were obtained by acetylation, O-glycosyl reactions and methylation along with a novel tetra-O-acetyl-β-D-glucopyranosyl derivative. All these novel derivatives and phytocompounds will be beneficial in triggering insulin secretion, reduce blood glucose level and reverse diabetes complications (Borges-Argáez et al. 2019). However, there are a smaller number of literatures that are available on the root extracts of plants that belong to genus *Aloe*, as these plants are mostly succulent, and they contain less concentration of phytochemicals that can be extracted. Even though, they contain less concentration, the phytochemicals present in root extracts are similar to leaf extracts and thus they exhibited antidiabetic activity.

18.5.9.3 Flower Extracts

Flowers are another exclusive part in the plants of the genus *Aloe* from where the phytochemicals can be extracted. It has been reported that the phytochemicals extracted from the flowers of A. barbadensis contain anthraquinone such as aloe-emodin and aloin are proposed to be beneficial for their antidiabetic effects (2007). Likewise, the dried flower extracts were obtained from *A. barbadensis* which was analysed and identified to contain caffeic, caffeoylshikimic, chlorogenic, 5-p-coumaroylquinic, 5-p-cis-coumaroylquinic, 5-feruloylquinic, ferulic acid, p-coumarin, apigenin, luteolin, isoorientin, saponarin, kaelpferol, 7-O-glucosides and lutonarin. In addition, they also contain anthranoids such as aloe-emodin, glycosylchromone aloeresin B, Aloin A and B along with polyphenols and flavonoids. The combination of all these phytocompounds in synergistic effect can lead to a reduction in the blood glucose level, elevates the production of insulin and reverses insulin resistance to exhibit improved antidiabetic activity (Keyhanian and Stahl-Biskup 2007). Moreover, the skin and flower of *A. vera* was used to extract phytochemicals via methanol as solvent. It is noteworthy that the flower extract contains phenolic compounds such as gentisic acid, quercitrin and epicatechin. These phytocompounds are proved to highly significant as an antioxidant agent which will eventually help in the inhibition of diabetic cells and proliferation of normal cells (López et al. 2013). Furthermore, the flower extract of *A. vera* has been reported to possess anti-inflammatory effects due to the combination of extracted phytochemicals, which will also be beneficial in their antidiabetic effects (Vazquez et al. 1996, Nejatzadeh-Barandozi and Enferadi 2012). Polysaccharides from *A. arborescens* are extracted from the skin juice, gel juice and flowers via ethanol precipitation, where the polysaccharides from the flowers are reported to be weakly acidic in nature. The result also showed that the extracted polysaccharides contain glucose, galactose, glucuronic acid, mannose and xylose. Thus, these sugar molecules can be beneficial for diabetic patients, which will be an easily soluble sugar that can be converted into ATP with less concentration of insulin (Chang et al. 2011). In recent times, the flower extract of *A. barbadensis* and *A. vera* is highly significant as antioxidant agents which will eventually lead to enhanced antidiabetic effects (Debnath et al. 2018, Haroon et al. 2018). However, it is necessary to carry out several studies to prove the exact antidiabetic mechanism of phytochemicals that are present in the flower extract of plants under genus *Aloe*.

18.5.9.4 Other Extracts

Other than the extraction of phytochemicals from the leaf, root and flower of *Aloe* genus, the whole plant extracts are widely used for antidiabetic activity, as they contain large quantities of significant phytochemicals that are responsible for antidiabetic effects. *Aloe vera* (Arunkumar and Muthuselvam 2009), *A. barbadensis* (Boudreau et al. 2013b), *A. arborescens* (Jia et al. 2008), *A. ferox* (Wintola and Afolayan 2011) and *A. schweinfurthii* (Salawu et al. 2017) are the plants that belong to genus *Aloe* which are used to extract the phytochemicals from the whole plant. These whole plant extracts with a wide variety of phytochemicals are highly useful in exhibiting antidiabetic activity via synergistic effects (Cock 2015). However, it is difficult to evaluate the antidiabetic effect of individual phytocompounds from the whole plant extract which will be a major drawback in using these type of extracts.

18.5.9.5 Antidiabetic Nanoparticles from Genus *Aloe*

The phytochemicals extracted from the genus *Aloe* are recently employed in the formation of nanoparticles as reducing and stabilizing agent. These green synthesized nanoparticles are revealed to possess potential in reducing blood glucose, increase insulin secretion and exhibit antidiabetic effects. Zinc oxide nanoparticles are synthesized via aqueous extracts of *A. vera* gel proves to contain antioxidant property which eventually helps in reducing serum glucose. The phytochemicals present in the extract namely saponins, tannins, alkaloids, flavonoids, carbohydrates, terpenoids, gums, mucilages and phenolic compounds help as reducing and stabilizing agent for nanoparticle formation and also their antioxidant activity (Mahendiran et al. 2017). Likewise, the phytochemicals present in the flower extract of *A. vera* was also used to fabricate copper nanoparticles (Karimi and Mohsenzadeh 2015). These nanoparticles are proved to be highly significant as a potential nanomedicine to cure diabetes-related complications (Bhagwat et al. 2018). Similarly, silver (Dinesh et al. 2015), gold (Altaf and Jaganyi 2016), iron oxide (Ali et al. 2018), copper oxide (Kumar et al. 2015), carbon-based nanoparticles (Devi et al. 2018) was also synthesized by using extracts obtained from genus *Aloe*. However, extensive study has to be carried out in the future to evaluate the exact antidiabetic mechanism of *Aloe* phytochemical coated nanoparticles in reducing the complications of diabetes.

18.5.10 Antihyperlipidemic Activity

Recent studies show the antihyperlipidemic activity of *A. vera* and its positive effect in the prevention of fatty streak and development of atherosclerosis. Due to less insulin secretion or its action in the body, the increase in circulatory glucose levels is responsible for an increase in the free fatty acids (FFA's) by the action of hormone sensitive lipase from adipose tissue in the blood. The excess FFAs in circulation enter into the liver for the synthesis of Tri glycerides (TG) and further lipoprotein biosynthesis. Liver plays a vital role in glucose and lipid metabolism. In diabetes, its function is affected and results in liver steatosis (accumulation of lipids) (Seifter and England 1982). The supplementation of ethanolic extract of *Aloe vera* leaf gel (300 mg/kg body weight) in diabetic rats showed an increase in the plasma insulin levels from remnant or regenerated pancreatic β-cells, whereas blood glucose levels were brought to normal. In addition, *Aloe vera* extract administration also showed a decrease in the plasma lipids, liver cholesterol, and kidney TG levels (Rajasekaran et al. 2006). Authors concluded that, phenolic and saponin compounds present in the *Aloe vera* extract might be responsible for hypoglycemic and hypolipidemic effects.

In a randomized double-blind placebo-controlled clinical trial, efficacy of *A. vera* leaf gel was checked in hyperlipidemic type 2 diabetic patients, results showed the reduction in low-density lipoprotein (LDL) and total cholesterol levels (Huseini et al. 2012). In Zucker diabetic fatty rats administration of phytosterols isolated from gel of *A.vera* improve hyperglycemia and reduce visceral fat mass (Dana et al. 2012). Medicinal herbs like *Aloe barbadensis* Mill. or *A. vera* has shown to possess anti-hyperlipidemic and hypoglycaemic potential. Letrozole-induced polycystic ovarian syndrome rat model treated with *A. vera* gel shows increase in HDL cholesterol along with reduction in LDL and triglyceride levels. The phytoconstituents present in *A. vera* gel manage metabolic complications by improving lipid metabolizing enzyme activities, abnormal estrous cyclicity and glucose intolerance (Desai et al. 2012). A remarkable antihyperlipidemic effect was shown by a dried pulp extracted from leaf of *A. succotrina* in high-fat diet and fructose-induced hyperlipidemic Wistar albino rats. *A. succotrina* normalize serum lipid profile and ameliorate oxidative stress in liver without affecting relative heart weight (Dhingra et al. 2014). The selenium (Se) polysaccharide (Se-AVP) from *A. vera* was shown to have cardioprotective effect against myocardial I/R injured in rats, it was noted that (Se-AVP) act as endogenous antioxidant which protect rat hearts from oxidative stress-induced myocardial apoptosis (Yang et al. 2017).

The antioxidative and hypocholestrol effects of *Aloe vera* was assessed in randomly selected liver of male specific pathogen-free (SPF) Fischer 344 rats to 17 of four groups: Group A (control) was fed test chow without aloe supplementation; Group B was fed a diet containing a 1% (per weight basis) freeze-dried aloe filet; Group C was fed a diet containing a 1% (per weight basis) charcoal-processed, freeze-dried aloe filet; and group D was fed a diet containing a charcoal processed freeze-dried, whole leaf aloe (0.02% per weight basis) in the drinking water. Results show that a life-long intake of aloe had superior anti-oxidative action against lipid peroxidation in vivo, as indicated by reduced levels of hepatic phosphatidyl choline hydroperoxide. Additional anti-oxidative action was evidenced by enhanced superoxide dismutase (SOD) and catalase activity in groups B and C. furthermore, study revealed that hepatic cholesterol significantly increased in the control group in contest to the aloe-supplemented groups, which showed approximately 30% lower cholesterol levels, thereby an effective hypocholestermic efficacy (Lim et al. 2003). The effect on man triglyceride level was estimated which is due to inhibition of the hepatic production of chylomicron (Boban et al. 2006). Saponin and phenolic components of *Aloe vera* extract also exerted the antihyperlipidemic effect by decreasing the levels of total cholesterol, triglyceride and lipoprotein. High blood cholesterol is a major risk factor for heart disease and stroke.

A study was conducted on the effect of *Aloe vera* extract on the serum cholesterol level on male Calote sversicolor Daudin. The calotes versicolor Daudin were made hypercholesterolemic by oral administration of cholesterol (100 mg/kg body weight/day) suspended in ground nut oil using dropper. In 1 month cholesterol feeding experiment, the serum cholesterol level in normal controls (not given cholesterol) was 321.333 ± 16.621 mg/dl and in cholesterol fed animals 437.333 ± 8.066 mg/dl. To such animals when different doses of raw extracts of *Aloe vera* leaves were given along with cholesterol, there was significant decrease in serum cholesterol level. Four groups of Calotes were administered Aloe vera (L) extract in four different doses (3 mg/kg, 4 mg/kg, 5 mg/kg and 6 mg/kg/day) for 21 days. There was a significant increase in serum cholesterol levels at 1% level after feeding with high cholesterol diet. There was a significant decrease in serum cholesterol levels in all the *Aloe vera* (L) treated groups. Significance level is 5% for a dose of 6 mg/kg and other doses i.e. of 3 mg/kg, of 4 mg/kg and of 5 mg/kg show significant decrease at 0.1%, 0.5% and 0.2% level, respectively (Chandrakar et al. 2008).

18.5.11 Antiaging Effects of Aloes

Photochemoprotection has recently become valuable way to prevent aging. Although there are many active synthetic antiaging drugs that have been used for years, these drugs may exert safety risk on human health. Therefore, present era of treating an aged skin has been diverted towards natural biomaterials (Rajashree and Rose 2018). The plant Aloe has also been studied to protect the skin again UV rays (Surjushe et al. 2008).

Aloe vera gel was reported to improve skin hydration and to possess moisturizing effect for stratum corneum at different tested concentrations (0.1%, 0.25%, and 0.5%) (Chandan et al. 2007). Mucopolysaccharides (MPS) present in Aloe gel is responsible for the water-holding capacity exerted by the gel to the skin. *Aloe vera* can improve the elasticity of the skin through activation of the fibroblasts which responsible for the production of collagen and elastin fibers reducing skin wrinkles. In addition, amino acids in Aloe soften dry skin cells and increase its zinc content, and decrease pores sizes through its astringent effect (West and Zhu 2003).

Topical application of *Aloe vera* gel on the skin, yield to the production of the antioxidant protein metallothionein which scavenges hydroxyl radicals ($OH^{\bullet}$), inhibits superoxide dismutase (SOD) suppression and glutathione peroxidase (GSHx) in skin with subsequent reduction in interleukin-10 (IL-10) production (Byeon et al. 1988). Accordingly, *Aloe vera* gel can also prevent the UV and gamma rays-induced skin damages (Roberts and Travis 1995). Aloesin can act as a potential as a pigmentation-altering component for cosmetic uses through inhibition of the tyrosinase enzyme (Yagi et al. 2003).

Cho et al. 2009 studied the effect of 90 days dietary intake of *Aloe vera* gel supplementation at 2 different doses (1200 and 3600 mg/day) on thirty healthy female subjects over the age of 45. Their facial wrinkles measured using a skin replica were improved significantly in at the two doses, and facial elasticity determined by an in vivo suction skin elasticity meter were improved in the lower-dose group compared to their baseline status which was used as a control. In the photoprotected skin, the type I procollagen mRNA levels were insignificantly, increased at the two dose levels, the matrix metalloproteinase 1 (MMP-1) mRNA levels expression determined using real-time RT-PCR was significantly decreased in the higher-dose group. Type I procollagen immunostaining was substantially increased throughout the dermis in both groups. So, the gel significantly improved wrinkles and elasticity in photoaged human skin, with an increase in collagen production in the photoprotected skin and a decrease in the collagen- degrading MMP-1 gene expression. However, no dose- response relationship was found between the low-dose and high-dose groups.

Tanaka et al. (2015) investigated the capability of *Aloe* sterols (cycloartenol and lophenol) to stimulate human dermal fibroblasts in vitro. After 48-h co-culture with Aloe sterols, the production of collagen and hyaluronic acid increased in a concentration-dependent manner. Treatment of the human dermal fibroblasts with 2 μM Aloe sterols increased collagen and hyaluronic acid production by approximately twofold and 1.5-fold The gene expression levels of the enzymes responsible for the synthesis of collagen (COL1A1 and COL3A1) and hyaluronic acid (HAS2 and HAS3) was associated with a dose-dependent increase in their mRNA level after A 6-h incubation period with 0.02–2.0 μM cycloartenol and lophenol in human dermal fibroblasts.

The authors also investigated the effect of intake of *A. vera* gel powder containing 40 μg *Aloe* sterols on the skin conditions in 54 Japanese women with dry skin in a randomized, double-blind, placebo-controlled trial. An increase in arm skin hydration was observed at 8 weeks in the *A. vera* gel powder treated group, whereas a slight decrease in arm skin hydration was noted in the placebo group. However, there was no statistical difference between *A. vera* gel powder and placebo groups in skin moisture. In subgroup analysis, the change in the mean wrinkle depth was significantly lower in the *A. vera* gel powder group than in the control group. No observed harmful phenomenon during the treatment period. The study confirmed that daily oral *Aloe* sterol-containing *A. vera* gel powder significantly reduced facial wrinkles in women aged more than or equal to 40 years, and Aloe sterols stimulate collagen and hyaluronic acid production by human dermal fibroblasts.

Tanaka et al. (2016) performed a randomized, double-blind, placebo-controlled study for 12-weeks to evaluate the effects of oral Aloe sterol-supplemented yogurt on skin elasticity, hydration, and the collagen score in 64 healthy women (age range 30–59 years; average 44.3 years). The treated group revealed statistical differences in skin moisture, trans-epidermal water loss, skin elasticity, and collagen score between the from placebo groups. The gross elasticity (R2), net elasticity (R5), and biological elasticity (R7) scores of the Aloe sterol group significantly increased with time. In addition, skin fatigue area F3, which is known to decrease with age and fatigue, also increased with Aloe sterol intake. Ultrasound echogenicity revealed that the collagen content in the dermis increased with Aloe sterol intake. The results suggest that continued Aloe sterol ingestion contributes to maintaining healthy skin.

Rajashree and Rose (2018) reported an anti-aging gel formulated by blending three biopolymers which were Collagen (3%w/v), Chitosan (1.5% w/v) and *A. vera* gel (0.21% w/v). The prepared gel was characterized by good spreadability and high hydrophyllicity. Cell culture studies on the mouse fibroblasts cells (NIH3T3) were carried out using senescence-associated-β-gal as a biomarker. A. vera blended gel stimulated proliferation rate of the fibroblasts cells (NIH3T3) reversing of the process of senesce. The prepared gel helps in regeneration and rejuvenation of the skin and is considered as a promising anti-aging gel.

18.5.12 Wound Healing Activity

The sterols present in plants have been reported to exert healing properties thus justifying the uses of aloe for healing purposes. The sterols are identified to increase production of the cells of endothelial walls of arteries as they increase the production of proteins important in keeping the integrity of vessels intact (Moon et al. 1999). Furthermore, *Aloe vera* administration in rats showed that the plant has wound healing properties as it rids of the bacteria that may enhance the inflammation associated with wounds (Heggers et al. 1995). Also glucomannan and gibberellins cause increase production of collagen by causing increase activity of fibroblasts when aloe is applied topically or administered orally (Sahu et al. 2013).

Adhikari et al. (2018) validated the antibacterial influence of gel extract of *Aloe barbadensis* against multiple antibiotic resistant *Pseudomonas aeruginosa* that is responsible for wound development from affected patients. The result obtained from their study shows that *Aloe vera* gel could be used for the treatment of multiple antibiotic resistant *Pseudomonas aeruginosa* isolated from wound specimen.

Escobar-Sierra and Perea-Mesa (2017) evaluated the capability of chitosan and polyvinyl alcohol to absorb *Aloe vera* gel for effective control release in order to facilitate the rate of healing infected wounds and their effectual healing. The fabrication of the membrane involves different composition which involve Chitosan and

Polyvinyl Alcohol (PVA/CH) at 5 and 10% w/v and employing different PVA/CH relations of 30/70, 50/50 and 70/30 (v/v) and embed them in a 2% (v/v) *Aloe vera* solution to create hydrogels. The crosslinking matrix developed from PVA/CH led to the formation of good mechanical properties, enhance absorption capacity, and control the release of the active compounds present in the *Aloe vera* gel.

18.5.13 Laxative Effects

Anthrones in aloe are a class of compounds mainly responsible for producing laxation and purgation effects (Cock 2015). Also anthraquinones have been reported to have laxative and purgative abilities as they have been found to increases intestinal motility, mucus secretion while also enhancing intestinal water content. Alloin A and B have been observed to increase intestinal motility as they are reduced by colonic flora into active compounds which irritate the GIT wall and increase motility (Sahu et al. 2013).

18.5.14 Antiseptic Activity

Presence of salicylic acid, lupeol, urea nitrogen, phenols, cinnamonic acid, and sulphur attributes to the plant's antiseptic properties (Sahu et al. 2013).

18.5.15 Analgesic Activity

Aloe rupestris Baker's root has been used in making decoctions that are used to get relief from painful menstruation in infertile women. The decoctions are either administered orally or are injected in womb (Amoo et al. 2014).

18.5.16 Antiosteoporosis Activity of the Aloe vera

Osteoporosis is a skeletal condition caused by low bone density and disorganized bone architecture (Sun et al. 2017). This is a result of excessive rate of bone resorption activity of the osteoclast compared to the rate of bone formation of the osteoblast (Jahanian et al. 2016). Excessive research has noted that antioxidants and levels of ROS are significantly correlated to osteoporosis (Jia et al. 2012; Mody et al. 2001; Zhao et al. 2015). In one study, the antioxidant and antiosteoporotic activities of 25 compounds isolated from *Aloe* exudates based on TRAP's contribution on bone resorption of osteoclasts by producing ROS (Sun et al. 2017). It was noted that four anthraquinones, one phenolic derivative, seven chromones, and six pyrones possess significant suppression activity against TRAP at 10.0 μm. Other compounds isolated showed weak to inactive suppression activity against TRAP.

Aloe vera, together with cancellous bovine xenograft (XCB), was noted to stimulate alveolar bone growth (Kresnoadi et al. 2017). The combination of *A. vera* and XCB prevented bone resorption activity of the osteoclast and stimulated the growth of osteoblasts. Moreover, similar results were noted in an earlier study that combination of *A. vera* and XCB enable growth of bone cells increasing osteoblast activity and inhibiting osteoclast activity in alveolar bone (Kresnoadi and Rahayu 2011). It was also suggested that the role of *A. vera* is to prevent inflammation of osteoclastogenesis, thus preventing bone resorption. Furthermore, it was also noted that the antraguinon present in *A. vera* possess an anti-inflammatory which reduces osteoclast activity.

Aloin, an anthraquinone glycoside from *A. vera*, was also noted to stimulate osteoblast induction by increasing alkaline phosphatase (ALP) activity and mineralization (Pengjam et al. 2016a). It is believed that the initial osteogenic activity derived from aloin is brought about by the structure-activity of methoxyl group in anthraquinone derivatives (Lee et al. 2008). Aloin was also noted to promote osteogenesis in bone marrow-derived mesenchymal stem cells as evidenced by the increased ALP activity, enhanced mineralization, and expression of osteogenesis-related genes (Li et al. 2019). Moreover, it was noted that this compound exerts is a potent inhibition of bone resorption activity and osteoclastogenesis as shown in in vitro test bone pit assay (Pengjam et al. 2016b). Regulation of osteoclastogenesis by aloin was also noted through its unfavorable effects towards NFκB repression of miR-21 (Madhyastha et al. 2019).

18.6 Plant Disease Management

Luiz et al. (2017) found that Aloe polysaccharides and essential oils are considered as potential agents in controlling plant diseases by working as anti-microbial agents or by activating plants' defence mechanism, for example combinations of aloe polysaccharides and palmarosa essential oil is effective against *Xanthomonas fragariae* (bacterial angular leaf spot disease). Antibacterial activity of *A. barbadensis* leaf extract was evaluated against *Serratia marcescens, E. coli*, *B. cereus* and *P. aeruginosa,* results showed that maximum inhibitory activities was through hexane and methanol extract against *S. marcescens* and *B. cereus*. Broadly it was noticed that methanol extract inhibit the growth of all tested pathogenic bacteria while no inhibitory activities was shown by ethyl acetate extract (Dharajiya et al. 2017). An anti-adhesive effect was shown by polysaccharides present in Aloe gel by inhibiting the growth of *H. pylori* bacteria (Cellini et al. 2014). Isolated compounds (aloin, aloe emodin and chrysophanol) of *A. ferox* were studied by Kambizi et al. (2005) and noticed that alonin A and aloe emodin shows inhibitory activities against *Shigella sonnei, B. subtilis, Staphylococcus epidermidis, E. coli, B. cereus* and *S. aureus*.

The potential role of *Aloe* species' with respect to bioactivity, availability and safety makes it an interesting alternative as control agent especially used in preharvest and postharvest fungal diseases of vegetables and fruits. Ali et al. (1999) reported that fresh leaves extracts of *A. arborescens* and *A. barbadensis* have antifungal potential against *Fusarium moniliforme, Aspergillus niger* and *Cladosporium herbarum*. Research studies have shown that up to 20% of *Alternaria, Penicillium and Botrytis* spores survival was reduced by *A. vera* and it also inhibits up to 38% of *Colleotrichum, Fusarium and Rhizoctonia* mycelium growth (De Rodrýguez et al. 2005; Castillo et al. 2010).

Ortega-Toro (2017) analyzed anti-fungal potential of *A. vera* gel against six fungi causing plant diseases- *Botryotinia fuckeliana, Fusarium oxysporum, Colletotrichum gloeosporioides, Curvularia hawaiiensis, Alternaria alternate* and *Bipolaris spicifera*. Results showed that was most effective against *F. oxysporum*. RP-HPLC fractionated methanolic extracts of inner leaf gel of *A. barbadensis* were tested against nystatin resistant strain of the fungus *Aspergillus niger* and it was found that *A. barbadensis* was capable of inhibiting the growth of this resistant strain (Cock 2007). Research studies shows that fungal contamination were effectively controlled with highest *A. vera* ratio, treatment of *A. vera* gel with nectarine alone or with thymol addition inhibits fungal growth of *Penicillium digitatum, Rhizopus stolonifer* and *Botrytis cinerea*. Hence, application of Aloe significantly lowers two to threefold fungal infection than in non-treated nectarines. It was noticed that efficiency of Aloe gel to reduce infection was not improved by addition of thymol (Navarro et al. 2011).

Eight Aloe species gels (*A. vera* L., *A. arborescens* Mill., *A. claviflora* Strydenburg., *A. mitriformis* Mill., *A. aristata* Haw., *A. ferox* Mill., *A. striata* Haw. and *A. saponaria* A.) were evaluated for anti-fungal activity against *P. italicum*, *P. italicum*, *P. expansum* and *P. digitatum*. Results concluded that higher antifungal activity was observed for *A. saponaria*, *A. ferox* and *A. mitriformis* than *A. vera* which can be correlated with aloin content (Zapata et al. 2013).

18.7 Toxicological Profiling

It was observed that *aloe vera* gel or extract usually does not possess any toxicity although different cases of people are found having sensitivity towards them and suffered from liver problems. On using externally, many individuals have also experienced many skin problems like itching and swelling etc.

The adverse effects of aloe leaf and latex are:

- Oral ingestion of aloe spp. could be potentially toxic, and may cause abdominal cramps and diarrhea .
- Ingestion of non-decolorized liquid aloe vera is a possible carcinogen, Under the guidelines of California Proposition 65, orally ingested non-decolorized aloe vera leaf extract has been listed by the OEHHA, along with goldenseal, among

"chemicals known to the state to cause cancer or reproductive toxicity" (OEHHA 2015).
- They act as abortifacients, hence should not be taken by pregnant women. The compounds also pass to baby with the milk.
- In case of hemorrhoids aloe vera should be avoided.

Number of investigation has been carried out to check the toxicological properties of Aloe gel and leaf extracts on the basis of toxicity causing ability in humans and animals.

Side effects have been reported with the use of aloe in humans which present themselves as severe form of watery diarrhea associated with colicky abdominal pain and spasms may occur. Mild side effects occur with even a small dose of aloe but the overuse can lead to severe symptoms.

It has been reported that the overuse of anthraquinones can cause hepatitis as well as metabolic acidosis, albuminuria, malabsorption, weight loss and hematuria. Dehydration and hypotension associated with overuse of the plant follows the watery diarrhea due to the plant's laxative effects.

Hypoglycemia may occur as a result of overuse of the plant for a longer duration as the plant increase the insulin level in the body which although beneficial for diabetes can lead to serious side effects in healthy individuals by depleting of glucose levels inside the body and may cause symptoms of weakness and lethargy and excessive sleep and drowsiness.

Hypokalemia that may result due to electrolytes imbalances attributed to excessive diarrhea can lead to serious complications ranging from hypoalbuminemia and hematuria to neuromuscular and cardiac dysfunction which can ultimately lead to mortality especially along with the use of cardiac glycosides.

Ininflammatory conditions of the GIT aloe usage should not be encouraged. Aloe should also be avoided during pregnancy and in GIT symptoms such as nausea and vomiting that have not yet been diagnosed.

Experimentally, Aloe and their preparations have been reported to cause allergic conditions and hypersensitivity (Ernst 2000). Emodin exposure to rats have shown an increased incidence of renal tubule pigmentation and introduced nephropathy in mice (National Toxicology Program 2001).

On applying various forms of aloe extracts, various plant derived components and commercially available gels, by intraperitoneal or intravenous injections to mice, rats and dogs for single or eight repeated interval of 4 days the dogs has observed emesis and diarrhoea (Fogleman et al. 1992). Repeated administration of the material had led to increase in accumulation of macrophages and monocytes in the lungs of intravenously-treated animals and in the liver and spleen of intraperitoneally-treated. Intoxication approved with a clinical sign of a decrease in activity, abnormal gait and stance, piloerection, flaccid body tone, and tremors in mice. For dogs it included emesis, abdominal discomfort, decreased activity, and diarrhoea. The high (80 mg/kg per dose) and middle (40 mg/kg per dose) doses of intravenously treated mice and doses of 100 mg/kg to 200 mg/kg of intraperitoneally treated mice have resulted into early death problem.

Reduction in food efficiency and body weight as well as increase in kidney weight and testis weights was observed in a subchronic toxicity study of 88 Sprague Dawley rats when they were fed with Aloe whole leaf powder at doses of 2, 4, and 8 g/kg body weight (2.5%, 5%, and 10% Aloe in diet) for 90 days. Additionally, the introduction of pigmentation in renal tubular, increase in mesenteric lymph nodes, and lamina propria of the colonic mucosa were also observed compared to the controls (Zhou et al. 2003).

Examination was done on the growth, metabolic reactions and dietary intake of rats after ingestion of crude and decolorized Aloe gel for 1.5 and 5.5 month studies (Herlihy et al. 1998a, b) Marked changes in serum parathyroid hormone and calcitonin concentrations were observed, concluding that Aloe gel may alter calcium metabolism (Herlihy et al. 1998b).

18.7.1 Reproductive Toxicity

It was observed after a chronic oral ingestion of 100 mg/kg *Aloe vera* extract per day, for a period of 3 months have led to various reproductive losses (Shah et al. 1989). This was resulted into significant sperm losses, hematological damages, inflammation, and increased mortality in the study animals.

Similarly in a study by Nath et al. (1992), pregnant rats were given a dose with aqueous or 90% ethanol extract preparations of the plant orally for 10 days. The abortifacient activity of the plant was found to be at a high percentage in the studied animal in comparison to the controls.

It was advised not to take Aloe latex for pregnant women because of its cathartic effect, which may be a cause of severe uterine contractions and in turn increase the risk of miscarriage. Nursing mothers should also not ingest it because of the probability of inducing severe cramps and diarrhoea in the infant (Brinker 1998).

18.7.2 Genotoxicity

In a study genotoxicity of Aloe spp. whole extract-and decolorized extract were evaluated using the mouse lymphoma assay (MLA) (Guo et al. 2014). The results indicate deletions and/or mitotic recombination type of chromosomal mutations from both the treatments.

The genotoxicity of three components present in *A. vera* latex ie. emodin, danthron, and aloe-emodin was checked using the MLA, micronucleus test, and the Comet assay (Muller et al. 1996) all three compounds have shown increases in micronuclei and moderate increases in mutant frequency in L5178Ycells, at micromolar concentrations. Emodin also found to cause DNA damage in human lung carcinoma cells through the production of ROS, (Lee et al. 2006) induction of micronuclei in TK6 human lymphoblastoid cells, (Nesslany et al. 2009) and increase aberration of chromosomes in Chinese hamster ovary cells (Lee et al. 2006). Danthron also found potent carcinogen and induced DNA damage and apoptosis in SNU-1 human gastric cancer cells with the help of mitochondrial permeability transition pores and Bax-triaggered pathways (Chiang et al. 2011).

18.7.3 Carcinogenicity

F344/N rats were administered *Aloe vera* whole leaf extract in drinking water orally for 2 years and observed clear evidence of carcinogenic activity (NTP 2015). NTP Report 577 reported that the 13-week exposure resulted into. The 2-year study described significant related increases in the incidences of adenomas or carcinomas of the ileocecal and cecal-colic junction, cecum, and the ascending and transverse colon in male and female rats in the high-dose groups.

Hydroxyanthraquinones (HA) like danthron, aloe-emodin, and emodin were investigated for tumor promotion activities, like induction of cell proliferation and initiation of malignant transformation, in mice (Wolfle et al. 1990). A two or three-fold increase in DNA synthesis was observed in primary rat hepatocytes on exposure to danthron and aloe-emodin. Danthron also found to enhance malignant transformation of C3H/M2 mouse fibroblasts concluding that HA present in Aloe latex with hydroxy groups in the 1,8 positions may exhibit tumor-promoting activity.

18.7.4 Adverse Clinical Effects on Human

Dioscorides, a Greek physician of the first century A.D have first medically recorded the therapeutic use of Aloe (Fantus 1922). Afterwards, Aloe latex was widely utilized in herbal laxative preparations in many countries. Hence ingestion of latex in high doses and prolonged time resulted into a number of adverse effects and has been reported in clinical studies. Prolonged use manifested with electrolyte imbalance due to diarrhoea, vomiting, abdominal pain, hypokalemia and the development of a cathartic colon (the colon becomes atonic and dilated) with the risk of developing colon cancer (Van Gorkom et al. 1999).

There are various single clinical cases for different types of adverse effect has been reported however there are no published controlled toxicology studies in vivo reports are available. A female patient 1-week history of progressive jaundice, pruritus, alcoholic bowel movements, and abdominal discomfort, have resulted into severe acute hepatitis with portal and acinar infiltrates of lymphocytes, plasma cells, granulocytes along with bridging necrosis and bilirubinostasis when she began ingesting tablets of an unspecified extract of Aloe barbadensis Miller (500 mg/tablet) 4 weeks prior to inspection (Rabe et al. 2005).

A. vera also found to potentially interact with the drugs prescribed to the patients for medication. It was also found that the compound present in *A. vera* can cause a reduction in prostaglandin synthesis, which may hinder secondary aggregation of platelets. In a study Vazquez et al. (1996) showed that Aloe gel caused a 48% reduction in prostaglandin synthesis compared with a 63% reduction by indomethacin. It is evident by a case of a female patient in which she lost 5 litres of blood during surgery due to a possible herb-drug interaction between orally ingested *A. vera* tablets and sevoflurane, an inhibitor of thromboxane A_2 (Rabe et al. 2005). Interactions of Aloe gel have also been observed for hydrocortisone, antidiabetic agents, and UV radiation.

A case of acute oliguric renal failure and liver dysfunction occurred when a 47-year-old man ingested Cape Aloes (Luyckx et al. 2002). Hepatotoxicity is considered one of the most reported adverse effects caused by herbal dietary supplements (Guo et al. 2010). The first case of acute hepatitis due to the ingestion of *A. vera* compound was reported in 2005 in Germany (Rabe et al. 2005). Afterward, cases of Aloe-induced toxic hepatitis were reported in Turkey, (Kanat et al. 2006) United States, (Bottenberg et al. 2007) Argentina, (Curciarello et al. 2008), and Korea (Yang et al. 2010). A total of six females and two males were admitted to hospital for acute hepatitis after taking Aloe preparation over 3–260 weeks (Lee et al. 2014). Their clinical manifestation, liver biopsy, and laboratory findings supported the diagnosis of toxic hepatitis. All eight patients showed improved condition after discontinuing this medication. These cases emphasize the importance of considering phytopharmaceutical over-the-counter drugs as causative agents in hepatotoxicity. Adverse effects of Aloe whole-leaf powder have been reported at concentrations of 2 g/kg BW, and the LOAEL for aloin is estimated at 11.8 g/kg BW (Zhou et al. 2003).

18.8 Conclusion

Aloe is a genus with a number of beneficial and medically important species that have been used traditionally in several parts of the world especially Africa and India. Aloe species are one of the best studied medicinal plants with therapeutic properties due to presence of wide range of novel bioactive compounds with synergistic actions. The presence of the several pharmacologically important chemicals in the plants supports the clinical importance of the plants' usage within the specific doses that have been confirmed by the scientific data and are approved to be within the safety limits. This review article focuses on the present research advancement on Aloe species especially highlighting its mechanism of action in combating multiple diseases. The plants especially *Aloe vera* has been identified to exhibit a number of useful properties such as anti-inflammatory, anti-cancerous, anti-microbial, wound healing, immunomodulation, antiseptic and cosmetics use attributing to the presence of several importance constituents. Although, there are issues related to safety concerns much needs to be done for evaluateing the toxicological evaluation of Aloe species. Thus, implementation of some biotechnological techniques is required to cover a wide spectrum of scientific applications and to better understand the possible benefits offered by the significant Aloe plant.

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Chapter 19
Polyphenols: Classifications, Biosynthesis and Bioactivities

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Abbreviations

COX-2	Cyclooxygenase-2
DPPH	1,1-Diphenyl-2-picrylhydrazyl
GST	Glutathione S-transferases
IL-6	Interleukin-6
LDL-c	Low Density Lipoprotein-cholesterol
LOX	Lipoxygenase
NF-kB	Nuclear Factor kappa-light-chain-enhancer
QR	Quinone reductases
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
THF	Tumor necrosis
XO	Xanthine oxidase

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19.1 Introduction

Polyphenols are polyhydroxyphenols, which are structural class of compounds that are mostly composed of natural compounds, and sometimes synthesized or semi synthetic in nature (Cattani et al. 2012). These compounds have the characteristics of multiple phenolic structural units in them, hence, the name polyphenols. Polyphenols are mostly of plant origin and they are among the most studies class of phytochemicals. These natural phytochemicals are readily available in the entire plant kingdom and therefore find their way into human diet as they are found in whole grains, legumes, cereals, coffee, wine and tea. They are also found in fruits like grapes, pear, broccoli, apricot, orange, lemon, cherry and blueberry. More than 8000 of these polyphenolic compounds, including stilbenes, phenolic acids, lignans and flavonoids have been identified in whole plant foods (Pandey and Rizvi 2009). These phytochemicals are secondary metabolites found in plants, where they act as antioxidants, anti-inflammatory, antimicrobial, protect the immune system, defend against pathogens attack and ultraviolet radiations (Beckman 2000; Park et al. 2004; Widyarini 2006; Andarwulan et al. 2010; Chen et al. 2016; Hisanaga et al. 2016). Catechins, curcumin, cyanidin, ellagitannins, kampferol, myricetin, quercetin, resveratrol and rutin (Fig. 19.1) are among polyphenols with important bioactivities.

Catechins

Catechins ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) are group of procyanidins, which can be found in the leaves of *Camellia sinensis* (Türközü and Tek 2017). Studies have shown through to both epidemiological and experimental studies, that catechins are potent compounds against cancer, cardiovascular diseases, and aging (Arts et al. 2001). Catechins similarly protects the body against degenerative disease, with strong inverse relationship between the consumption of catechins and mortality through coronary heart disease (Arts et al. 2001).

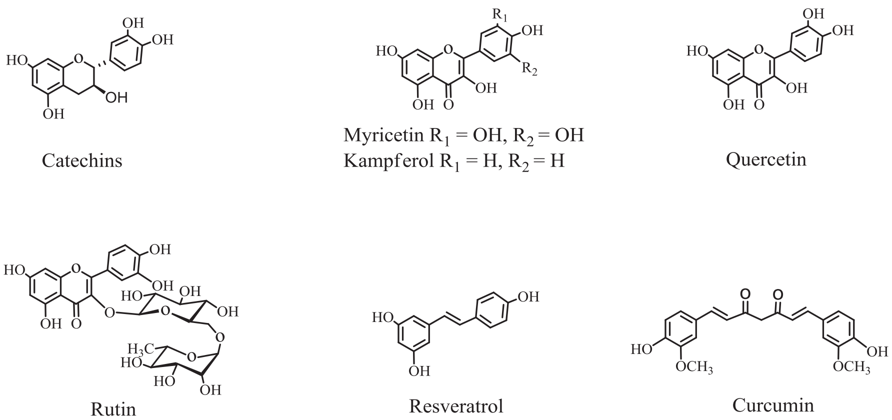

Fig. 19.1 Chemical structures of some polyphenols

They are potent antibacterial compounds when tested on Gram positive than Gram-negative bacteria (Park et al. 2004; Friedman 2007).

Quercetin

Quercetins are flavonoids with reported antioxidant and anti-inflammatory activities (Chen et al. 2016; Hisanaga et al. 2016). They are also important compounds in the prevention of cardiovascular diseases (Guillermo-Gormaz et al. 2015). Other activities of quercetin include their anti-influenza A virus activities, antiulcer, antiallergy, and anti-proliferative effects (Al-Jabban et al. 2015). The free radical scavenging activities of quercetin (Andarwulan et al. 2010) has been linked with the ortho-dihydroxy substitution in their B-ring and their enol moiety in the A-ring (Murota and Terao 2003). These structural features allows quercetin to form additional H-bond with 4-keto group (Murota and Terao 2003). Other bioactivities of quercetins include their anticancer and antidiabetic activities (Hashemzaei et al. 2017; Srinivasan et al. 2018).

Resveratrol

Resveratrol (5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) is a dietary polyphenol, belonging to the stilbenes group and naturally occurring in fruits and food products (Bhat et al. 2001; Harikumar and Aggarwal 2008) and active during response to injury, fungal attack, or other environmental stress (Baur and Sinclair 2006; Aguirre et al. 2014). Resveratrols are potent antioxidant compounds (Olas et al. 2001) and important in strengthening of the muscle cells (Dolinsky et al. 2012). They are used in the treatment of inflammation, regulation of body metabolism (Palsamy and Subramanian 2009), and the treatment of neurodegenerative diseases, diabetes (Shin et al. 2010), cardiovascular diseases, and cancer (Sun et al. 2008).

Rutin

Rutein is a glycoside of quercetin (3-O-rhamnoglucoside), the most abundant flavonol in vegetable and fruit and widely studies polyphenol (Sharma et al. 2013; Hosseinzadeh and Nassiri-Asl 2014). Rutein is similarly known as quercetin-3-O-rutinoside (Erlund et al. 2000). Rutein is important as an anticancer, anti-hypertension, and anti- hypercholesterolemic compound (Erlund et al. 2000). Studies has indicated that rutein increase the concentrations of High Density Lipoproteins (HDL) and decreases the levels of Low Density Lipoproteins (LDL-c) in diabetic patients (Sattanathan et al. 2010).

Curcumin

Curcumin is a 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, also known as diferuloylmethane. Curcumin is a natural polyphenol strongly found in rhizomes of the plant *Curcuma longa* and are also available in others species of Curcuma (Aggarwal et al. 2003). *Curcuma longa* is a medicinal herb which has wide traditional uses due to its anticancer, anti-inflammatory, antioxidant, antimicrobial and antimutagenic properties (Mahady et al. 2002; Reddy et al. 2005; Vera-Ramirez et al. 2013; Wright et al. 2013; Lestari and Indrayanto 2014). Curcumin is relatively safe in its medicinal usage (Wilken et al. 2011).

Kampferol

Kaempferol is a 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one flavonoid that is present in a wide variety of edible plants including cabbage, beans, tomato, grapes and strawberries (Calderón-Montaño et al. 2011). They are similarly found in *Moringa oleifera, Ginkgo biloba* and *Sophora japonica*. Kaempferol and their glycosides exhibit wide biological activities including analgesic, antiallergic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, anti-osteoporotic, anti-oxidant, cardioprotective, and neuroprotective activities (Calderón-Montaño et al. 2011). The chemical structure of kaempferol, which include the double bound at C2-C3 link with an oxo group at C4 and the presence of an hydroxyl groups at C3, C5 and C4' are reported to be important in its antioxidant activities (van Acker et al. 1996; Rice-Evans 2001).

Myricetin

Myricetin is a flavonoid that is structurally related to quercetin and kaempferol (Semwal et al. 2016). Just like kaempferol, myricetin may occur in its free and glycosidically-bound forms. Its found in beverages including teas, vegetables and fruits. Myricetin is a strong iron-chelating compound (Ong and Khoo 1997) with anticancer, antidiabetic, anti-inflammatory and anti-oxidant properties (Ong and Khoo 1997; Semwal et al. 2016).

19.2 Classifications of Polyphenols

Plant metabolites are classified into primary or secondary metabolites depending on the functions they play in the plant system. While primary metabolites play important roles in plants growth and their survival (Tijjani et al. 2018), secondary metabolites are synthesized as defense system against disease and herbivores (Mazid et al. 2011). These secondary metabolites are bioactive compounds, which are very useful as functional foods (Carović-StanKo et al. 2016). Polyphenols are among the largest group of secondary metabolites found in plants. Over 8000 structurally variants of polyphenols exist which are characterized by aromatic rings with one or more hydroxyl groups (Han et al. 2007). They are majorly classified into groups and subgroups based on the numbers of phenolic rings and structural elements attached to the rings (Butterfield et al. 2002). The major classes are phenolics, flavonoids, stilbenes and lignans (Fig. 19.2) (Pietta et al. 2003). Phenolic acids are subclassified into hydroxybenzoic acids including gallic acid or hydroxycinnamic acid and ferulic, coumaric, caffeic acid. The flavonoid subclass include anthocyanidins, flavanols, flavanones, flavones, flavonols and isoflavones.

Polyphenols are similarly classified into their various sources of origin, major biological functions and based on their chemical structures (Tsao 2010). Majority of these polyphenols that exist in plants are glycosylated with different carbohydrate units and acylated sugars at various positions on their basic polyphenol skeletons (Tsao 2010).

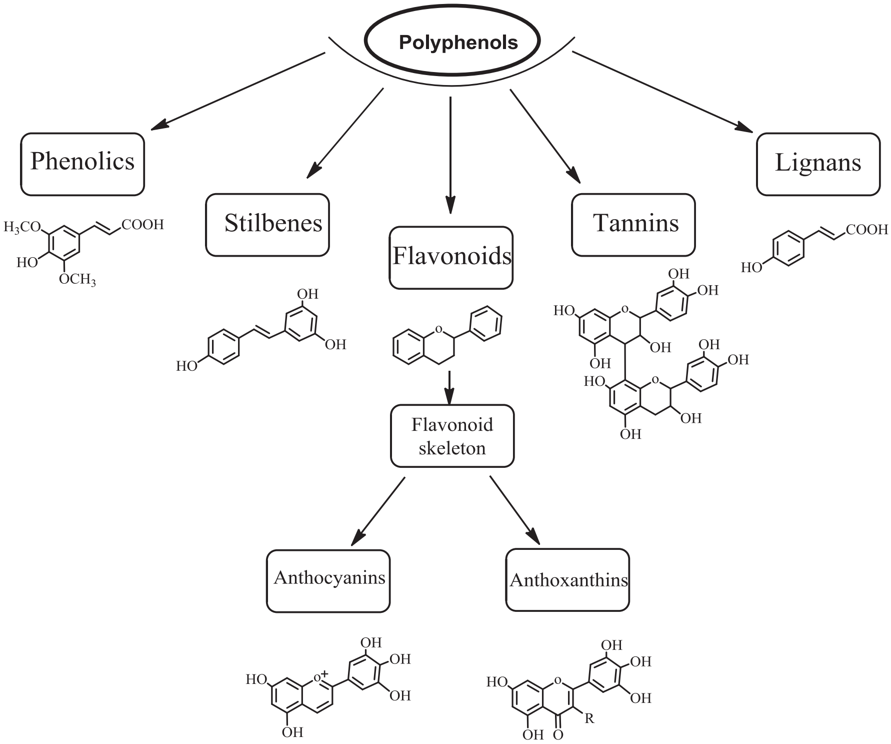

Fig. 19.2 Classifications of major polyphenols

19.3 Distributions of Polyphenols

Polyphenols are widely distributed in nature. The main dietary sources of polyphenols are from vegetables, fruits and beverages. Flavones and flavonols are polyphenols ubiquitous in plants and thus the most common within the flavonoid group of polyphenols (Bravo 1998). Catechins and epicatechin are monomeric flavan-3-ols and gallocatechin, Catechin derivative are majorly found in tea leaves and cocoa beans. They are the most abundant flavonoids in diet, which also include their oxidized products. They are common in dry legumes, cereals, wine, fruit juice, coffee, tea and chocolate.

Condense tannins are found as constituents of woody plants. Anthocyanidins are components of purple, blue and red pigments, which are distributed in some fruits, flower petals and vegetables. These anthocyanidins may also be present in plants as anthocyanins when glycosylated (Bravo 1998). Anthocyanins are water-soluble and are important pigments in plants where they impact colour ranging from vivid red to blue color (Khoo et al. 2017). Table 19.1 shows specific plant sources of polyphenol and the various classes of polyphenol found in them.

Table 19.1 Plant sources of polyphenols

S/No	Plant source	Class of polyphenol	Polyphenol	References
1.	*Oroxylum indicum* seeds	Flavones	Luteolin	Chen et al. (2003)
	Lycopersicon esculentum L.		Apigenin	Barros et al. (2012)
	Cichorium endivia L.			
	Medicago sativa L. leaves			El-Shafey and AbdElgawad (2012)
				Mishima et al. (2015)
2.	Citrus fruit	Flavanones	Hesperetin Naringenin	Mullen et al. (2008)
				Tomás-Navarro et al. (2014)
3.	*Ginkgo biloba* leave	Flavonols	Quercetin Kaempferol	Chan et al. (2007) and Calderón-Montaño et al. (2011)
	Moringa oleífera		Myricetin	
	Sophora japônica			
	Allium cepa			Rodriguez et al. (2008)
4.	*Rhus verniciflua* stoke	Flavanonols	Taxifolin Fustin	Kim et al. (2010)
	Larix gmelinii			Liu et al. (2014)
5.	Green tea, black tea, cocoa	Flavan-3-ols	Catechin	Subhashini et al. (2010)
			Epicatechin	Gadkari and Balaraman (2015)
			Epigallocatechin	
			Proanthocyanidins	Bravo (1998)
6.	Soybean *Vitis vinifera*	Isoflavones	Genistein	Wang and Murphy (1994)
			Genistin	Mazur et al. (1998)
			Daidzein	Kuligowski et al. (2017)
				Lotito and Frei (2006)
7.	Maclura pomifera (Raf.) Schneid.	Chalcones	Isoliquiritigenin	Tsao et al. (2003)
8.	Fruits	Anthocyanidins	Cyanidin Pelargonidin	Mazza (1995)
	Vegetables		Peonidin	Bravo (1998)
			Delphinidin	
			Petunidin	
			Malvidin	
9.	Peanuts	Stilbenoids	Resveratrol	Sanders et al. (2000)
	Cajanus cajan		Piceid	Burns et al. (2002)
			Cajanotone	Counet et al. (2006)
			Cajanamide	Zhang et al. (2012)
10.	*Curcuma longa*	Phenolic acids	p-Hydroxybenzoic acid	Manach et al. (2004)
	Punica granatum L			
	Fruits			
	Vegetables		Gallic acid	Seeram et al. (2006)
			Ellagic acid	
			Curcumin	

Some derivatives of hydroxybenzoic acid includes protocatechuic acid, gallic acid and p-hydroxybenzoic acid. Hydroxycinnamic acids, is however more common than hydroxybenzoic acid has some of its derivatives as caffeic acid, chlorogenic acid, coumaric acid, ferulic acid and sinapic acid. These derivatives are rarely found in free forms but they occur in fermented, frozen or sterilized foods. Berry fruits, apple, chicory, cherry, kiwi, coffee and pear are the foods with high levels of these phenolic acids (Manach et al. 2004).

Stilbenes, one of the sub-classes of flavonoids occur in smaller amount in human diet. Resveratrol (Fig. 19.1) which is one of the well-studied compounds of this group is largely found in red wine and grapes (Manach et al. 2004; Adlercreutz 2007; Pandey and Rizvi 2009). Chun et al. (2007) and Ovaskainen et al. (2008) reported dietary intake of polyphenols is estimated to be approximately 1 g/day. The availability of the important dietary components depend on their food source preparatory methods, their digestions in the gastrointestinal walls, their absorption rates as well as their metabolism in the body systems (Scalbert and Williamson 2000). In the absorption pathway of polyphenols, there must be colonic microflora or intestinal enzyme action in way of hydrolysis, which should be followed by conjugation in the intestinal cells and later glucuronidation, methylation or sulfation by the liver (Scalbert et al. 2002). Polyphenols, thereafter, accumulate in their target tissues to induce biological properties and the excretion of these phenol derivatives majorly occur in through urine and the bile (Scalbert et al. 2002).

19.4 Biosynthesis of Polyphenols

The shikimate pathway is critical to the synthesis of amino acids, which are further acted upon by specific enzymes for the biosynthesis of phenolics, lignins and flavonoids (Tijjani et al. 2018). Other polyphenols obtained from the shikimate pathway include phenolic compounds such as cinnamic acid and gallic acid (Tsao 2010). The aromatic ring B of polyphenols and the chromane ring are derived from phenylalanine obtained from the shikimate pathway while ring A are obtained from the condensation of three units of malonylCoA (Fatland et al. 2004; Tsao and McCallum 2009). Phenylalanine is deaminated by the enzyme phenylalanine ammonia lyase to trans cinnamic acid (Fig. 19.3). Cinnamate-4-hydroxylase catalyze the conversion of trans cinnamic acid to form *p*-coumaric acid. CoASH is added by the enzyme 4-coumaroyl CoA ligase to form *p*- coumaroyl CoA (Bohm 1998; Tsao and McCallum 2009). The *p*-coumaroyl-CoA formed then condenses with three molecules of malonyl-CoA to form chalcone catalyzed by the enzyme chalcone synthase. The enzyme chalcone flavanone isomerase isomerize chalcone into a flavanone which could be further converted to dihydroflavonols or to flavan-3, 4-diols (Bohm 1998; Tsao and McCallum 2009). The glycosylation of flavonoids by the enzyme glucosyltransferase provide addition of a sugar unit to the flavonoid (Bohm 1998; Tsao and McCallum 2009).

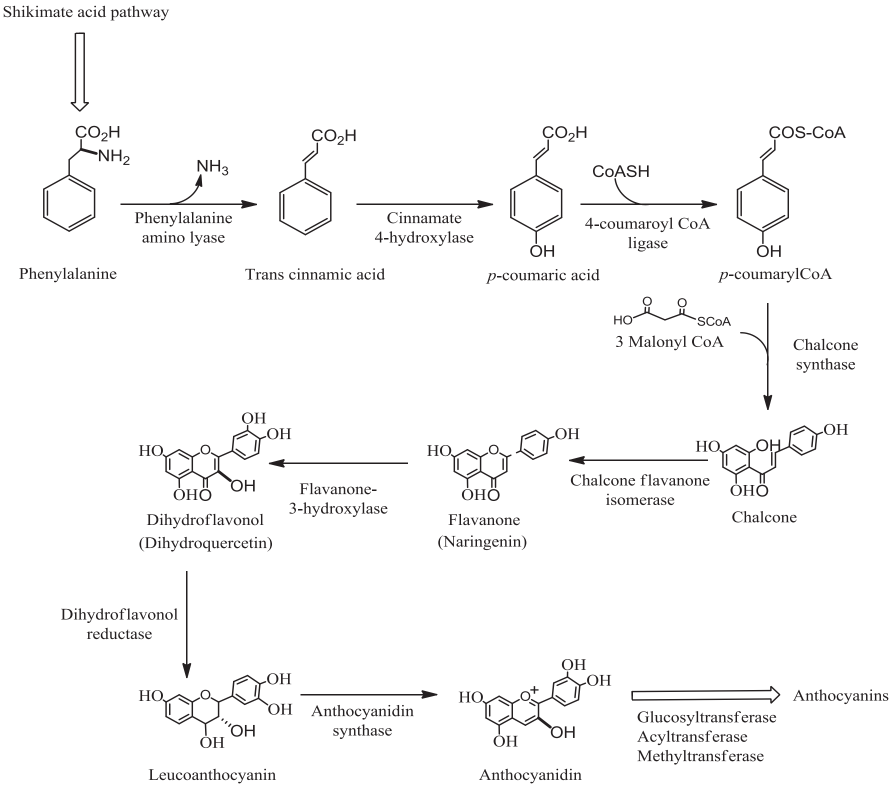

Fig. 19.3 Biosynthesis of polyphenols. (Bohm 1998; Fatland et al. 2004; Tsao and McCallum 2009)

19.5 Medicinal Properties of Polyphenol

Foods obtained from plants provide essential nutrients that are needed by human for growth and development and play important role in human health (Akbari et al. 2012; Bøhn et al. 2014; Rasouli et al. 2017). Over the last 16 years, a lot of studies have been carried out and reported on the importance of secondary metabolites on their medicinal importance and their ability to improve human health (Valdés et al. 2015). These phytochemicals were reported to have been synthesized by various plants, some for their protection against herbivores and predators (Tijjani et al. 2018). They have however played beneficial roles in the area of pharmaceutical and medicinal purposes Yue et al. (2016). Kossel (1891) was the first scientist who showed the significance comparism between the primary metabolites and secondary metabolites. Among these secondary metabolites, plant polyphenols are very crucial members and they've been identified to play significant role in human medicine as secondary metabolites. Recently, polyphenols have served as important prophylactic and therapeutic agents against diseases and have shown to have antioxidant properties, which showed their importance in the field of medicine. Many scientific studies have revealed that people who focused on specific diet, especially polyphenolic rich diets are at low risk of certain chronic diseases such as heart disease, diabetes, obesity, and cancer. Studies on plant polyphenols has been carried out with the intention of discovering protective compounds against certain diseases including their antioxidant, anti-inflammatory and anti-microbial properties. Their blood pressure reducing effects, immune response, skin protection and nutraceuticals have also been reported.

19.5.1 Antioxidant Properties of Polyphenols

The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are deleterious to the body system. If the ROS and the RNS produced from cellular reactions are at balanced levels, they serve as beneficial compounds for cellular functions. However, if they are produced at unbalanced levels, which sometimes occur, they lead to oxidative stress, which can cause degenerative disorders, especially to the vital organs in the organism (Valko et al. 2006; Pham-Huy et al. 2008). Many scientist have advocated for the use of antioxidant compounds, which are found in plant based food substances, some of which are polyphenolic compounds. The use of these naturally occurring antioxidant compounds have significantly increased and they've been employed in medical usage as well as in pharmaceutical companies, which serve as substitute for the artificial ones which have been suspected to be one of the major causes of cancer (Carocho et al. 2014). Heim et al. (2002) stated that the arrangement of functional groups, their configuration, substitution and the number of hydroxyl groups also influence the antioxidant activity of flavonoids such as the radical scavenging activity and or metal ion chelation ability. Flavonoids and phenolic compounds have been described by many scientists as phytochemicals with the highest antioxidant properties from plants (Ryu et al. 2006; Okpuzor et al. 2009; Mishra et al. 2013; Wang et al. 2016; Andreu et al. 2018; Zahoor et al. 2018).

Oki et al. (2002) examined the antioxidant capacity of anthocyanins and other phenolics from different cultivars of purple-fleshed sweet potato (*Ipomea batatas* L.) which is an edible and economic medicinal specie in Japan. Their assessment of the antioxidant activity using diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity shows that a positive antioxidant activity exist the cultivars used for their study. Similarly, they observed that chlorogenic acid showed a dominant DPPH radical scavenging activity in Miyanou-36 cultivar and Bise cultivar in the sweet potato. The other two cultivars; Ayamurasaki and kyushy-132 also showed antioxidant activities but anthocyanins were rather observed to show the DPPH radical scavenging activity in them (Oki et al. 2002).

Mishra et al. (2013) observed that leaf extracts of *Bauhinia variegate* contained flavonoid compounds which showed a great antioxidant ability against oxidative stress through ion binding ability, reducing power ability as well as radical neutralization (Mishra et al. 2013). Andreu et al. (2018) also observed that the cultivars with a significant amount of total phenolic compounds in the peel and pulp of the fruits of six cultivars of *Opuntia ficus-indica* (L.) and young and adult plants of cladodes expressed a significant effect against oxidative stress (Andreu et al. 2018).

Zahoor et al. (2018) also reported that the antioxidant property of 2-hydroxy-2-phenyle acetic acid (mandelic acid) and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-Chromen-4-one (quercetin), which are the major bioactive molecules in *Aesculus indica* (Wall. Ex Cambess) showed a significant effect in the reduction of oxidative stress which are caused by reactive oxygen species (Zahoor et al. 2018). *Polygonatum verticillatum* L. extract exhibit great antioxidant activity, which has been linked with their phenolic compounds present in the plant (Kumar Singh and Patra 2018).

Meng et al. (2018) evaluated the phytochemical profiling as well as the biological activity of the extract of *Camellia fangchengensis* tealeaves. The result obtained showed that flava-3-ol oligomers and monomers are potent antioxidant compound found in the plant and maybe responsible for the antioxidant activities express by the plant (Meng et al. 2018).

19.5.2 Blood Pressure Reduction Properties of Polyphenols

Polyphenols are present in foods such as soybean (Kim et al. 2008). Black soybeans which contain different colours as a result of the presence of chlorophyll, anthocyanin other pigments. The black colour is due to the presence of anthocyanins in the epidemis palisade layer of the seed coat (Kim et al. 2008). The soybeans are widely consumed in Asia due to its medicinal purposes. Cyanidin-3-glucoside, pelargonidin-3-glucoside and delphinidin-3-glucoside have all been identified in black soybeans seed (Choung et al. 2001). Black soybeans contain some phytochemicals such as isoflavones, phytic acid, saponin and phenolic that are all very effective in the prevention of many chronic diseases and provide a lot of health benefits (Zhang et al. 2011). Black soybeans have been reported to have the greatest antioxidants properties compared to other coloured soybeans and these antioxidant is due to the presence of phenolics, distributed mainly in the seed coats (Kim et al. 2006; Slavin et al. 2009; Zhang et al. 2011). The rich content of soybeans has been found to reduce the effects of cancer and other chronic diseases and metabolic disorders (Kusunoki et al. 2015; Zou 2016; Tan et al. 2016; Matsukawa et al. 2017). Black soybeans contains low concentration of sodium, which enables it to maintain blood pressure at a normal range. The presence of the anthocyanins in it has also been reported as a factor for its ability to reduce the risk of cardiovascular diseases in individuals (Zou 2016; Hooper et al. 2008). The fiber in Black soybeans helps to lower total cholesterol, Low Density Lipoprotein-cholesterol (LDL-c) in the blood and liver which are inversely related to heart disease and also inhibits oxidative stress by increasing antioxidant activity and improving lipid profiles in postmenopausal women (Byun et al. 2010).

19.5.3 Anti-inflammatory and Immune Effects of Polyphenols

Phenolic compounds have antioxidant roles to play which could be relevant in the prevention of carcinogenesis by altering the oxidative stress condition, which reduces inflammatory responses associated with carcinogenesis (D'Alessandro et al. 2003). Reactive oxygen species can damage proteins, DNA and RNA, as well as oxidize fatty acids in cell membranes thus increasing the risk of mutations. It is however believed that majority of the damages caused by ROS can be restored by the body's internal surveillance and its repair system (D'Alessandro et al. 2003). These ROS are also considered to be important in the activation of NF-kB and other transcription factors as they serve as endogenous mitogenic factors in a variety of normal processes (Kovacic and Jacintho 2001). One of the chemoprevention mechanisms of phenolic compounds is associated with their scavenging properties of deleterious reactive species (including hydroxyl radical, superoxide anion, singlet oxygen, peroxynitrite and nitric oxide) (D'Alessandro et al. 2003). Alternatively, polyphenols can inhibit ROS generating transcription factors closely linked to inflammation (e.g., NF-kB24) and enzymes such as xanthine oxidase (XO) and cyclooxygenase-2 (COX-2) as demonstrated by curcumin (Lin and Shih 1994; Zhang et al. 1999) or lipoxygenase (LOX) by polyphenols including curcumin, silymarin, and resveratrol (Hong et al. 2001) which mediate inflammatory processes (Le Corre et al. 2005). Polyphenols have also been reported to behave as detoxifying enzyme inducers, where they modulate gene expression which also include the induction of phase II enzymes, such as quinone reductases (QR) and glutathione S-transferases (GST) (Fiander and Schneider 2000). This action usually leads to protection of cells or tissues where they occur against exogenous and/or endogenous carcinogenic intermediates (Fiander and Schneider 2000). A number of works have been conducted on the effect of flavonoid and other phenolics on their immune boosting effects and anti-inflammatory functions (Middleton 1998; Locatelli et al. 2018). Kumar and Pandey (2013) reported the anti-inflammatory potentials of some flavonoid compounds such as apigenin, hesperidin, luteolin and quercetin. The anti-inflammatory properties for different cultivars of the extract of *Lonicera caerulea* L. was investigated, by focusing on the pro-inflammatory cytokines, using *in vitro* human monocytic cell line THP-1 derived macrophages stimulated by lipopolysaccharide (Vasantha et al. 2015). Their result revealed that Borealis cultivar of haskap berry exhibited anti-inflammatory effect, which is comparable to diclofenac drug. This anti-inflammatory effect was due to the presence of high concentration of phenolic compounds like flavonoid and anthocyanin (Vasantha et al. 2015). The polyphenolic compounds of *Gaillardia grandiflora* Hort. and *Gaillardia pulchella* Foug were similarly observed to possess anti-inflammatory effect with no toxicological effect in mice model. The newly reported compound, 8-hydroxyapigenin 6-*O*-β-D-apiofuranosyl-(1″′→6″)-*C*-β-D-$^4C_1$-glucopyranoside, from *G. grandiflora* and other known compound which include luteolin 6-C-ß-D-4C1-glucopyranoside 8-methyl ether, schaftoside, isoorientin, apigenin 6-C-ß-D-4C1- glucopyranoside 8-methyl ether, 6-methoxyluteolin isovitexin and hispidulin were also isolated and tested (Moharram et al. 2017).

Ma and his team examined the anti-inflammatory ability of the polyphenolic fractions of sixteen cultivars of Chinese blueberry against tumor necrosis-(THF-) and interleukin-6 (IL-6). The anti-inflammatory effect of these blueberry samples were tested using lipopolysaccharide induced RAW 264.7 macrophages; anti-inflammatory potential of the polyphenol fractions were in the same trend phenolic their phenolic acid contents (Su et al. 2017). Likewise the anti-inflammatory activity, and evaluation of total phenolic content as well as the total flavonoid content of different fractions of *Bidens engleri* and *Boerhavia erecta* were evaluated (Compaore et al. 2018). Dichloromethane solvent was observed to extract flavonoid compounds more and the fraction exhibited the highest anti-inflammatory effect, via COX-2 and LOX-15 inhibition.

Macrophages have been documented to maintain the balance in pro-inflammatory and anti-inflammatory activities by controlling the switches in immune systems. Polyphenols extracted from roasted cocoa beans (*Theobroma cacao*) lower pro-inflammatory cytokine secretion significantly in *in vitro* THP-1 cells and also promotes oxidative stress by suppressing inflammation which led to ATP production as a result of increase in oxygen consumption (Dugo et al. 2017). *In vitro* evaluation of anti-inflammatory effects of the ethanolic extract of rhizome in astilbin (dihydroflavonol) from Smilax glabra was conducted by Lu et al., (2015). The result obtained revealed the suppression of nitric oxide production, TNF-a, tumor necrosis in factor-a as well as expression of mRNA inducible nitric oxide synthase in tested cells (Lu et al. 2015).

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19.5.4 Anti-microbial Properties of Polyphenols

Polyphenols possess anti-microbial properties according to evidences obtained from studies using plant polyphenols. These properties of polyphenol are believed to be beneficial to health from the consumption of food rich in polyphenols such as vegetables, fruits and other plant-derived products. Food is the main source of polyphenols, which when consumed pass through the gut. Selma et al. (2009) suggested that such phytochemicals could modify the microbes in the gut and influence their biological activity. Similarly, it has been observed that the plant-derived phytochemicals could also convert the colonic microbiota to active bio-compounds, which can influence the host health (Selma et al. 2009). Such health issues underline one aspect of a wider, more important and complex relationship between microbes, plant products and animal species.

This might affect the interpretation of the phenolics in their consideration as real antibacterial chemicals in human life. Some phenolic compounds and flavonoids such as quinic acid, caffeic acid, gallic acid and chlorogenic acid have been proved to be potent antimicrobial compounds against typical microbial strains which affect the human respiratory system or urinary tract system, including *Candida* species (Chirumbolo 2010). Galangin, which is a flavonoid compound, has been reported to inhibit the replication of the bacterium *Klebsiella pneumoniae* (Gram negative bacteria) by suppressing the bacterial enzyme DNA-B helicase (Gordon and Wareham 2010).

19.5.5 Skin Protective Effect of Polyphenols

Over exposure to ultraviolet radiation can cause harm to the skin. It induces extensive production of reactive oxygen species and eventually causes skin damages (Ichihashi et al. 2003). However, there are several strategies applicable for skin protection. Phytochemical compound, especially phenolics and flavonoids is one of the most interesting choices that exhibit beneficial effects on UV-irradiated skin (Svobodová et al. 2003; Korác and Khambholja 2011). The antioxidant ability of flavonoids makes them to possess photo-protective effects, based on their iron chelating ability, averting the destructive effects against oxidative damage which can destroy protein and lipids on cell membrane and also modulate several signaling pathways such as the inhibition of xanthine oxidase which is considered as a source of reactive oxygen species that contributes to oxidative stress (Ferrali et al. 1997; Cos et al. 1998).

Authors have reported various methods for the treatment of skin diseases as well as skin disorders caused by ultraviolet radiations with use of antioxidant ability of phenolic compounds (Działo et al. 2016; Korác and Khambholja 2011). Ferrali et al. (1997) reported the skin protective ability of apigenin, a major flavone found in edible medicinal plants and beverages such as beer, chamomile tea and red wine. Apple peel, onion skin and *Hypericum perforatum* leaves also contain flavonol, such as quercetin and has been proved to inhibit skin damage induced by UVB, in hairless mice. Similarly, extracts form *Ginkgo biloba* L. (EGb 761), which contains high content of quercetin derivatives has been proved to reduce sun burn symptoms in *in vivo* studies using UVB irradiated-skin mice model (Casagrande et al. 2006).

Sillymarin which is an extract of flavono-lignans obtained from the milk thistle of the plant *Silybum marianum* (L.) Gearnt fruit contains silybin, a major active component in the plant (Bijak 2017). Silymarin has been reported to stimulate the repair of DNA damage caused by induced-UVB and prevent cell apoptosis in UVB-exposed human epidermal keratinocytes and fibroblasts in *in vitro* study (Katiyar et al. 2011). Another photo-protective isoflavone compound reported is genistein, extracted from soybean and with the ability to inhibit UV induced DNA damage in the skin of human and therefore prevents photocarcinogenesis as observed in *in vitro* experiment (Moore et al. 2006). Wang et al. (2010) reported that genistein has the ability of maintaining antioxidant enzyme activities and modulate mitochondrial oxidative stress in human fibroblast on UVB induced senescence.

Equol is an isoflavonoid metabolite of the genistein or isoflavone daidzein produced from the gut of microflora (Setchell and Clerici 2010). Topical application of equol can inhibit DNA photodamage, prevent UV induced erythema associated with edema and prevent skin cancer when acting as sun-screen in hairless mice (Widyarini 2006). Additionally, Choi et al. reported that ultraviolet UVB induced photo aging in *in vivo* using hairless mice, in the evaluation of skin protective effects of spent coffee revealed the coffee ground extract made up of flavonoids were able to show protection on mouse skin by the down-regulating of matrix metalloproteinases (Choi et al. 2016). Kano et al. evaluated the photo-protective effects of isoflavones extracted from soy milk on the photo-damage of ovariectomized hairless mice skin in a 28 days administration. Their report revealed that there was an increase of isoflavone in the blood and skin of mice, which on the long run can scavenge reactive oxygen species generated by UV irradiation and the effect can also exert estrogenic activity. This in turn led to photo-protective effect on the skin of the mice (Kano et al. 2016).

19.5.6 Cardioprotective Activities of Polyphenols

The cardioprotective effects of phytochemicals (phenolics and/or flavonoids) which occur naturally in medicinal plants have been reported (Kumar and Pandey 2013; Craig 1999; Garjani et al. 2017; Dludla et al. 2017; Cook and Samman 1996). For example, the cardiotoxicity of doxorubicin, an extensively used anticancer drug for breast cancer, leukemia and lymphoma with side effects including pericarditis, arrhythmias, myocarditis and acute heart failure (Razavi-Azarkhiavi et al. 2016). The cardio-protective effects of many phenolic compounds on the adverse effects of doxorubicin on heart have also been studied (Razavi-Azarkhiavi et al. 2016). They are recommended as antioxidant as solution to the side effects of this anticancer drug. The role of many phenolics and flavonoids in cardioprotection has been attributed to their ability to inhibit generation of ROS, apoptosis, mitochondrial dysfunction, NF-kB, p53 and DNA damage in an *in vitro*, *in vivo* and clinical study. These phenolics and flavonoids (kaempferol, luteolin, rutin and resveratrol) showed efficacy against cardiotoxicity induced by doxorubicin but do not have effect on the activity of antitumour of this drug (Repo-Carrasco-Valencia et al. 2010; Morrison 2012; Han et al. 2012). Isorhamnetin, another interesting compound with report of its cardioprotective effect against doxorubicin toxicity and served as anticancer efficacy for the drug (Sun et al. 2013). Plant extracts contain phytochemicals, which possess cardioprotective effects. Example include phenolic composition of the methanolic extract from aerial part of *Centaurea borysthenica* Gruner and *Centaurea transcaucasica* Sosn Ex Grossh) plants with cardiomyocytes protective roles (Korga et al. 2017).

Alhaide et al. (2017) evaluated the cardioprotective effects of four varieties of date palm fruits and the total phenolic, total flavonoid, *in vivo* myocardial infarction and *in vitro* antioxidant capacity. The cardioprotective effects in the *in vivo* myocardial infarction study were attributed to the high flavonoid and phenolic contents in the fruit extracts, which led to the mobilization of the circulating progenitor cells from the bone marrow of the myocardial infarction animals, so as to promote tissue repair from ischemic injury (Alhaider et al. 2017).

Tian et al. (2018) studies on the cardioprotective role of polyphenolic extract of apple peel and apple flesh polyphenol in *in vivo* mice model on cardiovascular risk factors revealed that the extract of apple peel exhibited higher cardioprotective capacity than the apple flesh and this could be due to higher phenolic content and higher flavonoids in apple peel (Tian et al. 2018). Another research on the aerial part of aqueous extract from *Marrubium vulgare* L. against ischemia reperfusion injury *in vivo* on their cardioprotective ability shows that the aqueous extract from this plant provided cardioprotective potentials against cardiac injury on the animal (Garjani et al. 2017). Two main compounds isolated from *Aspalathus linearis* (Burm.f.) R. Dahlgren (i.e Aspalathin and phenylpyruvic acid-2-O-ß-D-glucoside) were observed to be potential cardioprotective compounds in myocardial infarction case caused by chronic hyperglycemia (Dludla et al. 2017). Polyphenolic compounds such as cinnamic acid, ferulic acid, gallic acid, gellanic acid, quercetin and syringic acid were discovered to be the major flavonoids and phenolic in the different fractions obtained from seeds extract of *Syzygium cumini* (L.) Skeels (Syama et al. 2017). Their cardioprotective role in *in vitro* H9c2 cardiac cell lines such as angiotensin converting enzyme modulation, HMG-CoA reductase, LDL oxidation and tertiary butyl hydrogen peroxide induced oxidative stress shows that the fractions obtained were observed to attenuate oxidative stress in H9c2 cardiomyoblasts and this was also demonstrated by molecular docking studies which revealed the correlation between the major phytochemicals compounds and key enzymes for the prevention of these cardiovascular diseases. Gao et al. (2007) reported the interest in the use of puerarin as a cardioprotective candidate which acts by protecting myocardium from ischemia and reperfusion damage by activating protein Kinase C through its ability to open Ca^{2+}-activated K^{+} *in vivo* in Sprague Dawley rats (Gao et al. 2007).

19.5.7 Anticancer Activities of Polyphenols

Polyphenols are reported with anticancer activities. The ethanolic extract of two members of the Zingiberaceae family; *Zingiber officinale* (rhizome) and *Curcuma longa* L. were reported to be natural promising anticancer compound for the fight against malignant melanoma due to the potential anticancer property on murine melanoma B164A5 cell line (Danciu et al. 2015). Their report also suggested *Curcuma longa* based on the increasing anticancer property of its extract, to be due to high concentration of polyphenolic compounds present in the extract (Danciu et al. 2015). Many biomedical researches have indicated flavonoid to be a potential apoptosis in various cancer cells (Zhishen et al. 1999; Brusselmans et al. 2003, 2005). Reports have been made on the anticancer role of quercetin, which is a major flavonol member against breast and prostate cancers (Kumar and Pandey 2013; Brusselmans et al. 2005).

Flavonoids (Gliricidin7-O-hexoside and Quercetin7-O-rutinoside) isolated from medicinal fern (*Asplenium nidus*) have been suggested to be potential anticancer compound against human carcinoma Hela and human hepatoma HepG2 cells (Jarial et al. 2018). Similarly, studies on the apoptosis inducing ability of quercetin in *in vivo* and *in vitro* studies using 9 different cancer cell lines, such as acute lymphoblastic leukemia MOLT-4 T-cells, pheocromocytoma PC12 cells, colon carcinoma CT-26 cells, human lymphoid Raji cells, ovarian cancer CHO cells, and colon carcinoma CT-26 cells among others, shows that quercetin can significantly induce apoptosis of every tested cell lines significantly at $p < 0.001$ compared with control group (Hashemzaei et al. 2017). For the *in vivo* studies using mice carrying MCF-7 tumors and mice bearing CT-26 tumors, the tumor in quercetin treated mice were significantly reduced in size and volume at significantly at $p < 0.001$ when compared with the control, with prolonged survival period (Hashemzaei et al. 2017).

Curcumin is a natural phenolic compound, it exert its anticancer effect on skin cancer and has been described as a compound that can influence cell cycle by acting as a pro-apoptotic agent (Działo et al. 2016). Antiproliferative effect of curcumin was investigated by Abusinina et al. on melanoma cancer in *in vitro* studies, using B16F10 murine melanoma cells. Their report indicated that curcumin served as an inhibitor to melanoma cell proliferation by acting as a non-selective cyclic nucleotide phosphodiesterase and this is related to epigenetic integrator UHRF1. They also suggested that curcumin which occur in food and diets can help in cancer prevention and contribute to gene expression via epigenetic control (Abusnina et al. 2011).

Curcumin mechanism involves the inhibition of the proliferation of selected cell lines and also induced apoptosis of cancer cells with dose-dependent response (Ide et al. 2018). The *in vivo* experiment on transgenic adenocarcinoma in mouse prostate model, with oral administration of curcumin for 1 month revealed that the curcumin regulated the transgenic enzyme expression, including AKR1C2, suppressed growth of prostate cancer cells, and decrease testosterone levels in prostate tissues of TRAMP mice (Ide et al. 2018).

19.6 Nutraceutical Application of Polyphenols

Cancer is one of the world's second leading deadly diseases, which has claimed many lives (WHO 2018). Reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) can as well serve as carcinogenic and mutagenic agents, which can later contributes to cancer development. The most proffered solution to treatment of cancer globally is through chemotherapy and this has been discovered to have many side effects (Huang et al. 2017). A flavonoid derived drug for the treatment of cancer known as flavopiridol is a phytochemical compound obtained from the plant *Dysoxylum gotadhora* (Buch.-Ham.), which is active for leukemia and lymphomas treatment (Shah et al. 2003; Cragg and Newman 2005). Some dietary supplements, made up of phenolic compounds, most especially flavonoids have long been reported to have chemopreventive ability and also have roles to play in management of cancer (Block et al. 1992; Zhishen et al. 1999; Brusselmans et al. 2003; Ahmed et al. 2016).

19.7 Conclusion

Polyphenols are class of aromatic compounds with polyhydroxyphenol structures. These compounds can be divided into several subclasses including phenolics, stilbenes, flavonoids, tannins, and lignan. These polyphenols are mostly natural compounds, which are readily found in plant-based foods while some are synthetic. Polyphenols have antioxidant, anti-inflammatory, antimicrobial, skin protection, immune system, blood pressure reduction, cardioprotective and anticancer properties. Polyphenols antioxidant properties can bring about the prevention and treatment of disease resulting from the imbalance in ROS and RNS that bring about oxidative stress and tissue degenerative diseases. Polyphenols are also promising nutraceutical agents; their inherent benefits should be exploited more for their bioactivity and nutraceutical applications.

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Chapter 20
Carotenoids as Functional Bioactive Compounds

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Abbreviations

Akt/PKB	Activated tyrosine kinases/protein kinase B
ALBP	Adipocyte Lipid Binding Protein
AP2	Adipose protein 2
C/EBPα	CCAAT enhancer binding protein alpha
C/EBPβ	CCAAT enhancer binding protein beta
cGMP	Cyclic guanosine monophosphate
COX 2	Cyclooxygenase 2
CPT1B	Carnitine palmitoyltransferase I
ERK1/2	Extracellular signal-regulated kinases 1/2

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GLUT-4	Glucose transporter type 4
HbA1c	Glycated haemoglobin
HDLc	High density lipoprotein-cholesterol
hsCRP	High sensitivity C-reactive protein
IL-1	Interleukin 1
IL-1β	Interleukin 1beta
iNOS	Inducible nitric oxide synthase
LDLc	Low density lipoprotein-cholesterol
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
PDGF	Platelet-derived growth factor
PGE_2	Prostaglandin E_2
PPARα	Peroxisome proliferator-activated receptor alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
PPARγ1	Peroxisome proliferator-activated receptor gamma 1
PPREs	Peroxisome proliferator response elements
ROS	Reactive oxygen species
RXR-α	Retinoid X receptor alpha
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble Vascular Cell Adhesion Molecule-1
T2D	Type-2 diabetes
TNF-α	Tumour necrosis factor alpha
UCP	Uncoupling protein
WAT	White adipose tissue

20.1 Introduction

Carotenoids are a class of orange, yellow and red-pigmented poly-isoprenoid hydrocarbons produced by prokaryotic cells, higher plants and concentrating in animal lipid (Najm and Lie 2008). About, 700 distinctive carotenoids exist in plants as well as some group of algae and fungi. In animals and plants, they act as pigments accountable for several and striking colors present in nature (Hammond Jr and Renz 2013). The word carotenoids was coined from the name carrot (*Daucus carota*) and together with anthocyanins, they are the most compounded group of non-artificial food colorants and they are documented to have more 750 different structures (Zakynthinos and Varzakas 2016). They contain about 15 conjugated double bonds and they are divided into two main classes with respect to their chemical structures: the pure hydrocarbons such as β-carotene and the oxygenated analogs like zeaxanthin, which are also called xanthophylls (Zakynthinos and Varzakas 2016). Furthermore, they are hydrophobic, soluble in organic solvents (methanol, acetone,

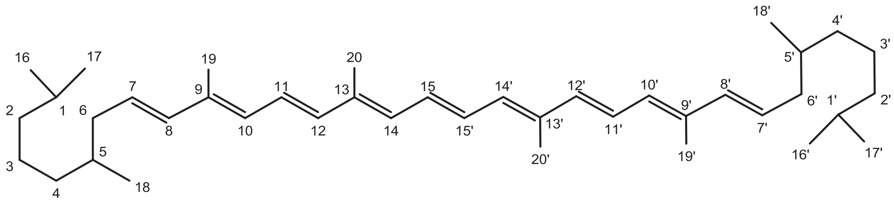

Fig. 20.1 Basic carotenoid structure

tetrahydrofuran, ethyl acetate) and regarded as non-saponifiable fats (Pradas-Baena et al. 2015; Uthayakumaran and Wrigley 2017). Carotenoids are the most widespread pigment in nature and they have received commendable level of attention because of their provitamin and antioxidant functions (Zakynthinos and Varzakas 2016). Majority of these poly-isoprenoid hydrocarbons like the carotene are utilized for the synthesis of vitamin A in the body. But other carotenoids like lutein and lycopene exhibit no activity of vitamin A (Wolak and Paran 2013). The major source of this bioactive compounds in the plasma of humans are vegetables and fruits (Heber and Lu 2002). Lycopene is extensively found in pink guava, pink grapefruit, tomatoes, papaya and watermelon while lutein is mostly found in persimmons, maize and leafy vegetables like kale, spinach and broccoli (Nagarajan et al. 2017). They are mainly used as feeds, cosmetics and natural colorant for food as well as important for photosynthesis and plant growth (Olson 1987; Zakynthinos and Varzakas 2016). The basic structure of carotenoids is shown in Fig. 20.1. The scope of this work highlights the classifications, distributions, biosynthesis, pharmacological activities and nutraceutic applications of carotenoids.

20.2 Classification of Carotenoids

Carotenoids are fat-soluble pigments with above 700 compounds, mostly containing more than 40 carbon and two terminal rings (Bell et al. 2000). Carotenoids are classified into two major groups. The first group includes carotene linear hydrocarbons with no oxygen molecule (Aizawa and Inakuma 2007) which tend to be cyclized at either ends of the compound. The second group comprises the oxygenated carotene derivatives (Fig. 20.2). Examples of carotene linear hydrocarbons includes α-carotene, *β*-carotene and lycopene while examples of oxygenated carotene derivatives include lutein, neoxanthin, violaxanthin, and zeaxanthin also known as the xanthophylls class of carotenes (Botella-Paviía and Rodríguez-Concepción 2006). The oxygenated group are usually having a carboxyl, epoxy, hydroxyl, keto or methoxy groups (Basu et al. 2001; de Quiros and Costa 2006).

Carotenoids may also be classified as cyclic or acyclic depending on the presence or absence of cyclic chains at the terminals. They may similarly be classified as primary or secondary carotenoids. Primary carotenoids include compounds

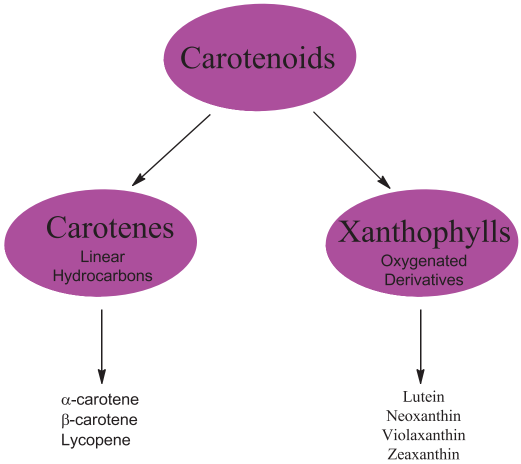

Fig. 20.2 Classification of carotenoids

required for photosynthesis of plants, like β-carotene, violaxanthin, and neoxanthin while secondary carotenoids do not possess photosynthetic activities and are localized in plants fruits or flowers, which include antheraxanthin, capsanthin, capsorubin, zeaxanthin, α-carotene, and β-cryptoxanthin (Lichtenhaler 1987).

20.3 Distributions of Carotenoids

Carotenoids are pigments, which occur widely in nature. They are hydrophobic, lipophilic and insoluble in water. They are however, soluble in organic solvents including chloroform, alcohol and acetone (Thane and Reddy 1997). Their soluble nature has enhanced their occurrence in different natural sources. They are found in both photosynthetic and non-photosynthetic organisms distributed in a broad class of plants, algae, bacteria and fungi (Delgado-Vargas et al. 2000). Natural sources of carotenoids include vegetables and colored fruits (Ambrósio et al. 2006). Lycopene are mostly found in tomatoes and its products (juices, ketchup, sauces and soups) (Tavares and Rodriguez-Amaya 1994). *β*-carotenes are most abundant in food sources expressing higher activities of provitamin A (Mezzomo and Ferreira 2016). β-carotene are abundant in *Canarium odontophyllum* Miq (Prasad et al. 2011). β carotene and α-carotene are also found in apricots, cantaloupe, carrots, pumpkin and sweet potato. β-carotene, phytofluene and phytoene are found in watermelon, grapefruit as well as in tomatoes (Paiva and Russell 1999). Lutein are abundant in mangoes, oranges, papaya, peaches, prunes, spinach and squash (Paiva and Russell 1999). β-cryptoxanthin are found in citrus fruits, papaya and peach (Sugiura et al. 2002). Zeaxanthin, the dihydroxylated form of β-carotene is found in corn and varieties of vegetables (Nishino et al. 2002). Zeaxanthin are found in corn, alfalfa, wolfberry and marigold flowers (Nelis and DeLeenheer 1991; Chang et al. 2010). Table 20.1 summaries some plant sources of carotenoids.

Table 20.1 Summary of some natural sources of carotenoids

Carotenoids	Natural Sources	Common names
α-carotene	*Daucus carota*	Carrot
	Citrus spp.	Orange
	Carica papaya	Papaya
	Musa acuminata	Banana
	Prunus armeniaca	Apricot
β-carotene	*Daucus carota*	Carrot
	Lactuca sativa	Lettuce
	Citrus paradisi	Grape fruit
	Prunus ameriaca	Apricot
	Brassica oleracea	Broccoli
β-cryptoxanthin	*Capsicum annuum*	Red pepper
	Prunus armeniaca	Apricot
	Mangifera indica	Mango
	Cucurbita spp.	Pumpkin
	Cucurbita moschata	Pumpkin
Lutein	Spinacia oleracea	Spinach
	Citrus spp.	Orange
	Zea mays	Corn
	Capsicum annuum	Yellow pepper
	Brassica oleracea	Broccoli
Lycopene	Solanum lycopersicum	Tomato
	Carica papaya	Papaya
	Citrullus lanatus	Watermelon
	Prunus ameriaca	Apricot
	Daucus carota	Carrot
Zeaxanthin	*Zea mays*	Sweetcorn
	Capsicum annuum	Red pepper
	Cucurbita moschata	Pumpkin
	Diospyros kaki	Persimmon
	Malpighia punicifolia	Acerola

Source: Southon and Faulks (2003) and Britton and Khachik (2009)

Carotenoids also occur in some microorganisms having the ability to produce them but not in animals, which obtain them through their various sources from the diet (de Quirós and Costa 2006). Astaxanthin are synthesize in large quantities by *Chlorella vulgaris*, *Phaffia rhodozyma* and *Haematococcus pluvialis* (Yin et al. 2013; Kim et al. 2016). Some microorganisms are similarly capable of synthesizing astaxanthin, canthaxanthin, lutein, lycopene, zeaxanthin, β-cryptoxanthin, β-carotene (Bhosale 2004; Bhosale and Bernstein 2005). β-carotene and zeaxanthin are produced by the microalgae *Dunaliella salina* and *Flavobacterium* sp. respectively (Masetto et al. 2001; Pisal and Lele 2005).

20.4 Biosynthesis of Carotenoids

Carotenoids are members of the tetraterpenoids containing 40 carbon atoms built by the arrangements of terpene units joined together through their head and tail (Bonnie and Choo 1999; Mattea et al. 2009). They are synthesized by plants, some algae, bacteria and fungi (Okada et al. 2008). The biosynthetic pathway were elucidated between the years 1950 to 1960 through classical biochemical approaches by the use of specific inhibitors and certain mutants blockers at different steps in the carotenoid synthesis pathway (Fraser and Bramley 2004). The biosynthesis in plant starts from the initiation step, where mevalonic acid is converted to isopentenyl pyrophosphate (IPP) and condensed with dimethylallyl pyrophosphate (DMAPP) by a head to tail fashion to generate geranyl pyrophosphate (GPP). Addition of two more molecule of IPP in two steps to GPP produces farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) through IPP isomerase and GGPP synthase respectively (Fig. 20.3). GGPP is the immediate precursor for the biosynthesis of carotenoids. Two molecules GGPP when condensed together head to head produce pre-phytoene pyrophosphate, which produces phytoene through the reductive dimerization of by the action of the enzyme phytoene synthase. Synthesis of phytoene is followed by dehydrogenation, desaturation, cyclization, hydroxylation, epoxidation and oxidation (Yano et al. 2005).

20.5 Pharmacological Activities of Carotenoids

20.5.1 Anti-aging Activity

The development and progression of atherosclerosis is regarded as one of the major manifestations of aging. It has been documented that the production of reactive oxygen species (ROS) by the inflammatory milieu of atherosclerotic plaque is one of the causes of atherosclerosis (Tavakoli and Asmis 2012). The level of ROS in aging blood arteries leads to endothelial damage and dysfunction, and this stimulates the progression of atherosclerosis (Selvaraju et al. 2012). Also, the combination of atherosclerosis and aging by ROS is a result of reduction of nitric oxide (NO) in the vascular tissue. Nevertheless, in normal vascular tissue, the concentration or bioavailability of NO is usually high. The atherosclerotic vascular aging is inhibited by NO produced in the endothelium by several mechanisms including maintenance of normal flow of blood in organs via flow/shear stress mediated vasodilation. The preserved vasodilation mediating blood flow in organs is one of the main mechanism of preventing anti-vascular aging (Celermajer et al. 1994). Also, NO stimulates the suppression of inflammation and thrombosis (Ungvari et al. 2010). Carotenoids like lutein, lycopene and others inhibits vascular aging as a result of their anti-oxidant activity, which elevates the bioavailability of NO in the vascular system (Fig. 20.4). Consistently, tomato extract of β-carotene and lycopene decrease

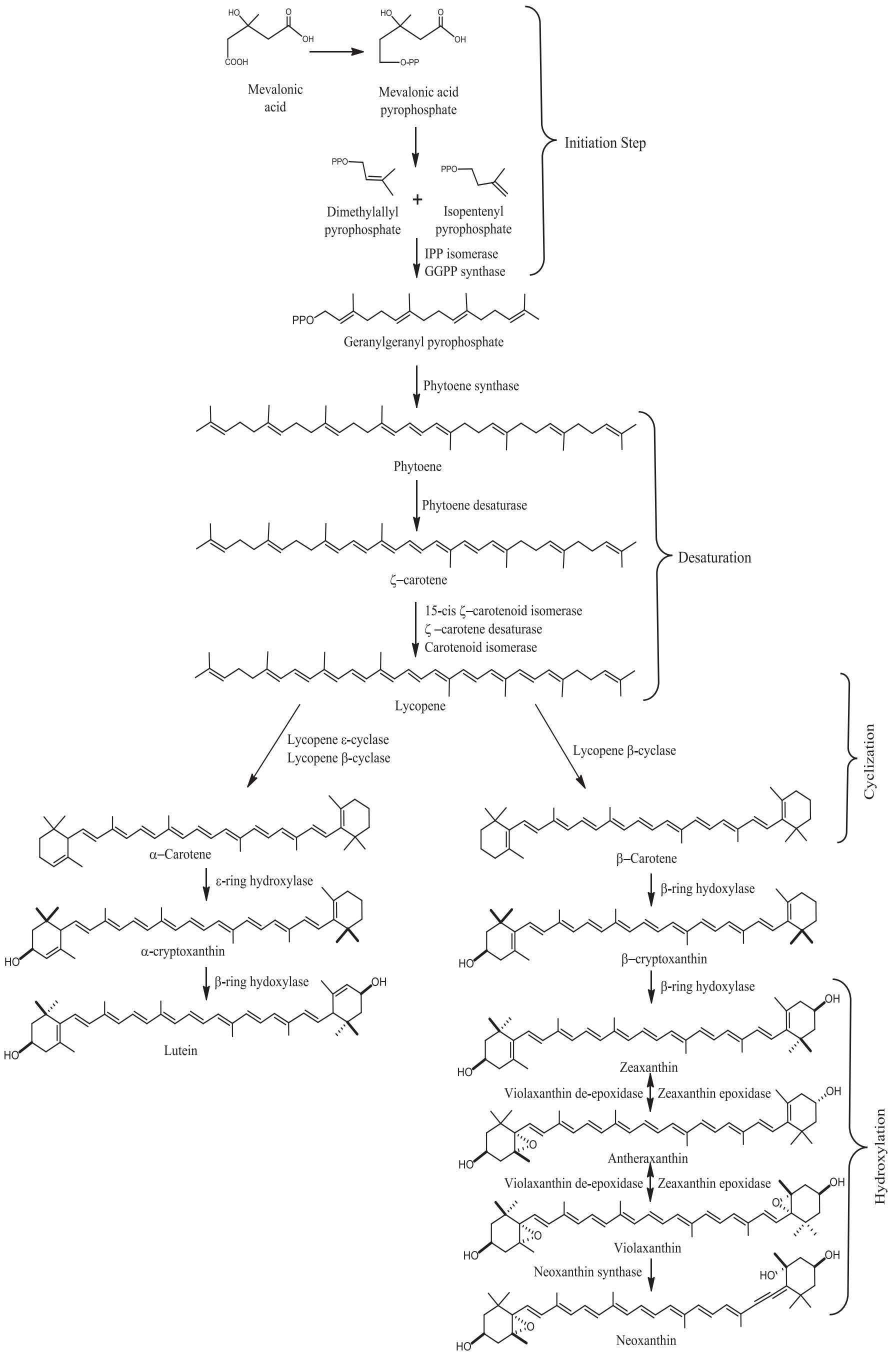

Fig. 20.3 Carotenoids biosynthesis pathway. (Source: Kato et al. 2004; Yano et al. 2005; Diretto et al. 2006; Kato et al. 2007; Apel and Bock 2009)

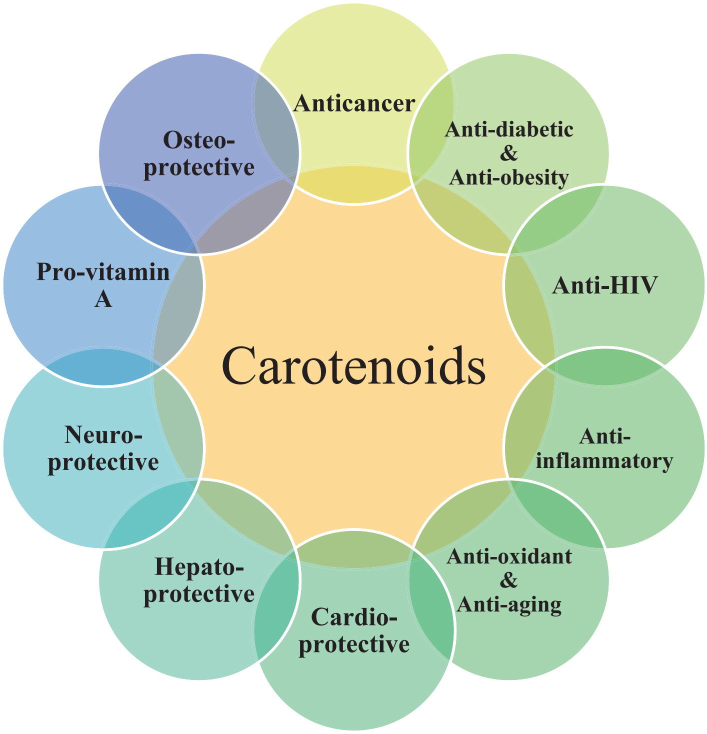

Fig. 20.4 Summary of pharmacological and biological activities of carotenoids

blood pressure with a corresponding increase of NO level in the plasma (Paran et al. 2009). In a similar study, lycopene pre-treated with human umbilical vein endothelial cell (HUVECs) reduced the formation ROS mediated by TNF-α. It also suppresses monocyte adhesion molecule expression (Di Tomo et al. 2012). Many other researchers have shown the activities of lycopene as an inhibitor of inflammation and ROS as well as in the amelioration of inflammatory mediated atherosclerotic processes (Wolak and Paran 2013). Amongst these is a work conducted on healthy male volunteers with lycopene supplementation. In this work, lycopene supplementation improves the function of endothelial system in these individuals with a corresponding reduction in sVCAM-1, hsCRP, and sICAM-1. Also, the atherosclerotic risk factors such as systolic blood pressure and lipid profile of the treated group improved (Kim et al. 2011). In another study done on young, healthy lean women and men with a mean age of 27 ± 8 years and mean body mass index of 22 ± 2 who were placed on high-fat meals on two occasions, high fat diet has been documented to cause postprandial oxidative stress. On one occasion, a processed tomato product (having high lycopene concentration) was included in the high fat diet while on the second occasion, processed tomato products were not included in the diet. There was an elevated postprandial concentration of lipid in both meals but the tomato meal resulted in a marked attenuation in the level LDL oxidation as well as the inflammatory marker and proinflammatory cytokine (interleukin 6) caused by high fat meal (Burton-Freeman et al. 2012).

Mostly lutein and lycopene among other carotenoids inhibit this process by reducing the level of ROS in living systems. This anti-oxidant function of lutein was shown in vascular smooth muscle cells (Wolak and Paran 2013). PDGF-induced intracellular production of ROS was reduced by lutein. It also ameliorated ROS-(H_2O_2)-induced ERK1/2 and activation of p38 MAPK (Lo et al. 2012). In another study carried out on vascular sections, lycopene protects oxidative stress-mediated endothelial injury. The endothelial cells pretreated with lycopene showed elevated viability, down-regulation of caspase-3 and p53 mRNA and reduced rate of apoptosis (Tang et al. 2009). Oxidation of LDL is one of the initial step in the process of atherosclerosis. Therefore, the oxidized LDL substances seen in the sub intimal region leads to chemical attraction of inflammatory cells (Stocker and Keaney Jr 2004). Lycopene in an exracted human LDL substances resist the activity of myeloperoxidase. This resistance activity might be one of the mechanisms that prevent oxidation of LDL and thus ameliorating the process of atherosclerosis (Chew et al. 2012).

20.5.2 Osteo-protective Activity

Carotenoids like β-cryptoxanthin have the ability to regulate the health of bone and inhibit osteoporosis (Uchiyama et al. 2004a; Yamaguchi 2008). The existence of β-cryptoxanthin at a concentration of 10^{-6} or 10^{-7} M leads to decrease in the activity of alkaline phosphatase and content of calcium in the femoral-metaphysial and femoral-diaphysial tissues in female rats (Uchiyama et al. 2004a; Yamaguchi 2008). Oral treatment with β-cryptoxanthin stimulates anabolic action of bone constituents in aged female rats' femoral tissues. Thus, this bioactive ingredient may have an activating action on loss of bone mass and leads to osteoporosis as well as bone fracture (Uchiyama et al. 2004b). Furthermore, β-cryptoxanthin has an efficacious anabolic action of calcification of bone in femoral-metaphysical and femoral-diaphysial tissues in both *in vitro* and *in vivo* studies (Yamaguchi and Uchiyama 2003; Uchiyama et al. 2004c). More so, the intake of reinforce juice which has higher β-cryptoxanthin than a usual juice, displayed a preventive action on loss of bone (Yamaguchi et al. 2006). In addition, they showed that long term intake of juice containing β-cryptoxanthin results to activation of bone formation and preventive action on reabsorption of bone in humans, which is favourable among menopausal women (Yamaguchi et al. 2006).

20.5.3 Anticancer Activity

About ten million cancer cases occur yearly and this rate is rapidly increasing globally. It has been reported that consumption of diet rich in plant and other natural products like fruits (eg. mangoes, legumes, peaches, cantaloupes, persimmons, apricots, papayas etc), vegetables, including carrots, sweet potatoes, arugula, watercress, broccoli, pumpkins, tomatoes as well as less processed carbohydrate rich staple foods daily could reduce the incidence of cancer by 20 percent (Basu et al. 2001). Additionally, diets that are rich in fruits and vegetables that are rich in carotenoids can reduce the risk, development and continuity of cancer (Rock 2009). Lutein, zeaxanthin, fucoxanthin, α-carotene, β-carotene, astaxanthin and β-cryptoxanthin are examples of carotenoids that displayed anticancer activity and may be essential in the prevention of cancer (Nishino et al. 2002). Due to their useful effects on human health such as stimulation of immune system and inhibition of cancer cells (which are all linked to their antioxidant role), scientific interest on carotenoids have increase over the years (Das et al. 2007). Lycopene is the main carotenoid that exists in the serum and it is reported to stimulate the connexion43 expression, a gene that codes for the gap junction protein that can cause upregulation of gap junction potentially preventing cancer cell proliferation (Zhang et al. 1991; Zhang et al. 1992; Rao and Agarwal 1999). Moreover, increased consumption of xanthophyll carotenoids found in human plasma including cryptoxanthin, lutein and zeaxanthin is reported to decreases the incidence of age-related disorders like cancer, cataract formation, macular degeneration and cardiovascular disorders (Bhosale and Bernstein 2005). Fucoxanthin is another interesting carotenoids that is abundantly found in *Undaria pinnatifida* (Liu et al. 2009). It was reported that it causes apoptosis of human HL-60 cancer cell by causing fragmentation of DNA and inhibition of cell proliferation (Hosokawa et al. 1999). In another *in vitro* study, it inhibits the proliferation of murine embryonic liver BNL CL.2 cells and human hepatoma SK-Hep1 cells (Liu et al. 2009). Fucoxanthin effectively decreases the motility of cancer cell lines of human colon like DLD-1, HT-29, and Caco2 (Hosokawa et al. 2004). Additionally, it causes the arrest of cell cycle at G0G1 phase in adenocarcinoma WiDr cells of human colon (Das et al. 2005) and hepatocarcinoma HepG2 cells of humans (Das et al. 2008).

PPARγ is a type of nuclear hormone receptor superfamily and acts as a ligand activated transcription factor. It is involved in several pathological and physiological processes, like lipid metabolism, insulin sensitivity, lipid metabolism and growth of cells in different organs and occurrence of many human disorders including dyslipidaemia, cancer, inflammation, obesity and atherosclerosis (Lehrke and Lazar 2005; Feige et al. 2006; Semple et al. 2006). Also, PPARγ ligands synthesized in the body include linolenic acid, arachidonic acid and linoleic acid as well as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2). Synthetic PPARγ ligands include the glizers also called known as the thiazolidinediones (rosiglitazone, ciglitazone, troglitazone and pioglitazone), which are ususally used in the treatment of type-2 diabetes. The ligands of the PPARγ (also called PPARγ agonists) forms a heterodimer with the RXR-α and react with its major response elements, PPREs, situated in the promoter region of the different target genes (Zhao et al. 2016). Emerging reports indicated that PPARγ is expressed in several tissues in human which includes the colon, stomach, breast, thyroid, lung, prostate and ovary, where it controls cell differentiation, proliferation and apoptosis. This feature makes PPARγ a vital target protein for the manufacture of new and efficacious anticancer drugs (Han and Roman 2007; Ondrey

2009). Carotenoids have been reported to mediate the function of PPARγ such as growth and apoptosis of cancer cells (Zhao et al. 2016). Hence, several reports have shown the carotenoids serve as a strong growth-inhibitory compounds in many tumour cell including colon, leukemia, breast, prostate, melanoma and lung cells (Gallicchio et al. 2008; Nishino et al. 2009). Some of the anticancer mechanism of carotenoid shows that it is involved in the PPARγ-signalling pathway. Carotenoids such as lycopene, phytofluene, β-carotene and phytoene instigate the transactivation of PPREs in MCF7 cell cotransfected with PPARγ. This effect was less compared to the activity of synthetic and knonw PPAR ligands like ciglitazone and 15d-PGJ_2 (Sharoni et al. 2004). Also, 3.8 μM fucoxanthin, which is a carotenoid isolated from edible seaweed (*Undaria pinnatifida*) combined with 10 μM troglitaone (a synthetic PPARγ agonist) resulted into an extensive induction of DNA fragmentation followed by reduced viability of human Caco2 colon cancer cells. However, individual treatment with troglitazone and fucoxanthin did not show a significant effect on the viability of Caco2 cells. In another study, 10–25 μM of lycopene exhibited no effects on the proliferation of PC3 prostate cancer cells. But, antiproliferative effect of PPARγ agonist like pioglitazone, ciglitazone and 15d-PGJ_2 was supported by 25 μM of lycopene, it also inhibited the expression of survival and growth-associated genes in PC3 cancer cells and controlled the apoptosis induction by PPARγ (Rafi et al. 2013). The proliferation of PC3 cancer cells by lutein was similar to that of lycopene. A mild decreased proliferation was induced by lutein, it also stimulated PPARγ agonist induced cell cycle apoptosis and arrest, changes the expression of apoptosis and growth-associated biological marker genes (Rafi et al. 2015). The expression of PPARγ in breast MCF7 cancer was stimulated by β-carotene (Zhao et al. 2016). The function of β-carotene in growth inhibition and apoptosis can be attenuated by an irreversible antagonist of PPARγ called GW9662. Consistently, β-carotene stimulated the production of reactive oxygen species and liberation of cytochrome C. This mechanism indicated that the expression of PPARγ and production of reactive oxygen species may be responsible for the anti-cancer activities of β-carotene (Cui et al. 2007). In another study, carotenoids down-regulated cyclin D1 expression and upregulated cyclin-dependent kinase inhibitor p21 and PPARγ expression (Zhang et al. 2011). Additionally, the inhibitory effects of carotenoids on K562-cell proliferation is supported by rosiglitazone. GW9662, a specific PPARγ inhibitor and PPARγ small interfering RNA (siRNA) activities are greatly suppressed by combination rosiglitazone and carotenoid. The activities of rosiglitazone and carotenoids on PPARγ siRNA and GW9662 remarkably ameliorates cyclin D1 down regulation and p21 up regulation (Zhao et al. 2014). The above data indicated that PPARγ signalling cascade is involved in the reduction of tumour cell apoptosis by carotenoids. But, it still remain unclear whether they can act as agonist of PPARγ and hence can stop the growth of cancer cells. The data investigated using PPARγ CALUX reporter cell line indicated that the γ-carotene, violaxanthin, neurosporene, lycopene, δ-carotene, phytofluene, β-carotene from tomato extracts stimulated expression of luciferase via PPARγ2 (Gijsbers et al. 2013). Thus, carotenoids appear to possess some PPARγ stimulating potentials (Zhao et al. 2016).

20.5.4 Anti-diabetic Activity

Recently, it was reported that carotenoids may be more effective in the management of diabetes mellitus (Sathasivam and Ki 2018) and most of these bioactive compounds are derived from diet and high concentration of β-carotene in plasma is inversely related to insulin resistance and the level of fasting blood glucose (Ylonen et al. 2003). The incidence of type-2 diabetes (T2D) has been reported to be greatly reduced in women and men by dietary carotenoids by many studies (Ylonen et al. 2003; Sluijs et al. 2015). Some studies have indicated that lutein, zeaxanthin and lycopene are examples of carotenoids that inhibit the development of diabetic retinopathy (Brazionis et al. 2009). Also, intake of dietary carotenoids is inversely associated with the level of HbA1c in plasma of humans (Suzuki et al. 2002).

Astaxanthin, one of the well-studied carotenoid, shows higher antioxidant properties than other carotenoids like the lutein, zeaxanthin and β-carotene and it has been reported to be effective in the prevention and management of diabetes (Yeh et al. 2016; Sathasivam and Ki 2018). The treatment of diabetes in db/db mice resulted to a reduced glucose tolerance, ameliorates level of blood glucose and stimulates the level of insulin in serum. Thus, the data shows that the antioxidant effect of astaxanthin can be effective in the preservation of β-cell function and morphology (Uchiyama et al. 2002). In another study, it was reported that in older Japanese and middle aged patients, the level of β-cryptoxanthin and α-carotene are related to lower T2D incidence (Sugiura et al. 2015). This studies indicated the relationship between the level of carotenoids in serum and smoking with 15 years of incidence of diabetes (Hozawa et al. 2006). Also, the concentration of zeaxanthin, α-carotene, lycopene, β-carotene and lutein were remarkably decreased in diabetic individuals (Ylonen et al. 2003). In mice feed on high fat and fructose, astaxanthin elevates the concentration of adiponectin and HDLc as well as decrease in the level of TAG and blood glucose (Hussein et al. 2007). Another important carotenoid is β-carotene, which its concentration in serum is inversely association with the concentration of HbA1c in serum as well as impaired insulin sensitivity (Hozumi et al. 1998; Arnlov et al. 2009). Lutein, a carotenoids, significantly reduces hyperglycaemia induced by streptozotocin and revealed a remarkable antioxidant action in the renal organs of diabetic animals (Katyal et al. 2013). Another vital carotenoids with anti-diabetic action is fucoxantin. In obese mice treated with carotenoid, the level of serum insulin and blood glucose was restored to normal. In this work, the genetic expression and level of mRNA of glucose transporter-4 (GLUT-4) was up-regulated in the cells of skeletal muscle (Maeda et al. 2009). In a similar work, fucoxanthin enhances the expression of GLUT-4 gene in skeletal muscles and it was stated that stimulation of PPARγ co-stimulator 1α regulates this process, which is associated with an up-regulation in the level of insulin receptor mRNA, with a corresponding high level of phosphorylation of Akt/PKB. All this play a vital role in the modulation of GLUT-4 translocation to the plasma membrane (Nishikawa et al. 2012). In addition to the studies carried out on fucoxanthin, it was reported that this carotenoid stimulates the gene expresstion of GLUT-4 and PPARγ (Maeda et al. 2006). Thus, it was indicated that it has anti-diabetic activity. In a KKAy mice model, it was shown that fucoxanthin can decrease high blood glucose, though, it was not effect in C57BL/6 J mice model (Hosokawa et al. 2010). A human study carried out by European Prospective Investigation into Cancer and Nutrition in Netherlands and a population work conducted in Queensland, Australia. In these studies, 37,846 women and men showed an inverse correlation between dietary β-carotene and the risk incidence of T2D. Also, it was stated that the concentration of β-carotene in serum is a vital determining index of metabolic syndrome results (Suzuki et al. 2011).

20.5.5 Anti-human Immunodeficiency Virus (Anti-HIV)

The attack of Human immunodeficiency virus (anti-HIV) can leads to production of ROS and progression of oxidative stress with corresponding suppression of the antioxidant system in the body. Therefore, the degenerating risk of HIV virus is evidently seen via nutritional supplements rich in antioxidant especially carotenoids (Nagarajan et al. 2017). It was reported that low level of provitamin A serum of human leads to elevated loads of HIV in humans with tuberculosis of pulmonary system compared to humans with high level of plasma carotenoids (van Lettow et al. 2004).

20.5.6 Anti-inflammatory Activity

Inflammation is the initial response of the defense system to irritation or infection and it is also known as the innate system (Sathasivam and Ki 2018). Nevertheless, some inflammatory responses can leads to several conditions including colitis, pneumonia, gastritis, arthritis, neuro-inflammatory diseases, and hepatitis (Pangestuti and Kim 2011). Hence, natural products, especially cartotenids are gaining many attentions and could be utilized as synthetic agents for the prevention and management of severe inflammatory disorders. This is linked to their inhibitory action on the activities of cyclooxygenase 2 (COX 2) and inducible nitric oxide synthase (iNOS) as well as their inhibitory action on the synthesis of prostaglandins E2, pro-inflammatory markers and NO (Rajapakse et al. 2008; Peerapornpisal et al. 2010). Astaxanthin at the dose of 100 mg/kg body displayed a higher anti-inflammatory action than that observed in 10 mg/kg of prednisolone, a synthetic anti-inflammatory drug (Ohgami et al. 2003). This carotenoid also reduces the action of NF-κB cascade by inhibiting the expression of proinflammatory genes as well as preventing the production of PGE_2 and NO. Additionally, it reduces the function of iNOS promoter by impairing the activity of IkB kinase (Lee et al. 2003). Some works using both *in vitro* and *in vivo* models have been carried out to determine the action of astaxanthin on inflammatory reactions induced by lipopolysaccharide (Ohgami et al. 2003; Shiratori et al. 2005). This carotenoid displayed a dose dependent anti-inflammatory action on LPS fed mice by reducing inhibiting NOS activity in RAW 264.7 cell lines as well as reducing TNF-α, NO, IL-1β and PGE_2. In a similar study, the expression of COX 2 and iNOS as well as the synthesis of NO in BV2 microglial cells stimulated by LPS are inhibited by astaxanthin (Choi et al. 2009). This xanthophyll carotenoid improved the microbicidal and phagocytic activity of neutrophils at 5 mM (Macedo et al. 2010). Furthermore, it suppresses oxidative injury to proteins and lipids in human neutrophils (Macedo et al. 2010). A reduction in the levels of NF-κB expression and improvement of expression of SHP-1 level was observed in cells pre-treated with astaxanthin (Speranza et al. 2012). A human study was carried out on cohort of young women that are healthy (Park et al. 2010). In this work, 2 mg of astaxanthin ingested by women for 8 weeks resulted into a reduced level of C-reactive protein in blood, thus, expressing it anti-inflammatory action. Furthermore, suppresses the synthesis of inflammatory markers as well as synthesis of ROS by down-regulating transcription factors of AP-1 and NF-κB. The data from this study indicated that ingestion of astaxanthin improves immune stimuli in young women that are healthy, reduces the levels of acute phase protein and damage of DNA (Park et al. 2010).

20.5.7 Anti-obesity Activity

Accumulation of high quantity of adipocytes leads to obesity and it has become a global health problem. This is due to its ability to increase the risk of life threatening disorders like obstructive sleep apnea, type-2 diabetes, and coronary heart diseases, among others. Adipocyte size and number are used as index for determination of adipose tissue mass (Zhao et al. 2016). Elevated storage of triacylglycerol (TAG) and high quantity of adipose tissues as a result of differentiation and proliferation leads to expansion of adipose tissue size. The differentiation of adipoctytes is a multiplex process which involve variety of transcription factors including early up-regulation of C/EBPβ expression. Then, the adipogenic factors like C/EBPα are acted upon by C/EBPβ to modulate the late stage of adipose tissue formation and it its deficiency, differenttiaon of adipocyte cannot not occur (Rosen and MacDougald 2006; Frey and Vogel 2011). The function of β-carotene in the adipose tissue has gain more attention. It exert its effect by inhibiting adipose tissues formation and differentiation (Zhao et al. 2016). Retinal, β-carotene and β-apo-8′-carotenal remarkably reduced the differentiation of 3T3L1 preadipose tissue cell line to adipose tissues via upregulation of RAR and PPARγ2 suppression (Kawada et al. 2000). In another study, rosiglitazone in combination with (all-E)-lycopene, β-carotene and other dietary constituents was used to conduct differentiation in murine C3H10T1/2 adipocyte. It was observed that β-carotene inhibited lipid formation that was stimulated by rosiglitazone and also suppresses the expression of differentiation markers in adipocytes like the C/EBPα, PPARα and PPARγ as well as other genes linked to metabolism lipid (CPT1B, AP2 and LPL). Thus, the modulation of genes involved in differentiation of adipocytes were stimulated by β-carotene (Warnke et al. 2011). Consistently, 20 μM of β-carotene in 3 T3-L1 adipocyte cell lines differentiation, stimulated gene expression associated with insulin sensitivity. This genes includes GLUT-4, C/EBPα, C/EBPβ, adiponectin, PPARγ1, PPARγ2, and ALBP. Also, TNF-α is inhibited by accumulated β-carotene and leads to the elevation in the expression of genes linked to sensitivity of insulin (Kameji et al. 2010). Hence, treatments with carotenoids may reduce the expression of PPARγ as well as other lipogenic and adipogenic genes in the initial stage of differentiation of adipose tissues. Additionally, diets rich in 9-*cis* β-carotene prevents the process of atherogenesis and fatty liver production in LDL receptor-knockout mice (Harari et al. 2008).

A physiological defense mechanism against obesity, diabetes and hyperlipidemia through uncoupling protein (UCP) families including UCP1, UCP2 and UCP3 has become a target of interest (Jezek 2002). The mitochondrial UCP1 is normally expressed mainly in the brown adipose tissue and is the major agent for antiobesity. Hence, its impairment leads to the development of obesity (Lowel et al. 1993; Miyashita 2006; Maeda et al. 2007a). A report shows that fucoxanthin, a carotenoid, remarkably lowers white adipose tissue (WAT) in mice and rats with a marked expression of UCP1 mRNA and protein in WAT. However, there was a minute expression of WAT UCP1 in mice fed on control diet. Additionally, the mixture of fish oil and fucoxanthin displayed a higher action for ameliorating the WAT weight gain when compared to feeding with fucoxanthin only (Maeda et al. 2007a; Maeda et al. 2007b). Furthermore, the intake of fucoxanthin daily leads to a remarkable decrease in body weight (Maeda et al. 2005). Also, astaxanthin prevents the elevation of adipose tissue and body weight due to high fat diets (Ikeuchi et al. 2007). Moreover, weight of liver, total cholesterol, TAG in liver, TAG in plasma are all reduced by astaxanthin and it may be concluded that it attenuates obesity (Jaswir et al. 2011).

20.5.8 Antioxidant/Pro-oxidant Activity

Carotenoids may promote oxidative stress inhibition and serve as antioxidants in all organism (Tian et al. 2007; Yeum et al. 2009). In human beings, carotenoids are components of the defense cascade (Stahl and Sies 2003). They inhibit single oxygen in the way to tocopherols and they scavenge free reactive oxygen species that are produced during photo-oxidative stress (Stahl et al. 2000). In addition, El-Agamey and Mcgarvey (2008)) reported that they quench free radicals through three major mechanism including electron transfer reaction (Eq. 20.1), addition and hydrogen atom transfer reaction (Eq. 20.2) and addition reaction (Eq. 20.3). Also, carotenoids have the ability to combine directly with free radicals like superoxide anion. A resonance-stabilised carbon-centred radical complex is formed then carotenoids reacts with free radicals. An example is there reaction with lipid peroxyl radicals (Smirnoff 2005). CAR: carotenoids; peroxyl radicals: ROO∗

$$ROO^{*} + CAR \rightarrow ROO^{-} + CAR^{*+} \tag{20.1}$$

$$ROO^{*} + CAR \rightarrow ROOH + CAR^{*} \tag{20.2}$$

$$ROO^{*} + CAR \rightarrow ROO - CAR^{*} \tag{20.3}$$

In another method, carotenoids accept electrons. For instance, they can accept electron from superoxide leading to the formation of radical anion. An example is given in the case of lycopene.

$$\text{Lycopene} + \text{superoxide}\left(O_2^{-*}\right) \rightarrow \text{Lycopene}^{-*} + \text{oxygen}\left(O_2\right)$$

Lycopene can act as an antioxidant by many mechanism (Erdman Jr et al. 2008). One of the major mechanism is by scavenging singlet oxygen ($^1O_2$). This is shown below:

$$\text{Lycopene+singlet oxygen}\left({}^1O_2\right) \rightarrow {}^3O_2 + {}^3\text{Lycopene}$$

An excited form of lycopene (^{3}Lycopene) has enough energy to excite other molecules and cause the generation of free radicals. Hence, single lycopene molecules normally scavenge two or more free radicals (Krinski 1992). Thus, lycopene does not display pro-vitamin A activities but it is a remarkable antioxidant and a single oxygen scavenger due to its unsaturated nature (Rao and Rao 2007). In the US, more than 80 of consumed lycopene are harnessed from products of tomato. Other sources like papaya, guava, apricots, watermelon and pink grapefruits are also considered as a dietary source. The lycopene content available for consumption in tomato products is almost equivalent to the quantity of β-carotene in tomato products (Paiva and Russell 1999). Nevertheless, lycopene among other dietary carotenoids has the highest antioxidant role (Rao and Agarwal 1999). The bioavailability of lycopene is enhanced by food processing and cooking (Gärtner et al. 1997). Also, food processing and cooking enhances the accessibility of lipophilic compounds which are required for lipid micelles formation including bile acids and dietary lipids (Stahl and Sies 2007). Processing of tomato products enhances the uptake of lycopene after ingestion (Gärtner et al. 1997). Additionally, other carotenoids like β-carotene other than lycopene have displayed antioxidant properties in *in vitro* and *in vivo* models. However, the absorption of carotenoids by animals as well as humans is limited, thereby limiting studying the antioxidant effects of carotenoids *in vivo* models (Paiva and Russell 1999). It was reported that α-carotene and lycopene have higher antioxidant function than β-carotene (Nishino et al. 2002). Furthermore, combination of other potent antioxidants like tocopherols (vitamin E) with carotenoids elevates their antioxidant role against free reactive species (Jaswir et al. 2011).

20.5.9 Pro-vitamin A Activity

About 50 of the 700 carotenoids that exist in nature have activity of provitamin A (Rodriguez-Amaya 1997; Okada et al. 2008). In humans, only three among them are the most potent precursors of vitamin A. these are β-cryptoxanthin, α-carotene and β-carotene (Thane and Reddy 1997; Park et al. 2009; Carrillo-Lopez et al. 2010). In the body, they are normally converted into retinol or vitamin A (Zeb and Mehmood 2004). Among the carotenoids containing foods, vegetables and fruits, β-carotene is the main provitamin A component (Imamura et al. 2006). Serious eye disorders like night blindness are prevented by β-carotene, the precursor of vitamin A (Takahashi et al. 2006). Additionally, carotenoids found in food are usually well digested to release these carotenoids from the matrix of food in order to act physiologically as a vitamin A (Carrillo-Lopez et al. 2010). Most of the carotenoids that serve as provitamin A must have the structure of β-ionone. In animals, they act as regulators of process related to photo-energy by trapping energy from light (Stahl and Sies 2005). Carotenoids like α-carotene, lycopene, β-carotene, lutein and β-cryptoxanthin are seen in plasma of human (Romanchik et al. 1995). From these carotenoids, β-carotene, β-cryptoxanthin and α-carotene are regarded as provitamin A carotenoids while others like lutein and lycopene are regarded as non-provitamin A carotenoids. In addition to the presence of β-ionone ring as a credential for provitamin A activity, proper position and number of methyl groups in the polyene chain is also required (Wirtz et al. 2001). The bioconversion of carotenoids involve the action of a cleavage enzyme called mono or dioxygenase. This enzyme breaks the double bonds in a polyene link into two retinal molecules. These molecules are then metabolized to other derivatives such as retinoic acids which are involved in the promotion of visual action, embryonic development for pregnant mothers, differentiation of epithelial cells, and body growth (Handelman 2001). But the requirement for vitamin A derived from carotenoids is minutes which is about 200 μg/day for a healthy adult a higher dose is toxic in human. It was reported that in low income nations, about 80% of vitamin A are derived from provitamin A carotenoids (Stahl and Sies 2003).

20.5.10 Cardio-Protective Activity

Oxidative stress and consistent severe low state of inflammation in the cardiovascular system, mainly leads to the formation of cardiovascular disorders. Low density lipoproteins cholesterol (LDLc) that were modified via oxidative stress are involved in the initial formation and progression of atherosclerosis, which finally leads to coronary heart disorders. Normally, atherosclerosis may be as a result of the production of foam cells serving as a source of reactive free radicals that result to LDLc oxidation. Hence, LDLc oxidation protection by antioxidants may prevents coronary heart diseases in humans. Putting into consideration that lycopene and β-carotene are majorly transported in LDLc, they are in a main position exhibits LDLc protection from oxidation (Sesso et al. 2003). An evidence based-population study is seen in the EURAMIC study, which indicated the relationship between acute myocardial infarction and adipose tissue antioxidant state from about 10 different countries in Europe in more than 1500 subjects (Riccioni et al. 2016). In their studies, a dose-response relationship between risk of myocardial infarction and adipose tissue lycopene was observed. Also, they indicated that lycopene clearly exhibited cardio-protective properties than observed in the result of β-carotene. Moreover, individuals with maximum stores of polyunsaturated fat showed highest protective role of lycopene (Rissanen et al. 2002). Thus, lycopene is directly associated to a decreased incidences of stroke, myocardial infarction, and lowered carotid intima-media thickness as well as, inversely related to abdominal aorta calcified plaques (Rao and Rao 2007). In a similar study carried on Swedish and Lithuanian population, it was reported that high cardiovascular risk and related mortality due to coronary heart disease are related to lower level of lycopene (Kaliora et al. 2006). Also, Lycopene prevents and inhibits cardiovascular diseases by decreasing total cholesterol concentration in the serum (Kaliora et al. 2006). Furthermore, the anti-atherogenic action of lycopene is related to anti-inflammatory functions, minor adhesion molecules expression, improved lipid homeostasis, interaction between endothelial cells and monocytes (Libby 2002). This is achieved by preventing the secretion of IL-1, which is the major factor in the process of inflammation, stimulating the production of other pro-inflammatory markers and the development of initial atherosclerotic plaques (Hu et al. 2008). Additionally, the level of nitric oxide is influence by lycopene supplying to vasodilation, thus, inhibiting the development of atherosclerosis and related disorders (Dwyer et al. 2004). In Los Angeles and Beijing atherosclerosis study, the degree of atherosclerosis is inversely related to lutein. It was further stated that zeaxanthin, which is inversely associated with pulse wave velocity, right common carotid artery and elastic modulus, may inhibits initial stage of atherosclerosis (Hozawa et al. 2009). Combined treatment of dyslipidaemia with drugs and 9-cis-β-carotene stimulates the high density lipoprotein increase in apolipoprotein of human (Hubacek and Bobkova 2006). Also, high level of β-carotene is related to elevated level of cGMP and bioavailability of nitric oxide which stimulate the down regulation of nuclear factor kappa B (NF-κB) dependent adhesion factors in cells of endothelium. The regulatory enzyme in the biosynthesis of bile acid is inhabited by 9-cis-β-carotene at mRNA level (Akbaraly et al. 2008). Thus, lowering the absorption of cholesterol in the small intestine and also reduced the expression of other genes (ABCG1, ABCG5, and ABCG8) that contributes to the metabolism of cholesterol. The cholesterol transporters are expressed in the hepatocyte and they play an essential part in the excretion of cholesterol, thus lowers atherogenesis. Recently, it study on the intake of vitamin C, D, E, B9, minerals and carotenoids and HbA1c relationship in non-diabetic population has been investigated. It was reported that regular intake of more than 6.8 mg/d carotenoid is inversely related to the level of HbA1c over a period of 10 years (Khaw et al. 2004). The present of carotenoid in plasma after

9 years remarkably reduces the risk of dysglycemia. The HbA1c reducing ability of carotenoids may be due to their antioxidant action, and this is nearly associated with glycosylation of protein (Riccioni et al. 2016).

20.5.11 Hepatoprotective Activity

The liver is a very important organ in the body with broad biological functions including production of digestive chemicals, detoxification, glucose homeostasis, protein synthesis. Furthermore, it plays an essential function in the biosynthesis of plasma protein, production of hormones and metabolism of stored glycogen (Sugiura 2013). In regards to carbohydrate metabolism, it is the main region of insulin clearance, and glycogen breakdown in the liver leads to increase in glucose production by the hepatocyte (Michael et al. 2000). Severe liver diseases is a global health problem and it is characterised by fibrotic and inflammatory process that causes continuous transformation from severe hepatitis to cirrhosis and then to cancer of liver. Chronic liver disorders is usually caused by type-2 diabetes, insulin resistance, abnormal metabolism of carbohydrate and lipid, virus, alcohol and xenobiotic (Loguercio et al. 2001). Consistently, oxidative stress plays a vital role in pathogenesis of hepatic injuries. In the liver, activated inflammatory cells like the Kupffer cells, macrophages as well as the cytochrome p450 of injured hepatic cells and mitochondrial enzymes are main source of ROS (Tilg and Diehl 2000; Parola and Robino 2001). High level of these ROS species do not only degenerate macromolecules like DNA, proteins, carbohydrates, lipids and other biomolecules but also induce transcription of biochemical modulators like cytokines, which regulate cellular and tissue events (Tilg and Diehl 2000; Parola and Robino 2001). Carotenoids, like other micronutrients with antioxidant activity, it may be essential in protecting cells against oxidative stress by effectively attenuating free radicals and singlet oxygen production (Fig. 20.4). Thus preventing continuous disease of the liver (Sugiura 2013). Carotenoids accumulates in the liver, where they are combined into lipoproteins to aid their transportation in extracellular fluid. They inhibits the formation of liver damage. β-carotene, lutein, β-crytoxanthin and lycopene are some examples of carotenoids they possesses antioxidiant properties againgst lipid peroxidation in rat hepatocytes (Chen and Tappel 1996; Whittaker et al. 1996). In a study, it was reported that the level of antioxidant micronutrients such as zinc, selenium and β-carotene were significantly reduced in group consisting of alcoholic patients compared to group consisting of non-alcoholic patients. The groups were further subdivided in respect to liver injury; the β-carotene indicated a continuous reduction in plasma with corresponding increase of liver damage (Ward and Peters 1992). In a similar study, group consisting patients with alcohol abuse with non-alcoholic cirrhosis had a significant decrease in the plasma level of total carotenoids such as β-carotene, zeaxanthin, lutein, cryptoxanthin, α-carotene and lycopene compared to control group (Van de Casteele et al. 2002). Carotenoids may be involve in the antioxidant defense cascade in biological systems when they occur in high levels in the hepatocytes. Thus, this could prevent the development and progression of hepatic damage (Sugiura 2013).

20.6 Nutraceutical Applications

In humans, carotenoids have been associated with the reduction of the risk of developing chronic diseases such as cancer, cardiovascular diseases, high levels of cholesterol, cataract, and macular degeneration as stated above, aside from the pro-vitamin A activity of some of these compounds. Recently, consumers are aware of their association among diet, health and the prevention of diseases. As a result, bioactive compounds, such as antioxidants, peptides, carbohydrates and lipids, present in food have become important for human nutrition and the reason for the development of functional foods and nutraceuticals in the food industry.

The term "nutraceutical" was coined by combining two terms, "nutrition" and "pharmaceutical", in 1989; nutraceuticals are defined as "a food (or a part of food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (Brower 1998). When the functional food promotes the prevention and/or treatment of a disease(s) and/or a disorder(s) other than anemia, it is termed "nutraceutical" (Trottier et al. 2010). It is important to note that functional foods may not be a universal remedy for poor health habits. Health benefits and novel markets in the food industry may be obtained by incorporating carotenoids into foods that do not contain high amounts of these natural pigments; also, when added to food, the bioavailability of carotenoids is improved compared to when they are consumed from their natural sources (Boon et al. 2010). Fruits and vegetables constitute the major sources of carotenoid in human diet. About 40 are present in a typical human diet (Rao and Rao 2007). Although, carotenoids are present in many common human foods, deeply pigmented fruits, juices and vegetables constitute the major dietary sources with yellow-orange vegetables and fruits providing most of the α-carotene and β-carotene, orange fruits providing α-cryptoxanthin, dark green vegetables providing lutein and tomatoes and tomato products lycopene.

Lutein is one of the two major components of the macular pigment of the retina and is present at a high concentration in the human serum (Naziri et al. 2014). Lutein is a member of the xanthophyll group, a subgroup of the carotenoid family and usually coexists with zeaxanthin. Lutein and zeaxanthin are widely distributed in brightly colored fruits and dark green vegetables (Granado et al. 2003; Arunkumar et al. 2013). Since the human body cannot synthesize lutein by its own, its requirement needs to be fulfilled from dietary sources such as dark green leafy vegetables like spinach, beans, and kale, vegetables like maize, and lutein containing fruits like kiwi fruit, grapes, oranges, etc. Natural lutein could be used as nutraceutical or nutritional supplement (Mitri et al. 2011). It shields the skin from damage and prevents LDL cholesterol by inhibition of oxidation. It delays lung aging and combats arthritis. It is also used as a characteristic yellowish-orange colored pigment for improving food and beverage color (Bartlett and Eperjesi 2008). Low lutein levels in plasma have also been associated with an increased tendency to suffer from myocardial infarction (Street et al. 1994), while a high intake of lutein has been inversely related with the risk of stroke (Ascherio et al. 1999).

Lutein offers a wide range of uses in human health and can serve as an ideal candidate for "nanofood," which may increase the bioavailability and photostability of lutein. Traditionally in food technology, encapsulation techniques such as spray drying and inclusion complexation are widely used to improve stability to oxidation and to increase the dissolution rate in water of the active compound (Santos and Meireles 2010).

Among carotenoids, beta carotene is the most commonly found substance in dietary supplements. Foods rich in β-carotene are green leafy vegetables, orange root vegetables, and yellow or orange fruits. Studies have found out that majority of the vitamin A in our diet comes from carotenoids like β-Carotene. β-carotene can also be manufactured as a synthetic compound in laboratories or also can be isolated from different fungi or algal sources. β-Carotene acts as a precursor for the production of vitamin A in our bodies. Thus it is also referred to as provitamin A. It protects cells from damage by inhibiting free radical and singlet oxygen-induced lipid peroxidation (Evans and Johnson 2010). It also has photo-protective properties, which increase the minimal erythema dose (MED), and protects against sunburn development and photosuppression of the immune system (Fiedor and Burda 2014; Grether-Beck et al. 2017).

Scleroderma is a condition characterized by a hardened skin. The condition also affects other connective tissues in the body. It is reported that this condition might arise when the levels of β-carotene in the body diminishes below the required quantity. Researchers pointed out that β-carotene products can help people with this condition. β-carotene has been also studied for treating sun sensitivity. This condition called erythropoietic protoporphyria, causes a painful severe sensitivity to sunlight. However, consuming large amounts of β-carotene will protect people with erythropoietic photoporphyria.

20.7 Conclusion

The pharmacological activities of various carotenoids have been shown to exhibit great beneficial roles in humans for the prevention and management of chronic diseases, including cardiovascular disease, atherosclerosis, cancer, diabetes, hypertension, oxidative stress, male fertility, neurodegenerative disorders. Thus, they are incorporated in food and drinks to serve as nutraceuticals and nutritional supplements.

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Chapter 21
Health Benefits of Organosulfur Compounds

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Abbreviation

COX2	Cyclooxygenase-2
CRDH	Cohen-Rosenthal Diabetic Hypertensive
DADS	Diallyl disulfide
DAS	Diallyl sulphide
DASO	Diallyl sulfoxide
DASO2	Diallyl-sulfone
DATS	Diallyl trisulfide
EAA	Essential amino acid
I3C	Indole-3-carbinol
iNOS	Inducible nitric oxide synthase
NAC	*N*-acetyl cysteine
NO	Nitric oxide
OSC	Organosulfur compound
PGE_2	Prostaglandin E_2

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SAC	s-allyl cysteine
SAMC	*s*-allyl mercapto cysteine
SEC	*S*-ethylcysteine
SFN	Sulforaphane
SMCS	*s*-methyl cysteine sulfoxide
SPC	*s*-propyl cysteine

21.1 Introduction

Organosulfur compounds (OSCs) are natural compounds that contains sulfur. They are unique because of their unsavoury or foul smelling odours, with few exceptions. This class of compound contains most of the Earth's sweetest compounds derivatives of sulfur, e.g. saccharin, a benzoic sulfimide artificial sweetener which is almost 400 times as sweet as sucrose. OSCs are present naturally in plants and animals. They are indispensable to life because they help in the prevention and treatment of several life-threatening diseases such as cancer, diabetes, cardiovascular

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diseases, neurodegenerative disorders, viral, bacterial and fungal infections. The natural world has abundant of OSCs. Among the 20 general amino acids, two (methionine and cysteine) are OSCs, while the antibiotics sulfa and penicillin medicines both have sulfur (Block 1978).

The OSCs can be categorized based on the presence of sulfur-holding functional groups; some of them are allicin, sulfanilamide, methionine, cysteine, lipoic acid, diphenyl disulfide, penicillin, dibenzothiophene etc. Natural sulfur compounds comprise a subcategory of natural substances that hold sulfur which are established in a variety of forms inside the bodies of every living organisms, for example, the essential amino acid (EAA), cysteine and methionine, glutathione, proteins, and a range of hormones, coenzymes, enzymes, and vitamins (Hoefgen and Hesse 2007). Cephalosporin and penicillin are life rescuing drugs, obtained from fungus. Gliotoxin is a sulfur holding mycotoxin made by a number of fungi groups under study as an antiviral agent. Composites like ajoene and allicin are accountable for the unpleasant smell of garlic, and lenthionine provides the taste to shiitake mushrooms. Evaporative organosulfur composites also gives slight flavour features to nuts, cheese, chocolate, wine, coffee, tropical fruit and flavours (Duan et al. 2012; Qian et al. 2011).

Vegetable that fits into the group Allium is really famous for their different smell and flavours, for which a variety of organosulfur composites are liable (Brewster 2008). Powdered extracts of garlic also present huge differences in their main organosulfur compounds along with their hunting activity and antioxidant power, based on their extraction technique and the extraction states (Kimbaris et al. 2006). Alteration and storage situations of garlic bulbs are significant factors that can have influence on composition of garlic's sulfur compound, as the majority of the evaporative compounds are discharged after the cutting of garlic and the beginning of alliinase stimulation (de Queiroz et al. 2014). Antibiotic outcomes of Allium species in opposition to microbes, fungi, viruses, and parasites have been explained in numerous studies until now. Unstable thiosulfinates of *Allium sativum* and *Allium cepa* has antimicrobial activities (Harris et al. 2001). Allicin is effective against a larger spectrum of bacteria in contrast to common antibiotics (Borlinghaus Borlinghaus et al. 2014). The group of Allium comprises around 500 species and the most extensively used are onions, garlic, leeks, chives, and shallots. Garlic is broadly farmed and eaten globally, and its favourable consequences have been recognized for thousands of years. It has been regarded as to enhance prolonged existence and to bestow endurance and physical power, and it has been utilized practically as an antiseptic, antipyretic, anthelmintic, antibacterial, and analgesic. The scientific society has currently turned out to be curious in the medicinal properties of Allium vegetables and their chemical components, predominantly with respect to their outcomes on the cardiovascular system and in anti-carcinogenic effects. Garlic holds carbohydrates, fiber, water, proteins, and fat in addition to EAA, vitamins, and minerals. When garlic is sliced, cut into pieces with knife, or compressed, the clove's covering is interrupted and S-allylcysteine sulfoxide is converted enzymatically into allicin by means of alliinase (Block 1985).

21.2 Garlic-as Major Source of OSC

Blackwood (2000) described that normal clove of garlic weighs between 3–6 g and has an average of 1 g of carbohydrates, 0.2 g of protein, 0.05 g of fibre, 0.01 g of fat and vitamins A, B1, B2, B3 and C. The Vitamin B1 is merged with the allicin and known as allithiamine which is effortlessly taken up by the intestine. Allicin does not subsist in garlic till it is mashed or cut (Blackwood and Fulder 1987). Damage to the bulb of the garlic stimulates the allinase enzyme (Stoll and Seebeck 1951) which metabolizes alliin to allicin (Block 1985). These Organosulfur compounds found in garlic have a number of properties that produce beneficial effects to life including antioxidant, anti-cancer, anti-inflammatory, anti-bacterial, anti-hypertensive, anti-hyperlipidemic, and anti-diabetic properties amongst others.

OSCs extracted and isolated from garlic are commonly studied in terms of their *in vivo* antioxidant activity (Yin and Cheng 2003). These are diallyl sulfide (DAS), diallyl disulfide (DADS), *S*-ethylcysteine (SEC), and *N*-acetyl cysteine (NAC). These compounds were evaluated in terms of its non-enzymatic antioxidant activity in a liposome system (Yin et al. 2002). It was found out that antioxidant protection can be best derived when these compounds are combined with α-tocopherol than α-tocopherol alone. Moreover, the antioxidant activity of these compounds was found to diminish with increasing temperature and pH except for diallyl sulfide and diallyl disulfide. This is of importance since the data on enzymatic antioxidant activity supports human and animal application for health consideration but not in application for food systems (Yin et al. 2002). Moreover, determining non-enzymatic antioxidant activity at low pH will be useful in a variety of application in food systems.

Other OSCs from different classes derived from garlic, like allyl cysteine, allicin, alliin, and allyl disulfide, were also examined in terms of their antioxidant properties. Alliin was found to scavenge super oxides while both allyl cysteine and allyl disulfide did not exhibit scavenging activity against superoxide (Chung 2006). Furthermore, allicin was also found to prevent the production of superoxide by xanthine oxidase system through thiol exchange mechanism. Allicin did not exhibit scavenging activity against hydroxyl radicals while alliin, allyl disulfide, and allyl cysteine did (Chung 2006). This finding is contrary to another study where allicin was found to be the main reason for the hydroxyl scavenging activity of garlic extract (Kourounakis and Rekka 1991). Antioxidant properties were also noted in the organo-sulfur-rich essential oils extracted from *Drypetes gossweileri* barks, *Pentadiplandra brazzeana* roots, and *A. cepa* and *A. sativum* bulbs (Ndoye et al. 2016).

Exogenous antioxidant activity of garlic-derived organosulfur, DAS, DADS, SEC, and NAC were also determined in ground beef meat and were found to significantly delay both oxymyoglobin and lipid oxidations in membrane phospholipids (Yin and Cheng 2003). These compounds possess potent protective effect towards biological membranes against lipid peroxidation both *in vitro* and *in vivo* (Borek 2001; Awazu and Horie 2008). The effect of cooking garlic and its antioxidant properties is still a subject of debate until now. In one study an increased antioxidant activity was found when garlic was boiled at 100 °C for 30 min (Shobana and Akhilender Naidu 2000) while in another study increased beneficial effects were found after heating (Benkeblia 2004). In another study, the antioxidants present in garlic were found to be thermally unstable (Lai and Roy 2012) and that allicin formation was prevented when the enzyme allinase is inactivated by heat (Banerjee et al. 2003; Schäfer and Kaschula 2014). Although OSCs are known to be antioxidants, their property is believed to be brought about and mediated indirectly through the sulfane-sulfur derivatives of these compounds (Colín-González et al. 2012; Toohey and Cooper 2014). Several mechanisms of these indirect effects were thoroughly reviewed by Goncharov et al. (2016).

21.3 Commonly Known Organosulfur Compounds

21.3.1 Allicin

Allicin is an organic compound, also known as allyl-2-propenethiosulfinate or diallyl thiosulfinate or S-allyl cysteine sulfoxide. It is a sulfur-containing volatile agent generally found in plants such as wild garlic (*Allium ursinum* L.), field garlic (*A. vineale* L.), elephant garlic (*A. ampeloprasum* L.), white garlic (*A. sativum* L.) and alpine leek (*A. victorialis* L.). It is one of the major organosulfur bioactive agents found in *Allium* plant species and is accountable for the pungent taste and smell (Salehi et al. 2019). Allicin is a predominant thiosulfinate compound typically responsible for 70% (w/w) of the entire thiosulfinates. It is a colourless oil compound with negligible polarity in water and not found in intact garlic clove, rather, it is naturally formed alongside with ammonia and pyruvate from S-allyl-L-cysteine sulfoxide (alliin precursor). It is an odourless precursor with a stable, non-protein amino acid via the alliin lyase (alliinase, α, β-eliminating endogenous lyase from *Allium* spp., EC 4.4.1.4) enzyme activity. It is noteworthy that the maceration or pulverisation of garlic cloves will lead to this type of enzyme activity (Marchese et al. 2016). Allicin was first reported to exhibit biological activity in 1944 by John Hays Bailey and Chester John Cavallito. Hence, decades of substantial research focused on exploring their biological properties began (Josling 2007). The extensive research has provided evidence that allicin possesses a wide range of biological activities such as anti-bacterial, anti-diabetic, anti-cancer, anti-angiogenesis and anti-hyperlipidemic effects (Chan et al. 2013; Chu et al. 2013; Fujisawa et al. 2009).

21.3.2 S-Allyl Cysteine

S-allyl cysteine (SAC) derived from garlic was found to significantly reduce the level of blood glucose in rats (Saravanan et al. 2009). The hypoglycemic effect of SAC was attributed to the sulfur compound by stimulating directly or indirectly the insulin secretion (Carson 1987; Augusti and Sheela 1996). Moreover, it was also suggested that this compound improves the utilization of glucose thus significantly decreasing blood glucose levels (Saravanan et al. 2009). SAC is also attributed to be responsible for activating the synthesis of thyroid hormones circulating in the blood which is responsible for triggering the synthesis of insulin (Saravanan and Ponmurugan 2012).

21.3.3 Sulforaphane

Sulforaphane from broccoli sprouts was also noted to prevent diabetes-induced fibrosis and to be an effective supplementary treatment for diabetes and its complications (Bahadoran et al. 2013). SMCS isolated from onions were also reported to possess an anti-hyperglycemic effect by stimulating utilization of glucose and production of other antioxidant enzymes (Kumari and Augusti 2002). Similar results were also observed in alloxan-induced diabetic rats where SMCS possessed hypoglycemic effects by triggering insulin secretion (El-Demerdash et al. 2005).

21.3.4 Cysteine

Cysteine is a sulfur-containing amino acid in plants that is present in the conjugated forms such as S-allyl cysteine, S-allyl mercaptocysteine, N-acetyl cysteine and S-ethyl cysteine (Poojary et al. 2017). Among conjugated cysteine forms, S-allyl cysteine is found in plants of Allium species such as onions (*Allium cepa* L.), garlic (*Allium sativum* L.), shallots (*Allium ascalonicum*), leeks (*Allium ampeloprasum* L.) and chives (*Allium schoenoprasumand* L.). Recently, it was stated that S-allyl cysteine was responsible for the anti-diabetic property of onions by stimulating the secretion of insulin from the pancreatic β-cells (Sabiu et al. 2019). Also, it has been reported in various literatures that S-methyl cysteine sulfoxide is accountable for the anti-hyperglycemic and antioxidant activity of onions (El-Demerdash et al. 2005; Kumari and Augusti 2002; Tamura et al. 2007). Likewise, S-methylcysteine sulfoxide was proven to stimulate the secretion of insulin (Augusti and Sheela 1996), while S-allylcysteine exhibited anti-diabetic effect via amelioration of free radicals and pro-oxidant species as well as stimulation of Nrf2 factors (Colín-González et al. 2012).

21.3.5 Sulfonylureas

Sulfonylureas are organo-sulfur-containing antibiotic derivatives that is orally administered and widely used to manage type 2 diabetes (DiStefano and Watanabe 2010). This substance increases secretion of insulin endogenously from the pancreas by binding with the plasma membrane receptor coupled to an ATP-sensitive channel located in the cell membrane of pancreatic cells (Lacroix and Li-Chan 2014). This process triggers the exocytosis of insulin from pancreatic β-cells due to calcium influx (Cheng and Fantus 2005).

21.3.6 Methionine

Methionine is an exclusive and essential amino acid with sulfur, that are predominantly found in proteins of meat, and are normally converted into homocysteine through transmethylation reaction (Finkelstein 2000). Further, it is found in plants such as soybeans, peanuts, sesame seeds, hemp seeds, kidney beans etc. It acts as a precursor for the production of glutathione which prevent the cells from oxidative injury and plays an essential role in the antioxidant system of the body (Caylak et al. 2008; Caylak et al. 2007). Among the biological activity of the sulphur-containing amino acid, it was reported that methionine conjugated with chromium reduces the index of insulin resistance, triglyceride, glucose and fasting insulin in insulin-resistant rat model (Ghiasi et al. 2010). In recent times, it was demonstrated that methionine conjugated with chromium administered for 15 days ameliorated blood glucose, altered lipid metabolism and provided hepatoprotective effects in alloxan-induced diabetic mice (Tang et al. 2015).

21.3.7 Lipoic Acid

Lipoic acid is an organosulfur compound with antioxidant properties. Plant species such as spinach, broccoli and potatoes possess a high concentration of alpha-lipoic acid (Karaarslan et al. 2013). Several reports showed that lipoic acids possess enhanced anti-diabetic property (Nagamatsu et al. 1995; Packer 1994; Reljanovic et al. 1999; Ruhnau et al. 1999). Karkabounas et al. (2018) revealed that daily supplementation of alpha-lipoic acid can reduce the concentration of glucose by elevating insulin secretion from the pancreas in type 2 diabetic patients via randomized double-blind study. Furthermore, Khabbazi et al. (2012) evaluated the oxidative stress, inflammation and serum lipid profile in hemodialysis patients after supplementation of alpha-lipoic acid and demonstrated that the lipoic acid supplementation reduced highly sensitive C-reactive protein, which is a responsible factor for cardiovascular and diabetic complications in hemodialysis patients. Furthermore, another study showed that lipoic acid supplementation reduced morphological alterations and elevated the regeneration process to prevent atrophy in type 1 diabetic rat skeletal muscle cells (Jurisic-Erzen et al. 2018). Oral alpha-lipoic acid supplementation has also been shown to reduce the glycemic status as well as oxidative stress-related deterioration in type 2 diabetes patients (Porasuphatana et al. 2012). Likewise, Sadyakova and Nazhmutdinova (2009) emphasized that the lipoic acid supplementation helps in improving diastolic function of the left ventricle and myocardial electrophysiological properties. Similarly, the effect of lipoic acid supplementation was evaluated in diabetic neuropathy and its association in early-stage diabetic foot syndrome patients. The study revealed that the lipoic acid possesses anti-neuropathic effect and leads to decrement in distal symmetric sensorimotor polyneuropathy (Volchegorskiĭ et al. 2008). More studies have also reported the preventive, curative and antioxidant effects of lipoic acid with respect to neuropathies and cardiomyopathies (Jamor et al. 2019; Liu et al. 2007; Mazloom and Ansar 2009; Papazafeiropoulou et al. 2019).

21.3.8 Other Organosulfur Compounds Possessing Anti-Diabetic Activity

Organosulfur compounds such as perfluorooctane sulfonic acid, penicillin, cephalosporin and sulfanilamide are currently being recognized to possess certain anti-diabetic property. Penicillin is a well-known antibiotic that is also an organosulfur compound. These organosulfur antibiotics are highly beneficial in the treatment of diabetic foot infections and to prevent further spread of disease-causing microbes (Lipsky 2016). Garg et al. (2017) reported that the anaerobic bacterial infections via species including *Bacteroides*, *Peptococcus*, *Eubacterium*, *Prevotella* species and *Peptostreptococcus anaerobius* in diabetic foot ulcer can be treated using penicillin as an antibiotic compared to metronidazole (Garg et al. 2017).

Cephalosporin is another organosulfur compound with antimicrobial property that are extensively studied as a better antibiotic agent for diabetic foot ulcer treatment (Amjad et al. 2017; Rastogi et al. 2017; Xie et al. 2017). In addition, Petrov et al. (2016) revealed that cephalosporin helps in the treatment of calculous pyelonephritis with their anti-bacterial effects and prevents their further complications due to type 2 diabetes. Perfluorooctane sulfonic acid belongs to the perfluoroalkyl substances and is secreted in diabetes patients. Lind et al. (Lind et al. 2014) reported that the detection of these compounds can prevent human islet function impairment by fatty acid secretion in diabetic patients. Cardenas et al. (2017) demonstrated that the determination of plasma per and polyfluoroalkyl substance concentration including perfluorooctane sulfonic acid along with glycemic indicator associations can be used to detect diabetes among high-risk adults in a diabetes prevention program trial. Sulfanilamide is a unique organosulfur compound with a structure similar to p-aminobenzoic acid with antibacterial efficacy (Liao et al. 2016). These antibiotic

Organosulfur compounds were reported to be beneficial as antibiotics for the treatment of diabetic foot ulcer, similar to penicillin and cephalosporin (Kandhare et al. 2014). However, certain reports states that both perfluorooctane sulfonic acid (Sant et al. 2017) and sulfanilamide (Yaribeygi et al. 2018) are toxic towards diabetic cells. Extensive research is thus required to evaluate the toxic mechanism of these compounds towards diabetic cells and to predict their toxic dosages and concentrations to use them as potential therapeutic agents against diabetes and diabetes-related complications.

21.4 Therapeutic Potential of Organosulfur Compounds

21.4.1 Anticancer Properties

Substantial literature has cited the cancer-reducing activities of organosulfur compounds including the arrest of cell cycle, apoptosis, and cell differentiation (Xiao et al. 2004, 2005; Herman-Antosiewicz and Singh 2005; Lanzotti et al. 2014). The cancer prevention activity of OSCs is brought about by its neutralizing activity against carcinogens (Ariga and Seki 2006). Moreover, the ability of organosulfurs to inhibit cancer cell proliferation is tightly correlated with the sulfur chain length (Cerella et al. 2011). OSCs were noted to possess inhibiting activity against various kinds of tumours like that of the prostate (Chandra-Kuntal and Singh 2010), pancreas (Ma 2014), skin (Wang et al. 2012), colon (Lai et al. 2012), small intestine, stomach (Azarenko et al. 2014), and bladder (Munday et al. 2008).

OSCs derived from garlic like allyl sulfides are known to possess anti-carcinogenic activity (Guyonnet et al. 2004; Milner 2009). OSCs from garlic are known to possess both therapeutic and preventive properties against cancer (Schäfer and Kaschula 2014). The integrity of sulfides in garlic should be ensured by avoiding unnecessary deterioration by inappropriate cooking and processing to avoid loss of its anticancer activity (Ariga and Seki 2006). Liver carcinogenesis in rats was also found to be inhibited by the administration of sulfur substances derived from garlic (Herman-Antosiewicz and Singh 2005). DADS showed a growth-inhibiting effect against human neoplastic cells (Sundaram and Milner 1996). Moreover, DADS also showed inhibition activity against the growth of *in vitro* human colon, lung, and skin tumour cells and found to be much stronger than isomolar quantities of *S*-(+)-Alk(en)yl-L-cysteines (Ariga and Seki 2006).

In another study, DADS were also established to have synergistic activity with a cancer suppressor eicosapentaenoic acid (Tsubura et al. 2011). Diallyl sulfoxide (DASO) and diallyl-sulfone (DASO2) were also found to possess a reductive activity against diverse, chemically-induced tumours in animal models (Yang et al. 2001). Furthermore, OSCs from garlic was found to inhibit proliferation and induce cell death in both culture and xenograft models of cancer cells (Shukla and Kalra 2007; Powolny and Singh 2008). DAS, DADS, and DATS derived from garlic also exhibited anticancer activities (Lai et al. 2012). Moreover, DATS was also found to induce apoptosis in human leukaemia cells by generation of ROS (Choi and Park 2012). Other OSCs derived from garlic and their mechanism against cancer is well reviewed by Omar and Al-Wabel (2010).

Ajoene, a garlic-derived organosulfur produced from allicin under specific conditions, also possess inhibiting activity against neoplastic growth (Apitz-Castro et al. 1992; Ariga and Seki 2006). This compound was found to exhibit antiproliferative effects against human tumour cell lines, blocking activity in early stages of mitosis of marsupial kidney cells, and tumour inhibition against sarcoma and hepatocarcinoma (Li 2002).

Sulforaphane (SFN) and other analogues like indole-3-carbinol (I3C) from *Brassica* genus were also found to possess anticancer activity (Tin et al. 2014; Gali-Muhtasib et al. 2015). SFN was found to exhibit anticancer activity against prostate cancer cells in humans by apoptosis via induction of the autophagy defense mechanism (Herman-Antosiewicz et al. 2006). Anticancer activity of SFN was also found against four cell lines of breast cancer by inhibiting its viability (Pawlik et al. 2013). In another study, SFN was found to stabilize microtubules in breast cancer cell lines thus suppress proliferation and mitosis (Azarenko et al. 2008). Meanwhile, SFN was found to amplify the oxaliplatin-induced cell growth inhibitions in colorectal cancer cells by inducing them in different apoptotic events (Kaminski et al. 2011). SFN stimulates apoptosis of tumour cells thus serves as a lead candidate for the development of multi-target strategy for the treatment of various diseases (Goncharov et al. 2016).

21.4.2 Anti-Inflammatory Properties

Anti-inflammatory properties were also found in OSCs. Allicin was found to inhibit production of cytokine messengers known to be a pro-inflammatory agent in a study of inflammatory bowel disease (Lang 2004). In another study, a compound derived from allicin was found to possess potential starting point as an anti-inflammatory drug with lesser side effects (Krishna and Yadav 2012). Four OSCs, *Z* and *E*-ajoene and ajoene oxidative sulfonyl derivatives were also isolated and found to possess anti-inflammatory properties (Lee et al. 2012). These compounds act as anti-inflammatory agents by inhibiting formation of nitric oxide and prostaglandin E_2 (PGE_2) and are considered to be a promising therapeutic agent for the treatment of diseases related to inflammation (Lee et al. 2012). Furthermore, ajoene was also found to prevent activity of cyclooxygenase-2 (COX2) and release of PGE_2 (Dirsch and Vollmar 2001). DAS was also found to reduce expression COX2 and release of PGE_2 (Chang and Chen 2005). Diallyl trisulfide (DATS), a garlic OSC, possess potential anti-inflammatory substance by inhibiting the mediators and cytokines in *in vitro* and *in vivo* models (You et al. 2013). Moreover, this compound also showed capability of reducing lipopolysaccharides-induced paw edema in mouse models.

The inhibition of inflammatory mediators of DATS, DADS, and DAS was also compared in terms of suppression of the expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). It was believed that the inhibitory effect can be related to the number of S atoms in the OSC (Liu et al. 2006). The order of inhibitory effect was DAS < DADS < DATS. This result was also confirmed when different OSCs were compared in terms of their inhibitory property and DATS was found to be the most effective (Trio et al. 2014).

SFN was also found to be useful as a therapeutic substance for treating inflammatory-related diseases (Chen et al. 2009). These results were observed by inhibiting expression of genes causing inflammation in endothelial cells. SFN was also found to have anti-inflammatory properties as a result of down-regulation of LPS-stimulated iNOS and TNF-α in mouse macrophages (Heiss et al. 2001).

Organosulfur-rich essential oils extracted from different medicinal plants from Cameroon were also noted to possess anti-inflammatory properties using albumin denaturation method (Ndoye et al. 2016). The results supported the medicinal use of different plants against skin disorders and inflammatory-related diseases in Cameroon. DADS found in garlic oil was also evaluated in terms of its activity against airway inflammation and found that it reduced the inflammation of the airway and mucus hypersecretion induced by OVA challenge (Shin et al. 2013).

21.4.3 Anti-Bacterial Properties

OSCs also possess anti-bacterial properties. This group of compounds has become attractive as a subject of anti-bacterial studies due to its unique capability of interrupting bacterial cell walls without harming human cells (Heldreth and Turos 2005). These compounds commonly found in garlic and shallots are most responsible for their anti-bacterial properties (Mikaili et al. 2013). Moreover, the anti-bacterial properties of these spices are believed to be results of the interaction of the sulfur-containing compounds with the thiol groups of microbial enzymes preventing growth microbial communities (Bakri and Douglas 2005).

DATS has also been used in China to treat bacterial infections which created an interest in sulfur-containing compounds (Lun et al. 1994). Patented allicin and other related sulfur-containing compounds showed anti-bacterial properties in blood products inhibiting selected microorganisms and increase shelf life of blood products (Goodrich 2003). Allicin also showed potent *in vitro* antibacterial activity against *E. coli*, *S. aureus*, *S. pyrogenes*, *P. mirabilis*, *P. aeruginosa*, *A. baumanii*, and *K. pneumonie* (Block 1992; Goncharov et al. 2016). Moreover, allicin was found to be the compound responsible for the microbial inhibitory action of essential oil extracted from garlic (Babu et al. 2011). In addition, allicin was reported to possess bactericidal activity against 25 saprophytic and pathogenic bacteria (Lanzotti et al. 2014). Although allicin was noted to possess anti-bacterial properties, the stability of ajoene and other OSCs makes it a better lead compound in crude extracts (Viswanathan et al. 2014).

Other OSCs from garlic-like DADS, DATS, ajoene, DAS, SAMC, and *s*-allyl cysteine (SAC) also possess anti-bacterial properties (Heldreth and Turos 2005). Another patented organosulfur, *N*-sulfonated monocyclic β-lactam also possessed antibacterial activity against *S. aureus* (MRSA) (Turos et al. 2002). Eighteen OSCs from *P. alliacea* L. were evaluated for their anti-bacterial properties and from those, thiosulfinates, trisulfides, and benzyl sulfonic acid were found to be the most bioactive against broad strains of bacteria (Kim et al. 2006). Moreover, the authors also found that fresh samples of this plant prepared by mild procedures showed significantly higher degree of anti-microbial properties compared to those prepared in harsher methods.

21.4.4 Anti-Parasitic Properties

Freshly crushed garlic has been known in ancient times to possess anti-parasitic properties. Moreover, Chinese are known to use alcoholic extracts of garlic cloves to treat diseases in the intestine (Sagdic and Tornuk 2012). Although garlic is commonly used to treat various diseases, studies on anti-parasitic activity of OSCs are limited. In one study, allicin was reported to inhibit growth of some parasites like *G. lamblia*, *L. major*, *L. colosoma*, and *C. fasciculate* (Ankri and Mirelman 1999). In another study, allicin showed strong inhibition of cysteine proteinases and the cytophatic effects of *E. histolytica* (Ankri et al. 1997). DATS also showed anti-parasitic activity against human and animal protozoan parasites (Lun et al. 1994). Low IC_{50} (0.8–5.5 μg/mL) values were found for six protozoans while higher concentrations were needed to inhibit growth of *E. histolytica* (14 μg/mL) and *G. lamblia* (59 μg/mL).

OSCs were also believed to be mainly responsible for the bioactivity against nematode parasites. In a study, the herbal formula of garlic was found to reduce the faecal egg counts of stronglyes and trychostrongylus species in treated sheep animals (Masamha et al. 2010). This anti-helminthic property is believed to be brought about by allicin although other literature suggests that it is the smaller metabolic products, DADS and DAS, which is responsible for this action. Meanwhile, in another literature, allicin was noted to be responsible for the anti-parasitic activity against cryptosporidiosis caused by a parasitic protozoan (Gaafar 2012). Moreover, allicin was also noted to cause morphological changes in male *Schistosoma mansoni* (Lima et al. 2011). In another study, it was also noted to possess anti-parasitic activity against *P. falciparum* and *T. brucei brucei* (Waag et al. 2010). Generally, garlic was suggested as potential treatment against parasitic nematodes in animals and humans (Ayaz et al. 2008).

It was also noted that other OSCs like DADS, DAS, dipropyl sulfide, methyl propyl disulfide, methyl propyl sulfide, allyl methyl sulfide, and dipropyl sulfide showed activity against *G. intestinalis* (Harris et al. 2000). In another study, thiosulfate was found to be a major component in anti-parasitic activity of garlic against immature leech *Limnatis nilotica* (Eftekhari et al. 2012; Bahmani et al. 2013).

Ajoene was also found to be anti-parasitic and cytostatic and this has been attributed partly to the inhibition of key enzymes of the metabolism of antioxidant thiol (Gallwitz et al. 1999).

21.4.5 Anti-Viral Properties

Garlic has been consumed conventionally to manage a number of communicable diseases (Adetumbi and Lau 1983; Fenwick et al. 1985a). The long-established anti-viral utilization of garlic consists of the management of common cold, measles, chickenpox, and influenza (Fenwick et al. 1985b). A small number of validating reports have been issued concerning these traditional anti-viral utilizations. It has been established that garlic extracts had *in vitro* antiviral activity against Herpes Simplex Virus (HSV) type 1 and influenza B virus. Immune-mediating activity of garlic has also been reported in humans and has been stated to improve the activity of natural killer cells (Kandil et al. 1987; Kandil et al. 1990).

21.4.6 Anti-Fungal Properties

Anti-fungal properties were also observed in some OSCs. Allicin was found to possess strong anti-fungal properties against 12 *Candida* spp. (Khodavandi et al. 2010). It was also suggested that the inhibition effect of allicin alone is best read after 24 h and not after 48 h. In another study, it was found that allicin exerts its anti-fungal properties within 2–12 h *in vitro* administration against *Aspergillus* spp. and it prolongs survival and inhibits fungal loading in mice infected with *A. fumigatus* (Shadkchan et al. 2004). Allicin present in essential oils was also found to possess synergistic anti-fungal activity with ketoconazole against species of *Trichophyton* (Pyun and Shin 2006). Allicin was also found to inhibit the production of aflatoxins in *A. parasiticus* (Ankri and Mirelman 1999). In addition, pure allicin was also found to inhibit *Microsporum, Cryptococcus*, *Trichophyton*, *Candida*, and *Epidermophyton* (Yamada and Azuma 1977).

Although various literature suggests the potential *in vitro* anti-fungal and anti-microbial properties are brought about by allicin, *in vivo* studies suggests that the effects are brought about by metabolic products due to the instability of allicin (Lemar et al. 2002). Allyl alcohol, a product of metabolic breakdown of allicin, possess a relatively significant anti-fungal activity against *C. albicans* (Lemar et al. 2005). Moreover, this compound exerts anti-fungal activity by inducing oxidative stress in *C. albicans*. In another study, DADS showed highly effective anti-fungal activity against *C. albicans* by triggering oxidative stress (Lemar et al. 2007). DADS was found to possess inhibition effect against four yeast strains (*C. albicans* 14053#, *C. albicans* 10231#, *C. tropicalis*, and *B. capitatus*) where the higher the concentration of DADS, the stronger the anti-fungal properties exhibited (Avato et al. 2000).

Moreover, it was suggested that the presence of trisulfide bond or an extra sulfur atom reduces the potential anti-fungal activity of allylsulfide since DATS showed a weak or absent inhibition effect.

21.4.7 Anti-Hypertensive, Anti-Hyperlipidemic, and Anti-Atherosclerotic Properties

OSCs are also believed to possess anti-hypertensive properties. In one study, consumption of garlic was found to be responsible for lowering of blood pressure (Qidwai et al. 2000). Moreover, in a more recent study, moderate dose of garlic was shown to be effective in its cardio-protective and anti-hypertensive activities against induced toxicities (Asdaq and Inamdar 2011). Deficiency in sulfur has been believed to play a role in the aetiology of hypertension and can be eased by consumption of organo-sulfurs from garlic (Goncharov et al. 2016). An excellent, exhaustive review on the use of garlic to treat hypercholesterolemia was also presented by Stevinson et al. (Stevinson et al. 2000).

The various biological properties of garlic have been believed to be brought about by the OSCs present in it and especially abundant in the fresh garlic (Duda et al. 2008; Asdaq and Inamdar 2011). It was suggested that this property was brought about by the conversion of allicin upon administration to known antioxidants *s*-allyl cysteine (SAC) and *s*-allyl mercapto cysteine (SAMC). Similar results were also found to verify that SAC significantly reduces lipid peroxidation and increases *in vitro* and *in vivo* antioxidant activities (Gorinstein et al. 2006). Moreover, SAC, SEC, *s*-propyl cysteine (SPC) were found to inhibit biosynthesis of lipids in cultured rat hepatocytes. It was also found out that organosulfurs from garlic were strong agents against LDL oxidation which offer further protection against complications brought about by cardiovascular diseases. In another study, SAC was also found to improve the lipid profile of rats (Zhai et al. 2018). *s*-methyl cysteine sulfoxide (SMCS) was also noted to reduce endogenous lipogenesis and increase catabolism of lipids (Kumari and Augusti 2007).

DADS was also found to enhance palmitate-induced inhibition of biosynthesis of cholesterol in rats (Gebhardt 1995). In another study, DADS analogues were found to reduce total lipids levels by inhibiting HMGR activity *in vitro* and *in vivo* (Rai et al. 2009). In a more recent study, DADS was found to possess hypo-lipidemic activity in diabetic rats (Sambu et al. 2015). DATS, on the other hand, also showed hypo-lipidemic effects by preventing synthesis of fatty acid and cholesterol (Lii et al. 2012). Ajoene was also found to reduce lipid accumulation and stopping adipogenesis and inducing cell death (Ambati et al. 2009).

The ability of garlic to reduce cholesterol levels is believed to be brought about by a metabolite of allicin, allyl mercaptan (AM) which reduces the function of a key enzyme involved in cholesterol synthesis (Cho and Xu 2000). Moreover, this compound was also found to decrease the systolic blood pressure, production of fatty acids, serum cholesterol, and triglycerides in rats fed with diet rich in cholesterol (Abramovitz et al. 1999; Ali et al. 2000). Allicin in garlic and onion was found to inhibit hypertension by eliciting NO-dependent relaxation in pulmonary arteries in rats (Ku et al. 2002; Schwartz et al. 2002; Sakai et al. 2003). Synthetic allicin administered intra-arterially also resulted in mesenteric arterial vasodilation in cats (Mayeux et al. 1988). Several studies also noted the ability of allicin to lower blood pressure in various models (Warshafsky et al. 1993; Eilat et al. 1995; Banerjee and Maulik 2002; Elkayam 2003). Meanwhile, blood pressure-lowering activity of garlic was also attributed to the vasodilation as a result of the liberation of H_2S from alliin (Benavides et al. 2007).

Allicin was also found to play an important role in the anti-atherosclerotic activity of garlic (Jain and Konar 1978). This compound affects atherosclerosis by serving in many mechanisms like in the modification of lipoproteins, and inhibition of uptake in LDL, and degradation of macrophages, and as an antioxidant agent (Gonen et al. 2005). Moreover, this compound was also found to possess anti-hyperlipidemic and hypoglycemic activities (Mathew and Augusti 1973; Sela et al. 2004). SAC was also found to improve glucose homeostasis and sensitivity of insulin (Zhai et al. 2018).

21.4.8 Anti-Diabetic Properties

OSCs including allicin, cysteine, methionine, dibenzothiophene, lipoic acid and sulfur mustard have exhibited enhanced anti-diabetic effects in both *in vitro* and *in vivo* models. Various volatile OSCs from garlic extracts were attributed to the reduction of insulin resistance (Padiya and Banerjee 2013). These are ajoene, allyl mercaptan, allicin, alliin, DAS, DADS, DATS, and SAC. Alliin was noted to contribute to the hypoglycemic activity although no clear mechanism was suggested (Nasim et al. 2011; Zhai et al. 2018).

Allicin has been reported to be the significant compound that is responsible for the anti-diabetic property of garlic species (Salehi et al. 2019). The first anti-diabetic efficacy of allicin was reported by using diabetic rabbits as *in vivo* animal model. The compound ameliorated fasting blood glucose in a dose-dependent fashion and the results revealed that allicin possesses better anti-diabetic efficiency than conventional anti-diabetic drug named tolbutamide (Augusti 1975). Further, the compound was also reported to attenuate diabetes-induced cardiovascular disorders (Huang et al. 2013; Yang Liu et al. 2012) as well as amelioration of type 1 diabetes in a rat model by stimulating insulin secretion (Osman et al. 2012). A recent study also showed allicin attenuated kidney damage in diabetic nephropathy-induced rats (Huang et al. 2017). Also, it was reported that allicin can be conjugated with captopril to form S-allyl-mercapto-captopril (CPSSA). This complex allicin compound exhibited potential to prevent body weight gain, ameliorated blood pressure and reduced blood glucose concentrations in Cohen-Rosenthal Diabetic Hypertensive (CRDH) animal models.

21.4.9 *Other Health Benefits of OSCs*

OSCs also possess other health benefits as suggested by different studies. Allicin together with adenosine was noted to be the most potent constituent of garlic with anti-platelet properties (Agarwal 1996). Ajoene was also found to inhibit the aggregation of platelets in various animals (Jain and Apitz-Castro 1987). This anti-platelet activity was believed to be a result of the suppression of the coagulation system by inhibiting thrombin formation (Mikaili et al. 2013). DATS was also found to possess anti-thrombotic properties against human leukaemia cells via apoptosis by the production of ROS (Choi and Park 2012). DATS was also found to inhibit platelet activity but DADS was found to be more potent (Bordia et al. 1998). Other sulfur-containing compounds from garlic were also noted to be effective in inhibiting platelet aggregation (MacDonald et al. 2004). Other thermochemical forms of allicin were also found to possess anti-thrombotic agents like allyl trisulfides, ajoenes, and dithiins (Nishimura et al. 2000).

Anti-viral properties of OSCs were also noted in various studies. Virucidal activity of OSCs from *A. sativum* were studied and ajoene was found to be the most potent while methyl allyl thiosulfinate was the least potent against several viral strains (Weber et al. 1992). Allicin was also noted to possess anti-viral properties against several viral strains (Ankri and Mirelman 1999) while in another study it was found that this compound protects against common cold virus (Josling 2001). DATS was also found to possess activity against human cytomegalovirus by inhibiting replication and expression viral genes (Zhen et al. 2006).

Neuro-protective properties are also exhibited by other OSCs. The neuro-protective properties of isothiocynate sulforaphane is believed to be brought about by its ability to trigger Nrf2/ARE pathway (Tarozzi et al. 2013). Moreover, this compound was also found to stimulate *in vitro* activity of ubiquitin-proteasome system and also activate protein degradation pathways in both peripheral tissues and the brain (Liu et al. 2014). SAC was also noted to possess multiple mechanisms to prevent the progression of Alzheimer's disease (Chauhan 2006; Ray et al. 2011a, b; Goncharov et al. 2016).

Organosulfurs were also noted to have hepato-protective effects. These compounds activate and increase the GSH-related antioxidant system activity in rat liver offering it protection from oxidants and oxidative stress (Wu et al. 2001; Agarwal et al. 2007). The liver protection property of these compounds is brought about by decreasing the oxidative damage in the liver (Gedik et al. 2005; Yun et al. 2014). These compounds are allicin, allinin, SAC, and DADS.

Other health benefits organosulfurs provide are pharmaceutical effect against arthritis by DADS (Williams et al. 2010) and DAS (Lee et al. 2009). SAC was also noted to possess therapeutic effects against neuro-degenerative diseases including stroke (Atif et al. 2009), ischemia (Ashafaq et al. 2012), Parkinson's (García et al. 2010), and edema formation (Numagami et al. 1996). Some studies on the various sources and benefits of OSCs were presented in Table 21.1.

Table 21.1 Previous studies on Organosulfur compounds from different regions

Sources	Region	References
Allium sativum	China	Shang et al. (2019)
Allium sativum	Korea	Kim et al. (2018)
Allium ampeloprasum	Korea	Kim et al. (2018)
Allium cepa	Korea	Kim et al. (2018)
Allium hookeri	Korea	Kim et al. (2016a, b)
Allium cepa	Malaysia	Zamri and Hamid (2019)
Allium ampeloprasum	Malaysia	Zamri and Hamid (2019)
Allium sativum	China	Liu et al. (2020)
Allium sativum	Argentina	Gonzalez et al. (2009)
Allium sativum	Japan	Ichikawa et al. (2006)
Allium sativum	Korea	Kim et al. (2016a, b)
Allium sativum	Japan	Zhu et al. (2016)
Azadirachta indica	USA	Balandrin et al. (1988)
Petiveria alliacea	USA	Kim et al. (2006)
Lentinula edodes	China	Liu et al. (2015)

21.5 Conclusion

Collectively, sulfur-containing plant-based secondary metabolites are known to have therapeutic efficacy throughout the centuries. Organosulfur compounds are very important part of human regular diet and contributed to health and well-being. Thousands of years ago, beneficial properties and therapeutic applications of OSC have been reported in folk and traditional system of medicine and practices. Clinical trials and animal studies confirmed its traditional claims for the suppression and treatment of various disease and disorders (possible mechanism). The present chapter covers the information on commonly known organosulfur compounds and their therapeutic activities including antiplatelet, immunomodulatory, anti-ageing, anti-inflammatory, antimicrobial, anti-parasitic, anti-hypertensive, anti-hyperlipidemic, anti-atherosclerotic, and antioxidant activities. In view of promising outcomes, additional efforts are required to explore its actual mechanism of action through clinical trials for specific diseases and disorders.

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Chapter 22
Health Benefits of Isoflavones Found Exclusively of Plants of the Fabaceae Family

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22.1 Introduction

The Fabaceae or Leguminosae family commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes a wide range of trees, shrubs and herbaceous plants, perennials or annuals, easily recognized by their fruits (legume) and their compound, stipulated

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leaves. The group, with 730 genera and over 19,400 species, is widely distributed and is the third-largest plant family in terms of number of species behind only to the Orchidaceae and Asteraceae (Judd et al. 2002). The largest genera are Astragalus (over 2400 species), Acacia (over 950 species), Indigofera (around 700 species), Crotalaria (around 700 species), and Mimosa (around 500 species), which contain around 9.4% of all flowering plant species (Magalion and Sanderson 2001). The family itself is the most common family found in tropical rainforests and in dry forests in American and Africa. It has traditionally been divided into three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae (Lewis et al. 2005) and includes almost all the major pulses like mung, urad, pigeon pea, moth bean, cow Pea, adzuki bean, etc. The plant species under Fabaceae family with medicinal importance includes Cassia, Senna, Mimosa, Crotoleria etc. which

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are being extensively used by the therapeutic drug industries, with high demands in the market of herbal medicine.

Recent molecular and morphological evidence supports the fact that the Fabaceae is a single monophyletic family (Lewis et al. 2005). The fact has been supported not only by the degree of interrelation shown by different groups within the family, but also by all the recent phylogenetic studies based on DNA sequences (Kajita et al. 2001). These studies confirm Leguminosae as a monophyletic group with close relation to Polygonaceae, Surianaceae and Quillajaceae families. Many cereals, several fruits and tropical roots of Leguminosae family have been a staple human food for millennia and their use is intertwined with human evolution (Burkart 1987). A number are important agricultural and food plants, including *Glycine max* (soybean), Phaseolus (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (liquorice). A number of species are also weedy pests in different parts of the world, including: *Cytisus scoparius* (broom), *Ulex europaeus* (gorse), *Pueraria lobata* (kudzu), and a number of *Lupinus* species (Sprent 2009; Wojciechowski et al. 2004).

22.2 Isoflavones: Biosynthesis and Mode of Action

Isoflavones are group of secondary metabolite formed by a symbiotic relationship with the rhizobium bacteria and the defence responses of leguminous plants (Yu et al. 2000). Isoflavons are synthesized as part of the phenylpropanoid pathway, the same biosynthetic pathway of flavonoid biosynthesis (Barnes 2010). Phenylalanine converts 4-hydroxycinnamoyl CoA by reaction with malonyl CoA. Chalcone synthase catalyzes the reaction of this intermediate to convert to 4,2,4′,6′-tetrahydrozychalcon (naringenin chalcone) and the combined enzyme reaction of chalcon synthase and chalcone reductase convert this intermediate to 4,2′,4′-trihydrozychalcone (isoliquiritigenin). Then, chalcone isomerase catalyzes the ring closure of the heterocyclic ring to form 7,4′-dihydrozyflavone (liquiritigenin) and 5,7,4′-trihydroxyflavone (naringenin). The B-ring is moved from the 2-position to 3-position by isoflavone synthase. Isoflavone dehydratase removed water to generate the 2,3-double bond in the heterocyclinc ring. The products generated by this reaction were daidzein (7,4′-dihydroxyisoflavone) and genistein (5,7,4′-trihydroxy isoflavone).

According to the xenohormesis theory, plants synthesize phytochemicals to withstand and adapt under stress (Table 22.1). Indeed, isoflavone biosynthesis depend on the environmental conditions in which the plant grows and is stimulated by stress. The stress-induced plant compounds have the ability to upregulate stress adaptive pathways in animals and humans. In the body, modulating pathways mediated by estrogen receptors (ERs) or various key enzymes involved in cellular signalling or metabolism and antioxidant potential exercise the precise biological effects of isoflavones (Cederroth and Nef 2009).

Table 22.1 List of Selected Plants with Reported Isoflavone Content

S/N	Scientific name	Local name	Habit	Part(s) used	Uses	References
1.	*Abrus precatorius* L.	Kuch	Climber	Seed, root	Paralysis, sciatica, stiffness of the shoulder joint, white leprosy, stimulant.	Rahman et al. (2015) and Saxena and Sharma (1999)
2.	*Acacia catechu* (L. f.) Willd	Khair	Tree	Bark	Astringent, anthelmintic, antidysentery, antipyretic, cures itching, inflammations, sore throat, bronchitis, indigestion, ulcers, boils, leukoderma, psoriasis, leprosy and elephantiasis, strengthens the teeth.	Rahman et al. (2015)
3.	*Acacia nilotica* (L.) Willd. ex Delile	Babla	Tree	Leaf, pods, flower	Astringent, tonic to the liver and brain, antipyretic, leukoderma, gonorrhoea, strangury, diarrhoea, cystitis, vaginitis, dysentery, ophthalmia, cough and insanity.	Rahman et al. (2015)
4.	*Albizia lebbek* (L.) Benth	Sirish	Tree	Leaf, seed	Ophthalmia, asthma, astringent, tonic to the brain, gonorrhoea, tubercular glands, leukoderma and leprosy.	Rahman et al. (2015)
5.	*Albizia procera* (Roxb.) Benth	Koroi	Tree	Leaf, bark	Insecticidal, ulcer, worms and scabies.	Rahman et al. (2015) and Rastogi and Mehrotra (1993)
6.	*Bauhinia acuminata* L.	Kanchan	Tree	Leaf, bark	Biliousness, bladder stone, asthma and leprosy.	Rahman et al. (2015) and Yoshikawa (2000)
7.	*Butea monosperma* (Lam.) Taub.	Palash	Tree	Bark	Aphrodisiac, laxative, anthelmintic, dysentery, piles, cold, cough, astringent, diarrhoea and stomatitis.	Rahman et al. (2015) and Velis et al. (2008)

(continued)

Table 22.1 (continued)

S/N	Scientific name	Local name	Habit	Part(s) used	Uses	References
8.	*Cajanus cajan* (L.) Millsp.	Arhar	Shrub	Leaf, root	Diabetes and jaundice.	Rahman et al. (2015) and Primiani and Pujiati (2016)
9.	*Caesalpinia bondus* L.	Nata	Shrub	Seed	Kidney disease and blood pressure.	Rahman et al. (2015) and Srinivas et al. (2003)
10.	*Caesalpinia pulcherrima* (L.) Sw.	Choto krisnachura	Tree	Whole plant, root, flower, wood	Tonic, stimulant, astringent, cholera, purgative, abortifacient, febrifuge, bronchitis, asthma, malarial fever, intestinal worms, coughs, chronic catarrh, emmenagogue, diarrhoea, dysentery and internally in skin diseases.	Rahman et al. (2015) and Srinivas et al. (2003)
11.	*Cassia fistula* L.	Badarlathi	Tree	Leaf, root	Burning sensation, leprosy, syphilis and malaria.	Rahman et al. (2015) and Sartorelli et al. (2009)
12.	*Clitoria ternatea* Linn.	Aparajita	Climber	Bark, leaf, flower	Irritation of the bladder and urethra, earaches and cough of children.	Rahman et al. (2015), Yanishlieva et al. (2001) and Chang et al. (2002)
13.	*Crotalaria alata* D. Don.	Jhunjhuni	Herb	Seed	Rheumatism.	Rahman et al. (2015) and Radwan et al. (2008)
14.	*Dalbergia sissoo* Roxb.	Sissoo	Tree	Wood, leaf	Abscess, astringent, haemorrhages, epistaxis, menorrhagia, bleeding piles and acute stage of gonorrhoea.	Rahman et al. (2015) and Sarg et al. (1999)

(continued)

Table 22.1 (continued)

S/N	Scientific name	Local name	Habit	Part(s) used	Uses	References
15.	*Desmodium gangeticum* (L.) DC.	Chalani	Herb	Root	Alterative, tonic, anthelmintic, aphrodisiac, astringent to the bowels, typhoid, fever, piles, asthma, bronchitis, dysentery, diarrhoea, biliousness, cough, chronic affections of the chest and lungs and whooping cough.	Rahman et al. (2015) and Grynkiewicz et al. (2005)
16.	*Desmodium triflorum* (L.) DC.	Kodalia			Antioxidant and antiproliferative activities	Rahman et al. (2015)
17.	*Erythrina variegata* L.	Madar	Herb	Leaf, root	Blindness, eye diseases, sores, whitlow, spleen complaints, stomach trouble, colic, diarrhoea, menorrhagia, breast pain, galactagogue, laxative, dysentery, wounds, abscesses, carminative, tonic, diuretic, cough, asthma and bilious complaints.	Rahman et al. (2015) and Sato et al. (2002)
18.	*Lablab purpureus* (L.) sweet	Sim	Climber	Leaf	Fresh leaves pounded and mixed with lime are rubbed over ringworms to cure.	Rahman et al. (2015)
19.	*Melilotus indica* (L.) All.	Banmethi	Herb	Root	The roots of this plant and *Amaranthus spinosus* are crushed together and taken with water, to stop bleeding through nose and mouth.	Rahman et al. (2015)
20.	*Mimosa pudica* L.	Ljjabati	Climber	Root	Fever and snake-bite.	Rahman et al. (2015)
21.	*Mimosa diplotricha* C. Wright ex Sauv.	Sadalajjabati	Herb	Leaf	Skin diseases.	Rahman et al. (2015)

(continued)

Table 22.1 (continued)

S/N	Scientific name	Local name	Habit	Part(s) used	Uses	References
22.	*Pisum sativum* L.	Motor	Herb	Seed	Refrigerant, appetizer, fattening, laxative, alleviative of bile, phlegm, burning of the skin and, emollient.	Rahman et al. (2015) and Pudenz et al. (2014)
23	*Pithocelobium dulce* (Roxb.) Benth	Gilapifol	Tree	Leaf	Febrifuge and enema, Saponins showed significant activity against carrageenin-induced oedema and formaldehyde-induced arthritis. Ethanolic extract of the leaf possesses strong antifungal and moderate antibacterial properties.	Rahman et al. (2015)
24.	*Saraca asoka* (Roxb.) de Wilde	Asoke	Tree	Bark, leaf, flower	Irregular menstruation, blood-purifying properties, stomach ache, excellent uterine tonic, haemorrhagic dysentery.	Rahman et al. (2015)
25.	*Senna sophera* L.	Kalkasundha	Herb	Leaf, root	Dyspepsia.	Rahman et al. (2015)
26.	*Senna alata* (L.) Roxb.	Dadmardan	Shrub	Leaf	Eczema	Rahman et al. (2015) and Rahman et al. (2015)
27.	*Senna occidentalis* Roxb.	Boro kolkeshundha	Shrub	Root	The root is made into a paste and given to nursing women for purification of the milk.	Rahman et al. (2015)
28.	*Senna tora* (L.) Roxb.	Chakunda	Herb	Leaf	Leaves and seeds are used as remedy for ring worm, skin disease and asthma.	Rahman et al. (2015)
29.	*Tamarindus indica* L.	Tentul	Tree	Fruit, leaf, bark	Burning sensation, heart disease, astringent and tonic, asthma, amenorrhea, fever, diarrhoea and topically for loss of sensation in paralysis.	Rahman et al. (2015), Nandave et al. (2005) and Sisa et al. (2010)

(continued)

Table 22.1 (continued)

S/N	Scientific name	Local name	Habit	Part(s) used	Uses	References
30.	*Uraria picta* (Jacq.) DC.	Sonkarjata	Herb	Whole plant, leaf, root, pods	Heart trouble, fractured bone, aphrodisiac properties, cough, chills and fevers, antiseptic, gonorrhoea, pods are useful in sore-mouth of children. Roots and leaves are used for typhoid and tetanus.	Rahman et al. (2015)
31.	*Vigna mungo* (L.) Hepper	Maskalay	Herb	Seed	Laxative, aphrodisiac, tonic, appetizer, diuretic, galactagogue, styptic, piles, asthma, leukoderma, scabies, gonorrhoea, pains, epistaxis, paralysis, rheumatism and affections of the nervous system, liver and cough.	Rahman et al. (2015)

Flavonoids are one of the most representative classes of secondary plant metabolism. They are divided into two main groups: the flavonoids and isoflavonoids. Flavonoids mixed biosynthesis products, include a basic molecular skeleton with fifteen carbon atoms ($C_6C_3C_6$). The isoflavones are structurally distinct from the other flavonoid classes because they contain a C15 skeleton based on 1,2-diphenylpropane, while the other classes have 1,3-diphenylpropane. Isoflavones (IFs) are yellow pigments derived from 3-phenyl-benzopyrone (3-phenyl-chromone) structure. They are found in plants, mostly as biologically inactive glycosides, namely 7-O-β-D-glycosides, 6″-O-acetyl-7-O-β-D-glucosides, and 6″-O-malonyl-7-O-β-D-glycosides (Tsao 2010; Bolca 2014). After ingestion, glycosides are not bioavailable to be absorbed through enterocytes (Setchell et al. 2002). They are hydrolyzed into bioactive aglycones by both intestinal mucosa and bacterial β-glucosidases from the gut microbiota. Only these forms are absorbed into systemic circulation directly or after subsequent metabolism in the bowel by intestinal bacteria (Setchell et al. 2002). Soybeans incorporate predominantly genistin, daidzin, and glycitin as inactive glycosides, which are hydrolyzed into their corresponding biologically active aglycones: genistein, daidzein (Fig. 22.1), and glycitein. Other isoflavones observed in legumes are ononin and sissotrin, with their aglycones, formononetin and biochanin A, respectively.

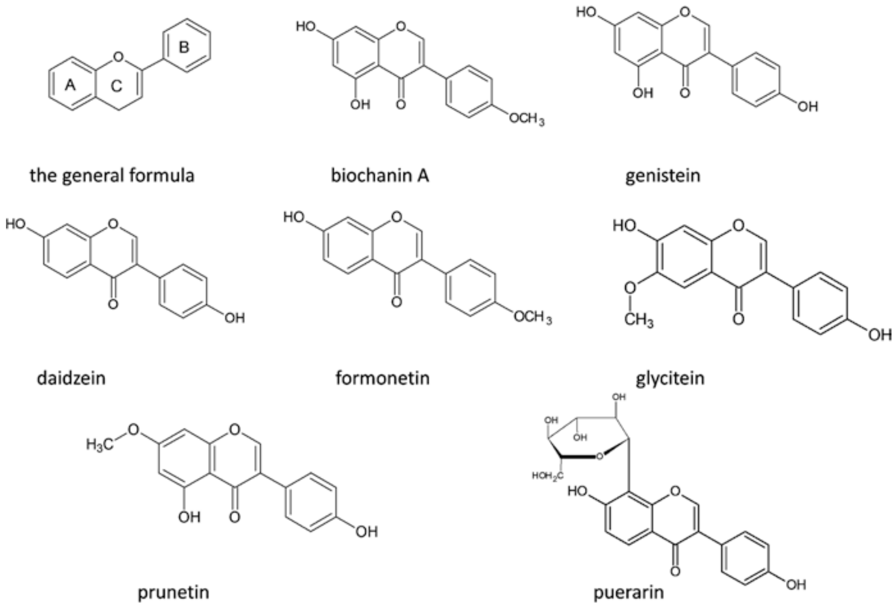

Fig. 22.1 The structural formulas of some of the isoflavonoids

22.3 Flavonoids and Plant Pathogen Resistance

The role of flavonoids in plant defence against pathogens, herbivores, and environmental stress are reviewed and their significant contribution to plant resistance is documented (Lu *et al.* 2017; Treutter 2005). The table below (Table 22.2) highlight several flavonoid compounds having anti-pathogenic activities found in plant organisms.

22.4 Isoflavones: Role in Human Health

Unlike the adverse effect of phytoestrogens, isoflavones has established positive role in human healthcare and several researchers investigated the beneficial effects of this important group of bioactive compounds in this regard.

Table 22.2 List of flavonoid compounds having anti-pathogenic activities found in plant organisms

Plants	Antimicrobial compound	Pathogen	References
Dianthus caryophyllus	Flavonol triglycoside of kaempferide	*Fusarium oxysporum*	Curir et al. (2005)
Dianthus caryophyllus	Kaempferol-O-rutinoside, Kaempferol-3-O-b-D-glucopyranosyl	*Fusarium oxysporum*	Galeotti et al. (2008)
Linum usitatissimum	Isoorientin, isovitexin, vitexin	*Fusarium culmorum*	Mierziak et al. (2014)
Triticum L. cv. Roblin	Flavonoids	*Fusarium graminearum*	Ravensdale et al. (2014)
Lotus garcinii	Catechin, epicatechin, rutin	*Fusarium graminearum*	Girardi et al. (2014)
Hordeumvulgare	Naringenin, kaempferol	*Gibberella zeae*	Bollina et al. (2010)
Mariscus psilostachys	Chalcones	*Cladosporium cucumerinum*	Gafner et al. (1996)
Arabidopsis thaliana	Quercetin	*Neurospora crassa*	Parvez et al. (2004)
Eucalyptus globules	Flavonols	*Cytonaema* sp.	Eyles et al. (2003)
Cicer bijugum	Isoflavonoids	*Botrytis cinerea*	Stevenson and Haware (1999)
Medicago truncatula	Isoflavone	*Erysiphepisi*	Foster et al. (2007)
Vitis vinifera	Quercetin-3-O-glucoside	*Plasmopara viticola*	Ali et al. (2012)
Austrian pine	Flavonoids	*Diplodia pinea*	Sherwood and Bonello (2013)
Glycine max	Isoflavone	*Phytophthora sojae*	Subramanian et al. (2005)
Solanum tuberosum	Glucosylated forms of flavonoids	*Erwinia carotovora*	Velasco et al. (2013)
Oryza sativa	Naringenin, kaempferol, quercetin, hydroxyquercetin	*Xanthomonas oryzae* pv. *oryzae, Pyricularia oryzae*	Song et al. (2013)
Lycopersicon esculentum	Flavonoids	*Pseudomonas syringae*	Vargas et al. (2013)
Citrus sinensis	Flavonoid glycosides, polymethoxylated flavones	*Candidatus liberibacter*	Hijaz et al. (2013)

22.4.1 Bone Health and Metabolism

22.4.1.1 Bone Health

Isoflavones exerts estrogen-like effect on bone by binding to ER-β and thus are expected to have positive beneficial effects for bone health including osteoporosis. *In vitro* and animal studies involving isoflavones and bone health suggest a positive relationship between isoflavones and positive action on both osteoblasts and osteoclasts. Through stimulating bone formation and inhibiting bone resorption, isoflavones can maintain bone health. Human studies, including observational and clinical trial studies also support favourable effects of isoflavones showing the results such as increasing bone mineral density and bone mechanical strength, and inhibiting bone turnover in postmenopausal women (Atmaca et al. 2008). Ingestion of isoflavones (more than 90 mg/day of isoflavones) at least 6 months has a significant effect for increasing spine bone mineral density in meta-analyses of randomized controlled trials however the effects on hip and leg bones are controversial (Taku et al. 2010). Long term safety and efficacy for isoflavones ingestion is needed to be confirmed.

22.4.1.2 Bone Metabolism

Bones having increased calcium retention are due to isoflavones. So, increasing bone mineral density. Both genistein and daidzein are effective in preventing bone loss, but daidzein is more potent of these two compounds. Zn, Mn and Cu are essential trace elements needed for optimal formation of bone matrix and bone mineralization. These elements act as cofactors for some specific enzyme. For example, Zn acts as the component of the ALP, which is involved in the formation of bone mineral matrix, and deficiency of Zn causes skeletal defects.

22.4.2 Beneficial Effects in Menopausal Women

Isoflavones helps reinforce estrogenic effect after endocrine fall in estrogen levels through binding to ER-α which then helps to reduce estrogen-related cancer risk. Through binding to ER-β, isoflavones induce estrogen-like effect and thus show positive health effects after menopause. In postmenopausal syndrome with hot flushes and night sweat, isoflavones prescription can decrease the symptoms of menopause (Setchell and Cassidy 1999). Dietary soy products have slightly and modestly reduced climacteric vasomotor relative to placebo (Bolanos et al. 2010).

22.4.3 Reduction in Cancer Risk

In vitro and animal studies has shown that isoflavones can reduce cancer risk through antioxidant and anti-tumorogenic effect, such as blocking ER-α protein and stopping carcinogenesis pathway through inhibiting PTK, tumor cell growth by suppressing DNA replication and various growth factors and controlling enzyme activities on the signal transduction pathway of carcinogenesis. Epidemiological studies also show that the soy isoflavones may be associated with a reduction in cancer risk. Soy intakes yield a reduced risk for prostate cancer and breast cancer in recent meta-analyses, among Asian population, although the dose-response relationship is not clear (Qin et al. 2006; Yan and Spitznagel 2009; Dong and Qin 2011). However, the protective effect of soy isoflavones is not significantly found among non-Asian populations who soybeans and soy-related products intake is not frequent (Yan and Spitznagel 2009; Dong and Qin 2011). After stratification for fermentation, non-fermented soy is only a significant preventive effect for breast cancer (Dong and Qin 2011). Soy food intake was associated with not only prevention of breast cancer, but also longer survival and low recurrence among breast cancer patients (Zhu et al. 2011; Kang et al. 2012; Zhang et al. 2012).

The effect of isoflavones for gastric cancer is more complicated and it depends on food processing. Both Korean and Japanese populations have high incidence rates of gastric cancer and frequently eaten a wide variable soy food. In recent meta-analyses, among Korean and Japanese population, non-fermented soy foods show a protective effect for gastric cancer, whereas fermented soy foods present no effect in reducing risk for gastric cancer (Kim et al. 2011). Fermented soy foods, including fermented soy sauce and soybean pastes such as Doenjang and miso, which Korean and Japanese are used to add for making foods with high salt content, and therefore fermented soy foods can be associated with higher risk of gastric cancer due to effect and high salt and N-nitroso compounds. There are few human studies using blood concentrations of isoflavones. In Korean study, isoflavones, including genistein, daidzein and equol have an effect of reducing gastric cancer risk, and the higher concentration of three isoflavones is much lower to facilitate gastric cancer risk (Ko et al. 2010). This protective effect against gastric cancer can be explained as an anti-inflammatory, anti-tumorogenic, and anti-oxidative effect of isoflavones. Although, the effect of isoflavones for other cancer prevention has little compelling evidences up to date, a Japanese recent study using a nested case-control reports an inverse effect for lung cancer (Shimazu et al. 2011) and possible preventive effects of isoflavones for other cancers are expected to be released in future studies.

22.4.4 Breast Cancer Prevention

Estrogen is known to induce breast cancer progression, and interventions, such as dietary modifications, that block or reduce estrogen production are likely to result in favourable prognoses for breast cancer patients. A population-based, prospective study confirmed that frequent isoflavone consumption was inversely associated with the risk of breast cancer (Yamamoto et al. 2003). In another study, researchers found that soy isoflavones did not have estrogenic effects in humans and concluded that such a diet may be safe for breast cancer survivors (Fritz et al. 2013), chemoprotective and can prevent recurrence in breast cancer patients. Numerous other plants of the Fabaceae family have been reported to possess anticancer activities. For instance, one study by Arul et al. (2018) demonstrated that the ethanolic extracts of *Pisum sativum* seeds exhibited cytotoxic activity against human breast adenocarcinoma

(MCF-7) cell lines using the MTT assay. The maximum percentage of growth inhibition was 75.1 ± 7.78%, which was obtained at 6 mg/ml concentration.

22.4.5 Anti-diabetic Effect

After menopause, women's glucose tolerance capacities deteriorate and accumulate central fat. As a consequence of these menopausal changes, many menopausal women have an experience in insulin resistance. Although some epidemiological and clinical trial studies have reported that isoflavones has a beneficial effect on insulin sensitivity and glucose metabolism, it is consistent in that isoflavones has slight decreasing insulin resistance observed after menopause. Recent meta-analysis of randomized clinical trials supports the weak effect of isoflavones for diabetes and shows no sufficient evidence in improving glycaemic condition (Ricci et al. 2010). Despite the limited evidences, fermented soy products may have better effects in preventing the progression of type 2 diabetes relative to nonfermented soy products (Kwon et al. 2010). In normoglycemic women, isoflavones is expected to have an association with reduced insulin resistance. In summary, the effect of isoflavones as a treatment medicine for diabetes has no compelling evidences, however, they seem to have a mild effect in preventing diabetes through reducing insulin resistance.

22.4.6 Antiaging Effects of Isoflavones

Red clover extract which is rich in isoflavones mainly, formonetin and biochanin A exerts a higher affinity for liposome formation in comparison to genistein and daidzein. Liposome-incorporated red clover extract resulted in a final product with significant antioxidant action as well as additional benefits in aging prevention (Klejdus et al. 2001; Kanouni et al. 2002).

Soy isoflavones demonstrated UVB protective effects due to their antioxidant activities (Chiang et al. 2007). In addition, soy isoflavone extract inhibited UVB-induced keratinocyte death and suppressed UVB-induced intracellular H_2O_2 release, decreased the epidermal thickness and inhibited COX-2 and proliferating cell nuclear antigen (PCNA) expression. UVB-triggered activation of p38, c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK1/2) which were inhibited by treatment with a soy isoflavone extract (Chiang et al. 2007; Chiu et al. 2009; Assefa et al. 2005).

Huang et al. (2010) evaluated the protective effects of soy isoflavone extract **f**raction 3 from soybean cake that contains the aglycone group (daidzein, genistein and glycitein) and acetylglucoside group (acetyldaidzin, acetylgenistin and acetylglycitin) on UVB-induced damage. The fraction inhibit UVB-induced death of human keratinocytes and reduce the level of desquamation, transepidermal water loss, erythema and epidermal thickness in mouse skin. Furthermore, topical application of the fraction 3 increased the activity of catalase and suppressed cyclooxygenase-2 (COX-2) and proliferating cell nuclear antigen (PCNA) expression in mice exposed to UVB.

Kim et al. (2004) investigated the anti-aging effects of dietary intake of isoflavones on photoaged (UV-irradiated) hairless female mouse skin for 4 weeks. The isoflavone oral administration resulted in better appearance and less wrinkling of the skin and higher amount of collagen deposit. The isoflavones also suppressed the UV-increased metalloproteinases (MMP-1) expression.

Isoflavones bind to estrogen receptors preferentially to 24 and transactivate 25 estrogen receptor-β that is the predominant receptor in the skin (Thornton et al. 2003a, b). In addition, isoflavones also have an effect on the skin, which is independent of estrogen receptor binding. Genistein, the soybean isoflavone is suggested to prevent photoaging through inhibiting the UV-induced epidermal growth factor receptor tyrosine kinase activity and its effect on several other signal transduction pathways in human (Kang et al. 2003). Genistein prevented UV-B irradiation induced skin damage when applied to the human reconstituted skin (Moore et al. 2006). Subcutaneous administration of genistein aglycone in mature ovariectomized rats has shown to improve skin (wound) healing and tensile strength with a greater effect than raloxifene and estradiol (Marini et al. 2010).

Equol, an isoflavandiol estrogen metabolized from daidzein, contains a chiral carbon and exists as enantiomers, S- and R- equol, where both enantiomers can specifically bind to 5α- dihydrotestosterone (DHT) stimulating androgen receptors (ARs) in the keratinocytes of the epidermis/dermis, fibroblasts, and sebocytes. Equol ability to stimulate collagen was tested on human dermal monolayer fibroblasts, where collagen deposition was quantified by collagen type 1 C-terminal peptide by ELISA assays. Equol effect on cell viability was similar to that of 17β-estradiol as shown by MTT assay. Equol could stimulate collagen deposition at different concentrations at significantly higher levels compared to 17β-estradiol (Lephart et al. 2005). Equol, also protects against UV induced skin damage (Widyarini et al. 2005).

Soy isoflavone emulsion could rejuvenate the structure of mature skin by topical application in a placebo-controlled study, through increasing the number of dermal papillae after 2 weeks, since flattening of the dermal-epidermal junction is most considered as important reproducible change in skin aging (Südel et al. 2005).

In a Brazilian study, estrogen and isoflavones were tested on 35 postmenopausal women for 24 weeks of topical application. Both drugs improved the histomorphometrical parameters; but estrogen was more effective than isoflavones (Moraes et al. 2009). Isoflavones can be absorbed effectively through the skin and thus, can exert good protection against photoaging and photodamage (Huang et al. 2008). In fact, Minghetti et al. 2006 suggested that topical administration could lead to circulating isoflavone levels sufficiently to have systemic effects. Bifidobacterium-fermented soy milk (BFS) extract containing genistein and daidzein was tested on the skin of hairless mice by topical application for 6 weeks and it improved the elasticity and viscoelasticity of mouse skin, increased hyaluronic acid content and increased hydration and thickening of the skin. Hyaluronic acid is a major component of skin where it is involved in tissue repair. Topical application of a gel formula containing 10% BFS to the human forearm for 3 months also improved skin elasticity (Miyazaki et al. 2004).

In a pilot study performed by Draelos et al. (2007), two groups of 20 healthy postmenopausal women were each instructed to consume their usual diet with or without 20 g/d of an isoflavone-rich soy protein. There were statistically significant improvements in facial-skin wrinkling, discoloration and overall appearance in the supplement group.

Treatment of 30 postmenopausal women with 100 mg/d of an isoflavones-rich soy extract for 6 months led to an increase in the epidermal thickness in 23 women and collagen amount in the dermis in 25 women with a reduction in the papillary index in 21 women. Further, the number of elastic fibres and dermal blood vessels increased in 22 and 21 women, respectively (Accorsi-Neto et al. 2009).

A study was conducted on 26 premenopausal Japanese women, they were maintained on supplements that provided 40 mg/d isoflavones for 3 months, this led to a statistically significant decrease in fine wrinkles with an increase in skin elasticity, especially in week 8, compared to the placebo group (Stevenson and Thornton 2007).

22.4.7 Cardiovascular Health

Isoflavones binding to ER-β at low endocrine estrogen level, have an agonist effect on estrogen in the cardiovascular system. Also, isoflavones can directly relax vessels by possibly enhancing the promotion of prostacyclin release and anti-inflammatory action and indirectly reduce plaques in vessels by inhibiting collagen-induced aggregation and platelet activation, although the clear mechanisms are not known (Cano et al. 2010). Physiologically, isoflavones are related in reducing lipid profiles such as low-density lipoprotein against global oxidation in endothelial cells. However, the role in total cholesterol and triglycerides is controversial (Cano et al. 2010). In the view of nutrition, many soy products contain high content of polyunsaturated fats, fibre, vitamins, and minerals and low content of saturated fat. Based on these mechanisms and nutritional point of view, isoflavones are beneficial to cardiovascular health by reducing cardiovascular burden in human. Recent meta-analyses using clinical trials to support the cardiovascular healthy role of isoflavones, reveals that isoflavones significantly increase flow-mediated dilatation in vessels and decrease arterial stiffness relative to placebos although heterogeneity across the studies was presented (Li et al. 2010; Pase et al. 2011).

Several phytochemicals that are extracted from the plants of the *Fabaceae* family exhibited exclusive cardiovascular effects. Randrianarivo et al. (2014) evaluated the toxic effects of extracts obtained from the seeds of nine different plants that belongs to the *Fabaceae* family of Madagascar region. It has been reported that all the seed extracts possess large quantities of alkaloids and saponins, where only the extracts of *A. masikororum* and *A. viridis* triggers a positive inotrope effect towards isolated auricles followed by complete atrial arrhythmia via progressive inhibition of contraction (Randrianarivo et al. 2014). Similarly, Atchibri et al. (2010) examined the bioactive extracts of *Phaseolus vulgaris* seeds and revealed that saponins, flavonoids, glycosides, alkaloids, steroids, terpenoids and tannins are present as the significant functional phytochemicals. Further, the study emphasized that the phytochemicals present in these seed extracts lead to their unique antioxidant, anticarcinogenic, antimutagenic and antihyperglycemic effects which will be useful in reducing cardiovascular complications (Al et al. 2010). Likewise, aqueous leaf extract of *Acacia tortilis* was evaluated by injecting them into rats for 7 days. The results revealed that the extracts contain flavonones, flavones, flavonols, coumarins, leucoanthocynanins, anthacyanins, tannins, tertiary and quaternary alkaloids. These phytochemicals help in reducing blood glucose, serum total cholesterol and low density lipoprotein level which will eventually reduce diabetes related cardiovascular complications such as myocardial infarction (Alharbi and Azmat 2011). Apart from other phytochemicals, the presence of isoflavone has been reported to elevate the cardiovascular effects of plant extracts from *Fabaceae* family. Isoflavones are the phytocompounds that belong to the group of flavonoids, which are present in most of the *Fabaceae* plants (Peluso 2006). Thus, Table 22.3 summarizes important isoflavones extracted from different parts of Fabaceae plants that exhibited enhanced cardiovascular effects.

22.4.8 Antimicrobial Activities of Plants of the Family Fabaceae: A Summary of Major Research Findings from 2009 to 2019

Arote et al. (2009), validated the antimicrobial effects of methanol extracts, petroleum ether extract, ethyl acetate extract and chloroform extract obtained from the leaves of *Pongamia pinnata* Linn, a species of tree in the pea family. The antibacterial activity was carried out using disc diffusion method against some enteric pathogenic bacteria which were *Salmonella typhimurium, Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumonia, Proteus vulgaris, Staphylococcus epidermidis, Pseudomonas aeruginosa, and Bacillus subtilis.* The result revealed that the methanolic extract exhibited the strongest antimicrobial activity when compared to other extract used. The high antimicrobial activity might be linked to the presence of saponins, tannins, steroids, flavonoids, glycosides, carbohydrates, alkaloids. Their study shows that extracts of leaves of *Pongamia pinnata* Linn. could be used for the management of enteric infection.

Prakash et al. (2009) evaluated the antibacterial influence of methanolic extract of roots of *Caesalpinia pulcherrima* which belong to Fabaceae family. The antimicrobial activity was evaluated using agar cup plate techniques against methicillin-resistant *Staphylococcus aureus* multi drug resistant *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*. The result obtained showed

Table 22.3 Different phytochemical extracts with isoflavones from *Fabaceae* family with potential cardiovascular treatment properties

Leaf extract			
Plant	Phytochemicals	Cardiovascular treatment	References
Schotia brachypetala	Phenols and flavonoids	Antioxidant property	Sobeh et al. (2016)
Trifolium pratense, *Trifolium repens* and *Genistella sagittalis*	Heterosides – daidzin, genistin and ononin. Glycosilated precursors – daidzein, genistein and formononetin	Diminish symptoms of menopause such as cardiovascular disorders and osteoporosis	Hanganu et al. (2010)
T. pannanicum and *T. pratense*	Phytocompounds of isoflavones	Estrogenic, antioxidative, antiangiogenic and anticancer activities	Kolodziejczyk-Czepas (2012)
T. palladium and *T. scabrum*	Different isoflavone compounds	Antiplatelet activities	Kolodziejczyk-Czepas et al. (2013)
T. pratense	Isoflavone malonyl glucosides	Prevents cardiovascular diseases	Toebes et al. (2005)
Lotus subbiflorus, *Lotus conimbricensis* and *Biserrula pelecinus*	*O*-methylated isoflavone named biochanin A	Neuroprotective, nephroprotective and cardioprotective activities	Barreira et al. (2017)
Glycine max	Phytoestrogenic isoflavones	Menopause-related cardiovascular complications	Lu et al. (2018)
Glycine max	Isoflavone-containing protein	Improves vascular function, cholesterol efflux and blood markers	Richter et al. (2017)
Lotus conimbricensis	Isoflavones	Age related cardiovascular disease	Visnevschi-Necrasov et al. (2016)
Lotus polyphyllos	Lupinalbins, genistin, sativan and vestitol	Prevent cardiovascular complications	Osman et al. (2015)
L. japonica	Genome to produce isoflavones	Prevent cardiovascular illness	Takos and Rook (2014)
Root extract			
Eriosema kraussianum	Pyrano-isoflavone	Prevent hyperglycemia related cardiovascular complications	Ojewole et al. (2006)
Millettia griffoniana	Griffonianone C	Menopause and estrogen related cardiovascular diseases	Ketcha Wanda et al. (2010)
Radix astragali	*O*-methylated isoflavone named calycosin	Prevention and treatment of cardiovascular disease	Gao et al. (2014)

(continued)

Table 22.3 (continued)

Leaf extract			
Plant	Phytochemicals	Cardiovascular treatment	References
Pueraria lobata	Isoflavones	Anti-inflammatory and antioxidant activities	Jin et al. (2012)
Trifolium pratense, roots of lobe Kudzu vine, *Ononsis spinosa* and *Glycyrrhiza glabra*	Isoflavone compounds	Prevention and treatment of cardiovascular disease	Sazdanić et al. (2019), Luo et al. (2017), Addotey et al. (2018) and Tyagi et al. (2018)
Seed extract			
Securigera securidaca	Isoflavone compounds	Improves vascular endothelium-dependent relaxation in hypercholesterolemia	Garjani et al. (2009)
Lupinus albus	Isoflavone-poor protein	Reduce hypercholesterolemic condition and elevate LDL receptor activity	D'Agostina et al. (2004)
G. max	Metabolic engineering to yield more daidzein isoflavone compound	Cardiovascular drug development	Yu et al. (2003)
G. max	Soybean cyst nematode influenced seed extracted isoflavone	Treatment of cardiovascular ailments	Carter et al. (2018)
T. Pratenses	Formononetin	Menopause mediated cardiovascular diseases in women	Budryn et al. (2018)
Soy and lotus seeds in rice porridge	Isoflavones	Antioxidant activity	Kim et al. (2019)
Other extracts			
Flowers of *Lotus japonicus*, *Nelumbo nucifera* and *Lotus conumbricensis*	Isoflavones	Treatment of cardiovascular diseases	Shimada et al. (2003), Deng et al. (2013) and Visnevschi-Necrasov et al. (2016)
Pueraria thomsonii, red clove, soy bean and Kudzu	Isoflavone	Prevention and treatment of cardiovascular diseases	Hirayama et al. (2011), Xu et al. (2006), Takahashi et al. (2012) and Delmonte et al. (2006)
Glycyrrhiza glabra, *Caragana changduensis, Maackia amurensis* and *Millettia ferruginea*	Isoflavone compounds	Prevention and treatment of cardiovascular diseases	Parvaiz et al. (2014), Shin et al. (1999), Sun et al. (2015), Maksimov et al. (1985) and Choudhury et al. (2015)

that the crude extract containing a concentration of 225 8 g/mL exhibited the highest zone of inhibition of 27 mm against *Klebsilla pneumonia* while concentration containing 75 8 g/mL exhibited the least antibacterial effect on *Staphylococcus epidermidis*. Their study shows that the methanolic root extract of *Caesalpinia pulcherrima* could be used as an antibacterial agent.

Sultana et al. (2010), reported the antimicrobial, cytotoxicity and free radical scavenging potential of methanolic extract of *Glycyrrhiza glabra* which belong to the family of Fabaceae. The result obtained shows that the extract of *Glycyrrhiza glabra* inhibited all the test microorganisms with the exception of *Pseudomonas aeruginosa.* The highest sensitivity was observed on *Staphylococcus aureus with* a zone of inhibition of 22 mm while cytotoxic activity had LC_{50} value of 0.771 μg/ml and free radical scavenging activity had IC_{50} values of 87.152 μg/ml.

Chanda et al. (2010) evaluated the antibacterial effectiveness of fruit rind and the seeds of nutraceutical plants which belongs to Fabaceae family. The antimicrobial assessment was performed using agar well diffusion techniques against four gram-negative and four gram-positive bacteria. The result shows that the crude extract obtained from the Fabaceae family exhibited a higher antibacterial activity on gram-positive bacteria when compared to gram-negative bacteria.

Arabi and Sardari (2010) evaluated the antifungal effect of a medicinal plant that belongs to the family of Fabaceae obtained from South and north of Iran. Some of the medicinal plants that belong to Fabaceae used during this study were *Astragalus stepporum, Taverniera cuneifolia, Dalbergia sissoo, Ammodendron persicum, Lathyrus pratensis, Sophora alopecuroides, Oreophysa microphyalla* respectively. The active component was extracted using the Percolation technique with 80% ethanol. The antifungal effect was performed using broth microdilution against *Aspergillus fumigatus* AF 293, *Candida albicans* ATCC 10231, and *Asperigillus niger* ATCC 16404 respectively. The result revealed that all the plants from Fabaceae utilized during this experiment exhibited an antimicrobial effect against at least one of the tested isolates. The study validates the significant of the Fabaceae plant as a therapeutic agent for the management of pathogenic fungi.

Aung (2011) assessed the antimicrobial effectiveness and the level of the phytochemical component present in *Sesbania grandiflora* L. from the family Fabaceae. The chloroform extract exhibit more antimicrobial activity against all the tested isolates most importantly on *Pseudomonas aeruginosa.* The antimicrobial activity demonstrated by the crude extract from the leaves of *Sesbania grandiflora* might be linked to the presence of various phytochemical constituents like α-amino acid, reducing sugar, phenolic compound, terpenoid/steroid, carbohydrate, tannin, flavonoid, saponin, glycoside, and glycoside.

Chaurasia and Saxena (2012), reported the antibacterial effectiveness of 4 different green beans. The aqueous extract from the various green beans were extracted from *Vicia faba* (broad beans; stem), *Vigna unguiculata* (cowpea; lobia), *Phaseolus vulgaris* (French beans), *Cyamopsis tetragonoloba* (cluster beans; guar) and were evaluated against two human pathogenic bacteria which were *Bacillus subtilis* (gram positive) and *Escherichia coli* (gram negative). The antimicrobial assay was performed using disc diffusion techniques. The effectiveness of the aqueous extract was evaluated with streptomycin serving as a control. The result obtained shows that the aqueous extract obtained from the various beans exhibited antimicrobial activity against all the tested bacteria. The antibacterial was shown to be bacteriostatic. The antibacterial activity on *E. coli* varies from 9 mm to 15 mm while *B. subtilis* varies from 10 to 12 mm.

Küçükboyacı et al. (2012), utilized gas chromatography-mass spectrometry to determine various alkaloids available in the *Genista sandrasica* Hartvig & Strid which belong to Fabaceae family grown in Turkey. The percentages of ten quinolizidine alkaloid detected in the plant include anagyrine (40.49%), *N*acetylcytisine (6.48%), baptifoline (10.76%), and 13-methoxylupanine (13.12%) respectively. The antimicrobial effectiveness of the alkaloid extract of *Genista sandrasica* was carried out against the following microorganisms which include *Pseudomonas aeruginosa*, *Candida krusei*, *Staphylococcus aureus, Escherichia coli*, *Candida albicans,* and *Bacillus subtilis*. The minimum inhibitory concentration exhibited by the crude extract from *Genista sandrasica* against *S. aureus* and *B. subtilis* was 62.5 μg/mL and 31.25 μg/mL respectively.

Hossain et al. (2012), evaluated the biological activity of *Cassia senna* leaves including its antimicrobial effectiveness which belongs to Fabaceae family. The crude extract present in *Cassia senna* leaves was extracted using chloroform, methanolic, n-hexane, ethyl acetate respectively. The crude extract was tested against 8-gram (−) bacteria, three fungi and 5-gram (+) bacteria using disc diffusion method. The result shows that n-hexane and chloroform fractions exhibited some level of antimicrobial effect when compared to the methanol extract without any antimicrobial effect.

Pahune et al. (2013) validated the antimicrobial effect of *Clitoria ternatea* Linn which is a species under a genus Clitoria, family Fabaceae. The main aim of their study was to evaluate the utilization of *Clitoria ternatea* Linn as a natural indicator as a permanent replacement to synthetic indicator. The natural indicator will be utilized in numerous acid-base titration. The result reveals that the equivalence points observed from the flower are comparable to the one by the standard indicators. Moreover, the same result was obtained from the weak base and weak acid titration most especially from the flower extract. Furthermore, the methanolic flower extract exhibits a significant antimicrobial effect against *Staphylococcus aureus*. Their research showed that natural indicator is more effective, simple, accurate and economical and eco-friendly when compared to the synthetic indicator most especially for acid-base titration.

Dzoyem et al. (2014) evaluated the effect of acetone leaf extracts of nine medicinal plant that belongs to the family of Fabaceae that has been under-investigated. The antimicrobial activity was performed using a serial microdilution against three Gram-negative and against three Gram-positive bacteria. Moreover, it was observed that 6 out of the 9 acetone extracts exhibited a significant antimicrobial activity with minimum inhibitory concentration values that varies from MIC 20–80 μg/mL against all the tested bacterial species. Among all the plant extract tested, *Crotalaria capensis* showed the highest activity against *Salmonella typhimurium* followed by *Indigofera cylindrical,* with minimum inhibitory concentration value that ranges from 20 μg/mL to 40 μg/mL respectively. Their study established that the plant species from Fabaceae could be utilized as an antimicrobial agent for effective promotion of health and productivity.

Tomar et al. (2014) validated the antimicrobial effect of the methanolic effect of the crude extract obtained from *Mimosa pudica* L which belong to Fabaceae family. The antimicrobial effect was performed using Minimum inhibitory concentration and agar well diffusion techniques. The crude extract of *Bacillus subtilis* MTCC-441, *Pseudomonas aeruginosa* MTCC-4673, *E. coli* MTCC-443, *Staphylococcus aureus* MTCC- 3160, and *Streptococcus pyogenes* MTCC-1926. It was observed that the crudes extract exhibited a board spectrum of antimicrobial efficacy against all the tested isolates. The zone of inhibition, which varies from 17.25 to 20 mm in diameter from the crude extract obtained from *Mimosa pudica* extracts against all the tested microorganism.

Darsini and Shamshad (2015) evaluated the antibacterial efficacy of organic extract from hexane, ethanol, aqueous and methanol of *Clitoria ternatea,* which belong to Fabaceae family. The antimicrobial efficacy was performed using an agar disc diffusion technique. The crude extract was tested against *Proteus vulgaris, Salmonella typhimurium, Candida albicans, Shigella dysenteriae.* The result obtained show that the methanolic extract exhibited the highest antimicrobial activity in comparison to other extracts from water, ethanol, and hexane respectively. The antimicrobial activity of these extracts might be linked to the presence of steroids, tannins, alkaloids, terpenoids, and flavonoids.

Santanaa et al. (2015), validated the medicinal properties of some plants from Fabaceae and were tested against Candida and fungal pathogens. The plant species tested were *Sclerolobium aureum* (Tul.) Baill, *Enterolobium ellipticum* Benth., and *Vatairea macrocarpa* (Benth.) Ducke. The plants were tested against dermatophytes, Leishmania (Leishmania) amazonensis and yeasts. The result revealed that 50% of the total plant screened among the 26 extracts exhibited minimum inhibition concentration of 0.12 to ≤31.25 g/ml while 50% of the total plant obtained from the 6 extracts showed an inhibitory concentration index which varies from 9.23 to 78.65 g/ml. Their study shows that plant from the Fabaceae family could be used as anti-Candida agent and antifungal.

Besong et al. (2016) wrote a comprehensive review of the medicinal importance of *Dialium guineense* which has been reported to be one of the most important plants in Fabaceae family. The plant has been reported to possess numerous medicinal properties which include anti-hemmorrhoidal, antioxidant, anti-plasmodial, vitamin supplement, anti-vibrio, molluscicidal, analgesic, oral care, anti-hepatotoxic, anti-ulcer, and most especially antimicrobial activity.

Aruna and Saravanaraja (2016), reported the antimicrobial activity of *Fabaceae* plant species available. The effect of the extract obtained from these plant species was tested against some indigenous bacterial species. The plant species from the Fabaceae family used during this study include *Pongamia pinnata, Clitoria ternatia, Abrus precatorus, Cassia auriculata, Crotalaria vergosa, Vigna mungo, Sesbania grandiflora, Mimosa pudica, Cajanas cajan, Acacia* *nilotica* and *Tephrosia purpuria*. The result obtained reveals that they all exhibits antimicrobial activity against all the indigenous bacterial isolates from water, soil and samples. Furthermore, greater antimicrobial activity was observed from all the tested plant extract against *E. coli* when least efficacy was observed on *Streptococci spp.*

Velmurugan and Parvathi (2017), evaluated the antifungal efficacy of *Phyllodium pulchellum* L. Desv which belong to the family of Fabaceae. The active compound in the plant was extracted using chloroform, aqueous and ethanol. The extract was tested against *Rhizhotonia solani, Colletotrichum falcatum, Aspergillus niger, and Pencillium notatum*. The result obtains revealed that the ethanolic extract exhibited the highest microbial activity against all the tested isolates when compared to other extracts. The higher antimicrobial activity might be linked to the presence of the flavonoid (71.33 ± 4.172 mg/g), tannin (30.23 ± 3.025 mg/g) and phenol (88.68 ± 2.081 mg/g) respectively. Their study shows that *Phyllodium pulchellum* could be utilized as an antifungal agent for the fungal pathogens.

In Pakistan, Khan et al. (2018), demonstrated that *Cichorium intybus* ethanolic extracts (18 μl/disc) inhibited the growth of *K. pneumoniae* (a gram-negative bacterium) and made the 22.5 ± 0.5 mm zone of inhibition followed by 22 ± 0.1 mm zone of inhibition against the gram-positive bacterium, *B. subtilis* and 19 ± 0.1 mm zone of inhibition against the fungus, *C. albican*. Similarly, the methanolic extract of *Medicago sativa* was found to inhibit *B. subtilis* (gram-positive bacterium), *E. coli* (Gram negative bacterium) and the fungus, *C. albican*.

Heydari et al. (2019), evaluated the antimicrobial and anti-inflammatory activities of methanol extracts and n-hexane, ethyl acetate, chloroform, and water fractions of five Lathyrus species, *namely Lathyrus armenus, Lathyrus aureus, Lathyrus cilicicus, Lathyrus laxiflorus subsp. laxiflorus*, and *Lathyrus pratensis*, growing in Turkey. The results showed that ethyl acetate fractions of the tested species exhibited higher antimicrobial activity than the other extracts. Among all of the tested extracts and fractions, the highest anti-inflammatory activity was detected in water fractions. Furthermore, water fractions of *L. pratensis* showed better anti-inflammatory activity than acetylsalicylic acid and diclofenac sodium, which were used as standard drugs in this assay.

22.4.9 Anti-parasitic Activities

Abdalla and Koko (2018), wrote a comprehensive review based on 53 studies carried out during the period of 1986–2016. The review work was based on the anti-parasitic activity of medicinal plants native to Sudan sourced from previous research work documented from short communications, research papers, MSc and Ph.D. theses, published books, review papers. This data was collated from Science Direct, the Pubmed science web of Knowledge, Google. The result of the search and study shows that 49 plant species belonging to 29 families were screened and evaluated for their anti-parasitic effects during this period. The result obtained shows that family Fabaceae was the most screened which consists of 10 species and exhibited an inhibitory effect against *Plasmodium falciparum*.

22.5 Isoflavones: Extraction Procedure

Extraction of isoflavones can be performed using methanol, ethanol, acetonitrile, acetone or their mixtures with water as extraction solvents. Isoflavonoids are present in plant material in free forms, called aglycones, but they are present mostly as glycosides. Due to the relative instability of the glycosides, the extraction method must be carefully considered in order to preserve the original isoflavone profile. Several studies have shown that isolation at high temperatures causes changes in isoflavone composition due to glycoside decomposition (Rostagno et al. 2009). Decarboxylation of malonylglucosides to acetyl glucosides and ester hydrolysis of malonyl and acetyl glucosides to underivatized glucosides are the two most common conversions of glycosides occurring during the extraction process. It is also possible for any conjugated isoflavone to generate the aglycone form by cleavage of the glucosidic bond. Some glycosides, including malonyl and acetyl isoflavones, are particularly unstable (Rostagno et al. 2009). Due to these potential chemical alterations, the use of drastic temperature and pressure conditions and long extraction times may cause degradation of isoflavonoids conjugates, changing the isoflavone profile of the samples and limiting the information obtained. In addition, chemical hydrolysis leads to a marked increase in the concentration of aglycones present in the sample at the expense of the glucosides and hence increase in the available amount of aglycones to be extracted. So, mild extraction techniques like maceration or negative pressure cavitation extraction are often favoured in order to extract the conjugated forms of isoflavonoids intact. For extraction of aglycones, however, more drastic methods, such as microwave-assisted extraction or accelerated solvent extraction, may be performed.

The principles of Green Chemistry, formulated in 1998 by Anastas and Warner, involve friendly products and processes (Gałuszka et al. 2013; Perez and Escandar 2016). The most important areas of GAC associated with the extraction of plant isoflavones are: automation and simplification of the process; increasing operator safety; reduction of sample size, solvent volume and waste production; elimination of toxic reagents and minimization of energy and time.

22.6 Distribution of Isoflavone in Different Plants Parts

22.6.1 Leaf Extracts

Isoflavones are widely extracted from the leaves of Fabaceae plants as they are easy to extract and are present in large quantities (Ameer et al. 2017). *Schotia brachypetala* is a South African endemic tree that belongs to the *Fabaceae* family, where the isoflavones are extracted from their leaves using hydro-alcohol as solvents. The result revealed that the isoflavones are present in high quantity. Other phytochemicals that are present in the extracts are flavonoid glycosides, dihydrochalcones, galloylated flavonoid glycosides, anthocyanins, procyanidins, hydroxy benzoic acid derivatives, methyl traces, hydrolysable tannins and acetylated flavonoid derivatives. The synergistic effect of all these phytochemicals in the extracts exhibited enhanced antioxidant activity, both *in vitro* and *in vivo* models which will be beneficial in reducing and preventing cardiovascular diseases (Sobeh et al. 2016). Similarly, the liquid chromatography-mass spectroscopy (LC-MS) analysis of isoflavones that are extracted from the dried blooming aerial parts (herbal leaves) of six different Fabaceae family plants. The result revealed that the combination of isoflavone compound is different in each plant, where *Trifolium pratense*, *Trifolium repens* and *Genistella sagittalis* contains a high concentration of heterosides such as diadzin, genistin, ononin and glycosylated precursors such as daidzein, formononetin and genistein. The study also reported that the presence of different isoflavone combinations in leaves of Fabaceae family makes them a better candidate for extracting isoflavones with positive cardiovascular effects. Further, it was also reported that the leaf extract of *T. pannanicum* ad *T. pratense* contains large quantity and different type of isoflavones that possess enhanced estrogenic, antioxidative, antiangiogenic and anticancer activities which are beneficial in reducing menopausal complaints and preventing cardiovascular diseases (Kolodziejczyk-Czepas 2012). Also, it is noteworthy that the leaf extract of *T. palladium* and *T. scabrum* also possess a unique combination of isoflavones that lead to their enhanced antiplatelet activities to prevent cardiovascular diseases (Kolodziejczyk-Czepas et al. 2013).

Moreover, isoflavone malonyl glucosides are extracted from the leaves of *T. pratense* (red clover) (Toebes et al. 2005) and these isoflavone type are reported to be useful in preventing cancer, cardiovascular diseases and gynaecological problems (Watanabe et al. 2002). The leaves of nine Fabaceae plants such as *Biserrula pelecinus*, *Lotus conimbricensis*, *Lotus subbiflorus*, *Ornithopus compressus*, *Ornithopus pinnatus*, *Ornithopus sativus*, *Scorpiurus muricatus*, *Scorpiurus vermiculata* and *Scorpiurus vermiculatus* were used to extract isoflavones. The result revealed that the isoflavone profiles of each plant are different and the high content of *O*-methylated isoflavone named biochanin A in *lotus* species and *B. pelecinus* will help in the neuroprotective, nephroprotective and cardioprotective activities of these plants (Barreira et al. 2017). In addition, phytoestrogenic isoflavones extracted from the leaves of *Glycine max* (soy beans) help to elevate the calcium and chloride level in premenopausal women, which will eventually reduce the menopause-related cardiovascular complications (Lu et al. 2018). Moreover, it has been reported that the isoflavone-containing protein extracted from the leaves of *G. max* proves to be significant in improving the vascular function, cholesterol efflux and blood markers, along with a slight increase in blood pressure, among adult patients with cardiovascular disease risks (Richter et al. 2017). Furthermore, isoflavones were also extracted from the leaves of lotus species such as *Lotus conimbricensis* which acts as a cure for age related cardiovascular disease (Visnevschi-Necrasov et al. 2016), *L. polyphyllos* to extract lupinalbins and genistin type of isoflavones to prevent cardiac diseases (Osman et al. 2015) and the genome present in *L. japonicus* uplift the production of isoflavones in leaves and exhibited unique mechanism to prevent cardiovascular illness (Takos and Rook 2014). It is noteworthy from all these reports that the leaves of *T. pratense* (red clove) and *Glycine max* (soy bean) are widely used to extract isoflavones, which exhibits improved cardiovascular effects depending on their available combination and quantities.

22.6.2 Root Extracts

Similar to leaves, phytochemical extracts of roots from plants that belong to Fabaceae family contains large quantities of isoflavones and are beneficial in the treatment of cardiovascular diseases. The root extracts of *Eriosema kraussianum* have been reported to possess two significant pyrano-isoflavone. These unique isoflavones are injected into hyperglycemic rat, which reduced their glycemic condition depending on the dose. Further, high concentration of these phytocompounds leads to biphasic effects towards portal veins of rats and long-lasting relaxation of venous muscle strips. This study proved that the root extract of pyrano-isoflavone helps in reducing and preventing hyperglycemia related cardiovascular complications (Ojewole et al. 2006). Likewise, the root extracts of *Millettia griffoniana* yielded a unique isoflavone named griffonianone C and are proved to alter the expression of various genes that are responsive to estrogen in the vena cava of ovariectomized rats. These isoflavones can be useful in preventing women with menopause-mediated cardiovascular diseases (Wanda et al. 2010). Moreover, the root extracts of *Radix astragali* are reported to contain calycosin, which is an *O*-methylated isoflavone, as a major bioactive chemical. These isoflavones are widely useful in the treatment of inflammation, cancer, stroke and cardiovascular diseases (Gao et al. 2014). In addition, the root extracts of *Pueraria lobata* contain large quantities of isoflavones, which exhibited improved anti-inflammatory and antioxidant activities which will be beneficial in reducing cardiovascular complications (Jin et al. 2012). In recent times, the root extracts of *Trifolium pratense*

(Sazdanić et al. 2019), roots of lobe Kudzu vine (Luo et al. 2017), *Ononsis spinosa* (Addotey et al. 2018) and *Glycyrrhiza glabra* have been studied. All these plant root extracts contain different quantities and combinations, depending on the place of growth, nutrient availability and several other factors. However, the isoflavones present in all these root extracts are proven to be useful for the treatment and prevention of cardiovascular diseases.

22.6.3 Seed Extracts

The seed extracts of plants from the Fabaceae family were also reported to possess isoflavones with cardiovascular effects. It is noteworthy that the hydroalcoholic seed extracts of *Securigera securidaca* possess a high concentration of isoflavone which possess enhanced vascular reactivity, decrease lipid levels, peroxidation, atherosclerotic lesion and improves vascular endothelial-dependent relaxation in hypercholesterolemia (Garjani et al. 2009). Similarly, the proteins extracted from the seeds of *Lupinus albus* are proved to be natural isoflavone-poor legume. However, these protein extracts are proven to reduce hypercholesterolemic condition and elevate low density lipoprotein (LDL) receptor activity in human hepatoma cell lines (HepG2) which will be beneficial in reducing the hyper-cholesterol related cardiovascular illness (D'Agostina et al. 2004). Further, the isoflavone levels in the seed of *G. max* has been increased via metabolic engineering of phenylpropanoid pathway genes and expressing maize C1 and R transcription factors. These modifications lead to decreased genistein and increase the daidzein levels in the overall isoflavone level. These molecular engineering methods were useful in the accumulation of isoflavone in the seeds of soy bean which will yield more isoflavones to formulate drugs for cardiovascular diseases (Yu et al. 2003). In recent times, it has been revealed that the presence of isoflavone in seed extracts of *G. max* is influenced by genotype and environment as well as the infestation of seeds by nematodes. The direct relationship between soybean cyst nematode and seed isoflavone concentration are emphasized to be useful in elevating the stability of isoflavones, which will be beneficial in yielding better quality isoflavones for the treatment of serious cardiovascular ailments (Carter et al. 2018). Further, the estrogenic activity of *T. pratense* seed extract was evaluated recently and the result revealed that the concentration of isoflavones, especially formononetin, plays a major role in their estrogenic activity which reduces menopause related cardiovascular diseases in women (Budryn et al. 2018). Moreover, the addition of soy and lotus seeds with isoflavones in the rice porridge increases its nutritional value and increases the antioxidant activity which can be taken as a food to strengthen the heart valves and improve cardiac functions among cardiovascular patients (Kim et al. 2019).

22.6.4 Other Plant Parts Extracts

Apart from leaves, roots and seeds, the phytochemical extracts from other parts of Fabaceae plants contain isoflavones. The flowers of *Lotus japonicus* (Shimada et al. 2003), *Nelumbo nucifera* (Deng et al. 2013) and *Lotus conumbricensis* (Visnevschi-Necrasov et al. 2016) contains different compounds of isoflavones. Likewise, the flowers of *Pueraria thomsonii* (Hirayama et al. 2011), red clover (Xu et al. 2006), soybean (Takahashi et al. 2012) and Kudzu (Delmonte et al. 2006) were also proved to contain high concentration of isoflavones. In addition, the plant parts of *Glycyrrhiza glabra* (Parvaiz et al. 2014), rhizomes of *Belamcanda chinensis* (Shin et al. 1999), plant parts of *Caragana changduensis* (Sun et al. 2015), heartwood of *Maackia amurensis* (Maksimov et al. 1985) and *Millettia ferruginea* (Choudhury et al. 2015) were also being proved to contain isoflavones in their extracts. These isoflavones will also be useful as a nutritional additives or pharmaceutical agent and curative agent for the treatment of cardiovascular ailments.

22.7 Future Scope

It is apparent that the study of isoflavones and their effects on human health will become an ever-increasing topic in the future because of their therapeutic potentials. As our wisdom of specific isoflavones excels, there will undoubtedly be the opening of newer dimensions in newer application forms that will precisely benefit human beings. Research endeavours should be focussed on detailed understanding of different dietary isoflavones so as to harness all the beneficial effects. Studies related to these compounds in an exhaustive and comprehensive way will enhance pharmaceutical exploration in the field of carcinogenic disease prevention. This detailed information will be extremely helpful for the pharmacognosist, ethno botanists, botanist and pharmacologist for the collection and identification of the plant for their research work. Health benefits of isoflavones undoubtedly justify the interest towards a definite direction, but the possible controversial outcomes of some clinical and epidemiological studies reaffirm the need of further investigations and subsequent fine tuning.

22.8 Conclusion

Isoflavones are molecules displaying various biological activities with relevance to plant physiology and development. They have an active protective role against harmful abiotic factors and their interactions with other plants and microorganisms. To assess the clinical effects of select foods with a plethora of bioactive compounds containing isoflavones as epidemiologic and cell line evidence, new vistas of health-related research is gaining momentum. Isoflavones are also among the potent dietary factors that offer necessary protection against cancer, heart disease and osteoporosis. Much research is waiting to clearly define the pharmacological effect of dietary isoflavones. In the context of the present as well as forthcoming researches, the role of analytical methods applied for assessment of isoflavones appears to be very crucial.

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Chapter 23
Medical Foods and Infant Formulas

Andrew G. Mtewa, Mushagalusa F. Kasali, Tamirat Bekele, and Bonniface Obura

23.1 Introduction

Medical foods are specially prepared food products that are intended to be consumed by patients in order to mitigate effects of or a particular disease as a prescription. The term "medical foods" is usually mistaken for "nutraceuticals", "functional foods", "medical nutrition" (Davey et al. 2015) and nutraceuptides which are basically designed to supplement dietary needs of patients on medication. They are formulated from different plant bases of which grains constitute a large portion. These foods are regulated to avoid causing toxicity in patients and all analytical test documents and records need to be available and traceable at all times. Shelf life and clinical trials are important components that require care and precision for the sake of validity of safety and efficacy profiles of the products. These foods have been very helpful during and in post-war recovery periods in refugee camps.

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23.2 Types of Medical Foods and Infant Formulas

Medical foods and infant formulas are found in different types according to the bases they are made from. The bases could be used singularly or in combination. Medical foods and baby foods can be made from fruits such as mango, kiwi, cherries, pumpkins, purees, pawpaw, apples, bananas and avocadoes. They can also be prepared from vegetables such as turnip, carrots, beets, tomatoes, asparagus, cucumber, bitter melon, peas, broccoli and egg plants. Sometimes, meats such as fish, eggs, wild game, chicken and beef and dairy such as cheese, yogurt and whole cow milk form a very good base of medical foods and baby formulas. Grains are the most common form of baby medicals foods and baby formula. The bases include rice, ground nuts, sesame, barley, wheat, flax, millet, corn, kamut, oatmeal and germ.

23.3 Ready-to-Use Foods (RUFs)

Ready-to-Use Food (RUF) refers to any energy dense food that is produced and can be eaten straight from the packaging, without the need for cooking or other preparation (ACF 2011; Schoonees et al. 2013). The low moisture content of RUF ensues that the product remains viable and free from microbial contamination during its shelf life (Schoonees et al. 2013). RUF is a broad term that encompasses food categories such as; ready to use therapeutic, supplementary and complementary foods.

23.3.1 Ready-to-Use Therapeutic Foods

Ready-to-use therapeutic foods (RUTFs) are food products with high energy and nutrient content designed to deliver the body's daily energy and nutrient requirements thus promoting rapid normal growth (ACF 2011; UNICEF 2013). RUTFs play a major role in the management of non-complicated severe acute malnutrition (SAM) in children aged six (06) months and above.

The first RUTF was developed in 1996 and in 2007 a joint statement released by WHO, WFP, UNICEF and the UN Standing Committee on Nutrition endorsed the product for use as part of programmes using the approach 'community-based management of acute malnutrition' (UNICEF 2013; WHO & UNICEF 2007). The endorsement of RUTF was premised on series of results from clinical trials that demonstrated the acceptability, efficacy and effectiveness of RUFT for the treatment of acute malnutrition when compared to F100 (Briend et al. 1999; Diop et al. 2003).

Currently RUTF is the preferred choice in the management of children with uncomplicated severe acute malnutrition. However, F100 is still the mainstay treatment for children who are too young (under 6 months of age) for solid food, or who have a medical condition requiring liquid food intake for the first part of their treatment (ACF 2011).

23.3.1.1 Composition of RUTF

The composition of RUTF is very similar to that of F100 therapeutic milk, the diet recommended by WHO in the recovery phase of treatment of SAM, except for the added iron (Collins et al. 2006; WHO & UNICEF 2007). RUTF is therefore designed so that it can be used as a complete replacement for food in the early stages of recovery during treatment of SAM. Ready-to-use therapeutic foods are fat-based products with extremely low water content unlike the water-based F100. The low water content of RUTF creates an unconducive environment for bacterial growth even when the product is accidentally contaminated thus ensuing it remains viable during its long shelf life without refrigeration (Collins et al. 2006).

The majority of RUFTs are formulated as lipid-based pastes or solid compressed bars (biscuits) containing a paste of peanuts, sugar, milk powder and micronutrient mix, with a low moisture content and a long shelf-life (ACF 2011). In addition, most formulations contain legumes, cereals, oil, and dairy products such as dried skimmed milk or whey protein concentrate (Osendarp et al. 2015). Table 23.1 presents a typical nutritional composition of RUTF.

23.3.1.2 Formulations of RUTF

The majority of RUFTs are formulated as lipid-based pastes or biscuits containing a paste of peanuts, sugar, milk powder and micronutrient mix, with a low moisture content and a long shelf-life (ACF 2011). Examples of products formulated as pastes include; Plumpy'nut, chiponde, peanut based-RUTF, Eezeepaste-NUT while BP-100 is an example of products formulated as biscuits.

The paste products can be consumed by children as young as 6 months. Solid RUTF products require soaking in clean water to form a porridge that can be eaten by young children, or consumed directly as biscuits by older children (Schoonees et al. 2013). Children should be provided with clean safe drinking water to drink at will since RUTF do not contain water (WHO & UNICEF 2007).

23.3.1.3 Indications and Dosage

In management of SAM, 10 to 15 kg of RUTF is administered over a period of 6–8 weeks to facilitate recovery (WHO & UNICEF 2007). The quantity of RUTF required by each individual child is dependent on the body weight, with a target of providing dosages that deliver 200 kcal/kg/day (ACF 2011).

Table 23.1 Nutritional composition of RUTF

Proteins	10–12% total energy
(%) Moisture	2.5% maximum
Lipids	45–60% total energy
Energy	520–550 kcal/100 g
Minerals/metals	
Calcium (Ca)	300–600 mg/100 g
Potassium (K)	1110–1400 mg/100 g
Iron (Fe)	10–14 mg/ 100 g
Magnesium (Mg)	80–140 mg/100 g
Phosphorus (P) excluding phytate	300–600 mg/100 g
Copper (Cu)	1.4–1.8 mg/100 g
Sodium (Na)	290 mg/100 g maximum
Zinc (Zn)	11–14 mg/100 g
Selenium (Se)	20–40 μg
Iodine	
Iodine	70–140 μg/100 g
Vitamins	
Vitamin E	20 mg/100 g minimum
Vitamin D	15–20 μg/100 g
Vitamin B2	1.6 mg/100 g minimum
Vitamin A	0.8–1.1 mg/100 g
Vitamin B1	0.5 mg/100 g minimum
Vitamin K	15–30 μg/100 g
Vitamin C	50 mg/100 g minimum
Vitamin B6	0.6 mg/100 g minimum
Vitamin B12	1.6 μg/100 g minimum
Niacin	5 mg/100 g minimum
Folic	200 μg/100 g minimum
Biotin	60 μg/100 g minimum
Pantothenic acid	3 mg/100 g minimum
Fatty acids	
Omega-3 fatty acids	0.3–2.5% of total energy
Omega-6 fatty acids	3–10% of total energy

Source: WHO & UNICEF (2007)

23.3.1.4 Advantages of RUTF

– Demonstrated efficacy and effectiveness in treatment of acute malnutrition when used according to protocol.
– It can be eaten direct from the packet without need for any dilution, processing or cooking.
– The products have long shelf lives due to the low water content making it less susceptible to contamination due storage.
– RUTF is readily acceptable by patients and can be used at home thus reducing the default rate of treatment and time spent in hospital

23.3.2 Ready-to-Use Supplementary Foods

Ready-to-use supplementary foods (RUSF) are similar in design to RUTF but are less nutrient-dense and only provide part of the body's daily energy and nutrient requirements. The milk powder in RUTF has been replaced by whey protein and soy protein isolates thus reducing the energy content and cost of RUSF (ACF 2011).

RUSFs are commonly formulated in the form of pastes (e.g. Plumpy'sup® and the locally produced peanut/soy spreads in Malawi). Plumpy'sup® comes in packets of 92 g and provide 500 kcal of energy. Supplementation with RUSF is not to substitute the normal diet but rather patients are expected to eat normal meals in addition to the product (ACF 2011).

23.3.2.1 Indication and Dosage

RUSF is used in the treatment of moderate acute malnutrition (MAM). The recommended dosage is much lower than with RUTF, at 75 kcal/kg/day (ACF 2011).

23.3.3 Ready-to-Use Complementary Foods (RUCFs)

Ready-to-use Complementary foods (RUCFs) contain an ingredient mix similar to RUTF and RUSF. Examples of products; Plumpy'doz® and Nutributter®. RUCFs are primarily used for prevention of acute malnutrition (ACF 2011).

When the goal of treatment is to avoid deterioration of nutritional status, RUCFs are administered in doses around 50 g per day (e.g. Plumpy'doz®) specifically targeting the general population of young children (6–23 months). Doses of around 20 g a day tend to be used for the prevention of micronutrient deficiencies e.g. Nutributter® (ACF 2011).

23.3.4 Fortified Blended Foods (FBF)

These are often used in the treatment and prevention of moderate acute malnutrition, and used as a supplementary food for pregnant and lactating mothers and certain other patients e.g. those with HIV/AIDS and TB (ACF 2011). The common base for these foods is a blend of milled cereals and soya, to which a micronutrient mix, milk powder, oil or sugar may be added e.g. Corn Soy Blend (CSB) CSB+, CSB++, wheat soy blend.

The ingredients in FBF are pre-mixed and the product can be made into a porridge for serving. Fortified blended foods can be availed to patients either as 'wet rations' or 'dry rations'. Programs utilizing the approach of 'wet rations' usually make the porridge on-site at a feeding centre from where patients are fed daily. With the 'dry rations' option, the porridge premix is given in one- or two-weekly rations to the care-giver of the patient for preparation at home (ACF 2011).

The traditional formulation of FBF consists of corn soy blend (CSB) with high levels of phytates which inhibit the absorption of several micronutrients. Thus, there has been a shift towards the use of newer products (e.g. CSB+, CSB++) which have been modified to reduce the level of phytates (anti-nutrients). The levels of phytates and fibre are reduced through dehulling the soybeans used in CSB+ and CSB++ (ACF 2011).

Advantages of FBF:

– It is of low cost
– Readily provides amino acids
– The blended flour ingredient is readily available

Disadvantages:

– The pre-mixing of the ingredients is a difficult procedure
– The product is bulky (i.e. it's relatively of a high weight for the number of calories provided compared to other products)
– It is of poor nutritional quality
– Requires an addition of oil which may not be readily available

23.4 Lipid-Based Nutrient Supplements

Products which derive most of their energy supply from fats are known as lipid-based nutrient supplements (LNS). The fat content in these products are made up of varying amounts of vegetable fat, milk powder, ground nuts or soya (ACF 2011). The RUTF, RUSF and RUCF products discussed earlier are some of the examples of LNS which have penetrated the therapeutic realm due to their low moisture content, less risk of contamination and long shelf life.

23.5 Product Fortification

Food fortification is the addition of nutrients at a level of higher than those found in the original food. Infants and young children are highly vulnerable to micronutrient deficiencies because of their high nutrient needs relative to energy intake. Micronutrient deficiencies in early life cause damage to brain development which is irreversible. Fetal and child malnutrition influences health throughout the life. A number of interventions exist to improve the micronutrient intakes in early life, such as fortification (Preedy et al. 2013).

23.5.1 Product Fortification by Mineral

Fortification of infant food with iron, calcium, phosphorus, magnesium is considered to incorporate an adequate supply of these nutrients. Several factors must be considered during this process in order to determine the appropriate amount of these mineral in the fortified food. These factors include the amount of the nutrient needed to prevent deficiency diseases, the cost and form of the fortified nutrients that will yield the highest bioavailability and consideration of the safety of adding these minerals together such as potentially adverse nutrient interactions which can occur between minerals (i.e. calcium and iron) (Abrams and Atkinson 2003).

23.5.1.1 Iron Fortification of Infant Formula

Iron is efficiently transported across the placenta during pregnancy so that healthy babies born at term would have adequate iron stores mainly in circulating erythrocytes, the liver and reticuloendothelial tissues. In the first 6–8 weeks of life the high hemoglobin level at birth which is 170 g/l falls to 125 g/l and after 2 months of age erythropoiesis increases and total hemoglobin also increases as the infant grows bigger. This high iron store at birth maintains iron supply during early age, between births to the age of 4 months, therefore little need for additional iron to support the synthesis of hemoglobin, myoglobin and iron bound enzymes. Iron deficiency is uncommon during the early months unless different situation such as preterm, excessive bleeding during neonatal or perinatal period occur (Moy 2000).

Iron is highly absorbed from breast milk than formula food. This may be due to higher binding of breast milk to fat globules which increase the absorption of iron, as compared to cow's milk. Cow's milk also contain high concentration of calcium which decrease the absorption of iron. For this reason breast fed infants are rarely develop anemia before 6 month of age. Infant formula contain lower iron and lower absorption necessitates to fortify the infant formula with iron especially after 4 months of age (Moy 2000).

Fortification of infant formula with iron is the most challenging because the iron compounds that have the best bioavailability interact most strongly with food constituents to produce undesirable organoleptic changes. During selection of iron compound as a food fortificant bioavailability, unacceptable changes to the sensory properties (i.e. taste, color, texture) of the food vehicle and costs are usually an important consideration. Based on this iron compounds currently used as food fortificants are broadly divided into three categories. These are; water soluble, poorly water soluble but soluble in dilute acid, water insoluble and poorly soluble in dilute acid (Allen et al. 2006).

Water soluble compounds are highly soluble in gastric juices and highest relative bioavailabilities than all the iron fortificants. However they are the most likely to cause adverse effects on the organoleptic qualities of foods especially on colour and flavor. During prolonged storage presence of iron in foods can cause rancidity and unnecessary flavor. Therefore they are limited to food that have relatively fast turnover such as cereal flours, dry foods such as pasta and milk powder and dry milk based infant formulas. Ferrous sulfate is the most widely used water-soluble iron fortificant, because of it is the cheapest.

Iron compound which are poorly soluble in water but soluble in dilute acid are soluble in gastric acid produced in stomach of healthy adult and adolescent and well absorbed from food. But the concern is the absorption in infant who secrete less acid and who suffer from a lack of gastric acid due to medical problems. Poorly water-soluble iron compounds, such as ferrous fumarate, have the advantage of causing fewer sensory problems in foods than the water-soluble compounds. Iron compounds that are insoluble in water and poorly soluble in dilute acid have lower bioavailability than water soluble and generally regarded as a last option. It consists ferric phosphate compounds, ferric orthophosphate and ferric pyrophosphate. They have less effect on the sensory properties of foods and because cheaper than the more soluble compounds.

The addition of vitamin C to infant formula increases iron absorption. The co-addition of ascorbic acid and iron in a 2:1 molar ratio increase iron absorption from foods 2- to 3-fold in adults and children. Sodium EDTA also increase iron absorption and unlike ascorbic acid, is stable during processing and storage. At low pH, sodium EDTA acts as a chelating agent and prevents iron from binding to phytic acid or phenolic compounds, which inhibit iron absorption (Allen et al. 2006).

23.5.1.2 Calcium

Calcium is required in large amount as compared to other micronutrients. Breastfeeding is the preferred feeding method for all infants in the first year of life which provide an estimate of 130 mg/day. Therefore, Rickets or calcium deficiency should not occur in breast-fed, full-term infants aged 7–12 months who have an age-appropriate solid food intake that includes calcium-containing foods. Calcium is well absorbed from human milk. Absorption from infant formulas are highly variable because of the various carbohydrate, protein and mineral sources of these formulas. The efficiency of calcium absorption from any food source is likely to depend more on the total amount added and its interaction with other food components (Allen et al. 2006).

It is difficult to clinically identify deficiencies of bone minerals in most children unless they are severe enough to cause recurrent fractures or rickets. Although vitamin D deficiency is the most cause of rickets, in some children very low calcium intakes may be partially or even primarily responsible for development of disease. The recommendation daily intake of calcium differs from country to country. United States, the calcium daily intake for infants aged 7–12 months is 270 mg/day and for infants aged 12–36 months is 500 mg/day. For infants fed cow's milk–based formulas, the recommendation would be increased because of the calcium is not as bioavailable as that of human milk. In the United Kingdom, The recommended calcium intake for infants aged 7–12 months is 525 mg/day and for infants aged 12–23 months decreases to 350 mg/day (Abrams and Atkinson 2003).

Infants who receive human milk without additional calcium sources cannot readily meet the required level. Rickets occurs in these infants in the 2nd years of life, especially when inadequate vitamin D status would lead to impaired calcium absorption below critical levels. Many children who are likely have low milk calcium intakes need supplemental calcium from solid foods. It is recommended that fortified food to provide 100–200 mg/day of calcium. This could provide 100 mg of calcium per 30-g serving of complementary food. This amount is safe, would effectively help prevent calcium deficiency conditions and could be readily incorporated into the products (Allen et al. 2006).

Bioavailable forms of calcium for the fortification of infant formulas and complementary foods include the carbonate, the chloride, the citrate and the citrate malate, the gluconate, the glycerophosphate, the lactate, the mono-, di- and tribasic phosphates, the orthophosphate, the hydroxide and the oxide. The absorption of calcium added is similar with the naturally available in foods which ranges from 10% to 30%. High levels of calcium inhibit the absorption of iron which can be reduced by addition of ascorbic acid (Allen et al. 2006).

23.5.1.3 Magnesium

Magnesium is one the abundant cation and is essential for life. Magnesium deficiency may cause unexpected death in the smallest and youngest members of each species. It may be due to congenital, as in premature infants, infants of magnesium-deficient mothers and infants with intrauterine growth retardation. Human milk provides 20–30 mg/day of magnesium and the solid food provides approximately 55 mg/day and magnesium intake during 7–12 months of age is approximately 75 mg/day. As the recommendation of the Food and Nutrition the RDA of magnesium was 80 mg/day.

Magnesium does not limited in most of the diet but provision of calcium supplementation of foods without magnesium is highly controversial. A ratio of 4:1 or lower of calcium to magnesium has been supported. Therefore, the addition of 40–60 mg/day of magnesium to a supplement is very unlikely to cause side effect on infant (Abrams and Atkinson 2003).

23.5.2 Vitamins

23.5.2.1 Vitamin D

Over 85% percent children living in America and Europe in seventeenth century had rickets. Until early nineteenth century physician had been used cold liver oil to treat this condition. Huldschinsky showed that exposure to ultraviolet radiation could cure rickets in early nineteenth century. Later the fortification of milk with vitamin D had been started and resulted an eradication of rickets from America and Europe. But in the 1940 and 1950 several outbreak of vitamin D intoxication were occurred in Europe and led to prohibition of fortification of food with vitamin D and the reappearance of rickets occurred. Again in 1957 the American Medical Association's Council on Foods and Nutrition confirmed the importance of vitamin D fortified milk in preventing rickets in children (Allen et al. 2006).

Vitamin D deficiency a major problem among those who live in the more northerly or southerly latitudes where UV radiation levels are lower during the winter months. For this reason, it is reasonable to consider adding of vitamin D to any complementary food if this is technically feasible. American medical association recommends milk as a vehicle and to contain 10 μ/quart (Abrams and Atkinson 2003).

23.5.2.2 Vitamin A

Breast milk from well-nourished mother is a good source of vitamin A. However, in some areas of the world children are weaned prematurely and must take fortified food with vitamin A to reduce the intake gap. In average levels of intake, human milk would meet 77–84% of RDI for infants aged 6–11 months and 69% of RDI for children aged 12–23 mo. The exact figure may be vary from place to place (Mora 2003).

Strategies that supply effective amount of vitamin A over long period of time would be useful to reach the population at risk. When the risk of vitamin A deficiency become the highest; fortification has to be used as part of public health and nutrition programs to prevent nutritional deficiencies. It has to be properly designed, manufactured, and commercially available at affordable prices to populations at risk (Dary and Mora 2002).

Oil is an ideal vehicle for vitamin A because of its fat solubility. Fortifying vegetable oil are simple and easy to implement at low cost. Oil also stabilizes retinol and delays the oxidation of vitamins. After oil other halogenated oil products such as margarine are the most suitable vehicle for vitamin A. This method is applied in many countries to mimic the nutritional value of butter. In some countries cereal flours and meals are fortified with vitamin A which is technically feasible and nutrient stability is good. However, there is a losses of vitamin A during transportation, storage and food preparation. Losses have been estimated at 30–50%. Sugar is also a good vehicle for fortification of vitamin A in some countries when other option is in appropriate. Some countries in Central America decided to fortify sugar because other options such as, oil/margarine and wheat flour were neither widely distributed nor consumed by the most needy individuals; dairy products were scarce; and corn flour, although widely consumed, was mostly produced at home (Dary and Mora 2002).

23.5.2.3 Folate and Other B Vitamins

The concentration of water-soluble vitamin including vitamin B in breast milk strongly related to the mother's dietary intake of these vitamin. For this reason it is important to ensure that adequate amounts of vitamins B are provided by complementary foods in populations where there is a high risk of maternal depletion. They can be divided into two groups. Group I Vitamins B include thiamin, riboflavin, niacin, vitamin B-6, vitamin B-12, pantothenic acid, biotin and choline. In this group low intake of mother or store results in lower concentrations in human milk and adversely affect the infant status and development. Group II include only folate in which maternal supplement has relatively little effect on the concentration in human milk. The child is relatively well protected from maternal deficiency (Allen 2003).

Thiamin deficiency causes infantile beriberi which is characterized by peripheral neuropathy, encephalopathy and cardiac failure. Low thiamine level in human milk could be improved by maternal supplementation. The main source thiamine is grain products but most of thiamin lost during the production of polished rice and white flour. In industrialized countries fortified cereal products are the main source of thiamine.

The B-complex vitamins are usually added directly to flour as single nutrients or as a premix or diluted with a small amount of flour at the mill before being added to the bulk. Riboflavin has a strong yellow colour and slightly bitter taste, however, the amount used for fortification the effect to white flour any colour or taste problems are likely to be minimal. The Coated forms of the water-soluble vitamins, such as thiamine and vitamin B6, are also available (Allen 2003).

In general according to USA Infant Formula Act (IFA) the adequate intakes (AIs) for infants, vitamins (A, C, D, K, and thiamine), mineral (calcium and iron) and choline are among many nutrient determined to be important to infant development which should be included in infant formula (Khan et al. n.d.).

23.5.3 Probiotics

Probiotics is a new word meaning "for life" and used to name the beneficial microorganism to human and animal when administered in sufficient quantity. These microorganisms maintain health by balancing disease-causing bacteria and contributing to normal flora. It consists mostly the strains of the genera of Lactobacillus and Bifidobacterium. Probiotics can be found in diary and nondairy product which usually consumed after the antibiotic therapy to replace the microbial flora destroyed by antibiotics.

Probiotics have several uses in maintaining health. Probiotics such as Lactobacillus and Bifidobacterium suggested to alleviate lactose intolerance, prevention and cure of viral, bacterial, antibiotic or radiotherapy induced diarrheas. Several randomized trials showed that probiotics significantly reduced the development of necrotizing enterocolitis. Necrotizing enterocolitis is a severe condition where a portion of the bowel dies. It's common in preterm infants. Some probiotic strains are present in mother's milk in small amount and some are commercialized product in which convincing data are available on safety and efficacy, can be recommended for medical use (Vandenplas et al. 2013).

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition committee showed that probiotic-supplemented formula to healthy infants does not raise safety concerns with regard to growth and adverse effects. However, administration of probiotic-supplemented infant formula during early life (≤4 months) does not show any consistent clinical effects. Because of a lack of data on the long-term effects of the administration of formula supplemented with probiotics especially the effects persisted after the administration of the probiotic has stopped, the Committee does not recommend the routine use of probiotic-supplemented formula in infants (Braegger et al. 2011).

23.6 Safety of Ready-to-Use Foods

Ready-to-use foods (RUFs) that have been produced according to good manufacturing practices (GMP) with the application of the Hazard Analysis and Critical Control Points (HACCP) program are generally safe for consumption (Manary 2006; Osendarp et al. 2015). Quality control is of paramount importance during the production of RUFs in order to ensure that the final product is free from contamination and of the desired quality.

Quality is built into the product rather than tested into it therefore robust quality control measures and systems are necessary at every stage of the manufacturing process. In the manufacture of RUFs, quality control measures are instituted to safeguard against contamination with aflatoxin and bacteria, oxidation of fatty acids and errors that result in content variation (Manary 2006).

Aflatoxin produced by the fungus *Aspergillus* can contaminate peanuts after harvest before they have been ground into a peanut butter. This can be prevented by storing the peanuts in a cool, dry environment and also by using chemical fungicides (Manary 2006). The maximum aflatoxin content in RUFs should be within the range of 10–20 ppb according to international standards. Consumption of RUF with high content of aflatoxin produces acute toxicities (CDC 2004).

Ready-to-use therapeutic foods are naturally free from bacterial contamination due to the low moisture content of the products therefore care must be exercised to prevent he introduction of water into the product during manufacture (Manary 2006). Potential sources of water during production include wet mixing bowls and containers thus it is recommended to limit the number of times the production line is cleaned with water and instead dry-wipe them clean. Typically, the production materials need to be cleaned with soap and water only once a week (Manary 2006).

The oxidation of fatty acids and some vitamins (vitamins A & C) can occur during the storage of RUTF and cause degradation of the product. It is imperative therefore that measures aimed at preventing the initiation of oxidation processes should be instituted during the production of RUTF (Manary 2006). For example, heating of oils during mixing processes should be done at temperatures below 45 °C to prevent oxidation of lipids. The use of airtight containers is and overly filled containers is recommended so as to limit the quantity of oxygen within the container thus preventing the initiation of oxidative processes.

The mineral ingredients used in RUTF formula are water soluble and do not form insoluble complexes when mixed together. Caution is exercised during production of the foods to ensure that the mineral composition of the food will not alter the acid base balance of children when administered for treatment of severe acute malnutrition (WHO & UNICEF 2007). It is recommended that the products have a moderate positive non-metabolizable base sufficient to safeguard against the risk of metabolic acidosis.

23.7 Shelf-Life Determination of Medical Foods and Infant Formulas

The shelf life of medical foods and baby formulas vary according to manufacturer's, market and regulators' needs, demands and requirements. Most of these foods are designed to have a shelf life of not less than 24 months. Shelf life is determined through research and development programs. They consist the identification of key stability and deterioration indicators. These include physical appearance of the products, chemical as well as microbiological susceptibility. Organoleptic analyses are one of the most important technical but basic activities that can be carried out to determine the taste-response relationship of the product and consumer's acceptability over time. Shelf life tests results are all compared against standards usually developed and provided by regulators. They are always required to be kept at all times.

23.8 Pre-Clinical, Clinical Trials and Regulations

23.8.1 Pre-Clinical, Clinical Trials and Regulations

Infants are vulnerable because of many organs are immature and undergoing rapid development. It should be given emphasis than food for adult who have developed homeostatic mechanisms to cope up with and developed protective mechanism. Therefore, it is important to assess the safety of infant formula by conducting pre-clinical and clinical study (Lönnerdal 2012).

23.8.1.1 Pre-Clinical Studies

Preclinical study is conducted before exposing the ingredient to human subject. It consists of in vitro study and in vivo study which conducted on laboratory animal. In USA the Committee on the Evaluation of the Addition of Ingredients New to Infant Formula recommends pre-animal tests if the ingredients to be added to infant formulas are not well known and if there is no adequate literature about them to determine their safety. Therefore, the complete chemical structure and functional groups and the purity and stability of the ingredients present in the matrix should be determined using well-established method such as high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and thin layer chromatography (TLC) (Lönnerdal 2012).

Toxicity studies should be conducted in animals to ensure the safety of the product in the formula. Rodent models (rats, mice) are mostly used for toxicity studies. But difference in metabolism and development limit their result. The rat has short reproductive cycle (21 days gestation) which allows for rapid cycle studies and assessment of generational effects. However, rat consume low fat diets (5% while human 45–55%) of their calories as fat. Piglet models are also used due to comparable in size to neonatal humans and metabolize fatty acids like humans. Piglets can be used for assessment of high-fat infant formulas. Generally investigators should choose healthy animals, animals that have never been exposed to any experimental procedure, male and female animals, and animals that are capable of completing the entire study (National Academy of Sciences 2004).

23.8.1.2 Clinical Trial

Clinical trial is conducted on human subject for different reasons. The first reason is extrapolation from animal studies are limited by difference of animal and human structure, development and physiology. Extrapolation from tissue isolate is also limited due to inability of such model to assess the function which needs coordination of different organ system (i.e. digestion and absorption of nutrient). Preclinical trial gives clue on the safety, absorption and the dose (amount of nutrient) that should be added to the formula. Therefore, to ensure the safety and efficacy of the medical food and infant formula clinical trials are mandatory (Frestedt 2017).

FDA describes the concept of 'healthy growth' including all aspects of physical growth and normal maturational development, including maturation of organ systems and achievement of normal functional development of motor, neurocognitive, and immune systems. These all growth and maturational developmental processes affects an ability of infant's to achieve his/her biological potential, and all can be affected by the infant nutritional status. Clinical trial include these end points in consideration and answers the following questions (National Academy of Sciences 2004).

Absorption, metabolism, distribution, and excretion, half-life of the food or food additive in the human body. It also used to assess interactions between the food or food additive and nutrients or medications, how the food or food additive affect the function of human organs and organ systems, possible adverse reactions in the general population of individuals who are likely to use the substance and in special populations (National Academy of Sciences 2004).

23.8.1.3 Regulation of Medical Food and Infant Formula

The regulation of medical food and infant formula fall under drug regulatory authority in many countries. For example, in USA it falls under the FDA's general regulations for Good Manufacturing Practices (GMPs) for conventional foods. These regulations are required to insure that the manufacturer include required ingredients, sanitary, to provide appropriate concentrations of ingredients in processed food. It also enables the community to be protected from potential safety hazards or inappropriate treatment claims for these products. Before the development of regulation medical food needs to be defined and classified. This enable to distinguish them from each other depending on intended use and required supervision, as well as from foods for special dietary uses, conventional foods, nutrient supplements, or drugs (Mueller and Nestle 1995; Food England 2007).

23.9 Role of Medical Foods During Crises

Medical foods have been playing a crucial role in times of crises. During wars and in post war periods, medical foods have been supplied to hunger stricken citizens in villages as well as in refugee camps to counter malnutrition which could lead to more health complications particularly in people with compromised deficiency. The UNICEF and the WHO (Ayisi 2011) have jointly been sourcing ready to use foods over the years from project peanut butter and valid nutrition of Malawi and other African local manufacturers in addition to international manufacturers in France, America, India and Europe.

23.10 Threats and Future Opportunities in Medical Foods and Infant Formulas

Medical food and infant formulas have proven to be one of the best ways through which disease can be managed using foods and food bases. The mostly used food base is grain including peanut, which is commonly grown in Africa. The beigest challenge with peanut and other grain based products has always been contamination with aflatoxin which sometimes are found above acceptable levels in final products. Opportunities to contain aflatoxin in peanuts still remain un exhausted and require concerted efforts and collaborations in the whole supply chain including researchers and manufacturers. Another challenge that could be there in the over use of medical foods and medical formulas is that they may encourage default from prescriptions and may also excercebate drug resistance. The relationship between over use for chronic conditions and drug resistance need to be explored for medical foods and baby formulas. The future in medical foods needs to focus on the inclusion of nutraceuptide studies of formulations that come from extracts from the kingdom animalia (including insects and larger animals). However, the most important aspects to be maintained shall have to remain efficacy and toxicity of the formulations among other properties.

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Chapter 24
Role of Nutraceuticals in Maternal Nutrition

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24.1 Introduction

Since the very beginning of existence, food and nutrition have been indispensable companions of humans. The early man searched the world for food and medicinal products from natural sources. He continued his quest for growing his food basket in the plant and animal realms, curing his illnesses and discomforts. The desire to achieve vitality and longevity also encouraged the early man in his immediate neighbourhood to experiment with anything possible. The early man became conscious through a process of trial and error, analysis and logical reasoning and inference (Pushpangadan et al. 2014). He made selections of a variety of biological materials to improve health, relieve pain or other physical and mental disorders. Therefore, the gathered knowledge was passed on to successive generations. Creative leaders of the generations that followed progressed incrementally and even added new skills to this traditional information network. Through our ancestors, this traditional wisdom has come down to us and we now call it traditional knowledge or ethnic knowledge. We consider this system of knowledge perpetuated by folklore, local health practices, tribal knowledge system, information structures based on family and culture, among others. Consequently, all the world's ancient cultures

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and civilizations had developed their own traditional food, health and medicine from their biological resources in the natural environment (Pushpangadan et al. 2012).

In recent years, especially over the past decade, the relationships between nutrition, physiological function and disease have advanced. The main challenge at the start of the twentieth century was to provide calories of consistent, sufficient value and shelf life to the population (Klaffke 2007). Nutrition is viewed with reference to the dietary needs of the body, according to WHO. A well-balanced diet is an adequate diet that is combined with regular physical activity contributing to good nutrition as well as a pillar of good health. However, disease susceptibility is increased as poor nutrition leads to reduced immunity. Poor immunity also results in physical and mental impairment, and reduced productivity (WHO 2014). In maternal and child health, nutrition plays a major role. The antagonistic birth outcomes have been associated with poor maternal nutritional status; however, many factors such as socio-economic, biological and demographic factors vary amid different populations that influence the complex relationship between maternal nutrition and birth outcome (Merialdi et al. 2003).

Dietary strategies designing is based upon understanding the relationship between maternal nutrition and birth outcomes that will enhance quality and birth outcomes over the long term and reduce the mortality, morbidity and cost of health care (Fall et al. 2003). A unique period in life span is pregnancy that has considerable potential to influence not only maternal health but also have a remarkable effect on the next generation's health. Among the pregnancy, maternal and child health nutrition is the main factor, so dietary intervention can impact maternal, fetal and infant health. The adverse outcomes of fetal genetic expression and pregnancy are associated with low maternal nutritional status, alongside metabolism and maternal body composition, and placental nutrient supply, which are the main factors that can have a negative or positive effect on fetal development (Cetin and Laoreti 2015).

24.2 Concept of Nutraceuticals

New concepts such as nutraceuticals, nutritional therapy, phytonutrients, and phytotherapy have emerged with this phenomenon (Berger and Shenkin 2006). These active or therapeutic foods and phytonutrients or phytomedicines play a positive role in improving health and immune function to prevent diseases, as well as promising to minimize side effects and maintaining ideal health (Ramaa et al. 2006). Today we recognize the wisdom of the often-quoted saying of the Greek physician Hippocrates, made almost 2500 years ago, "Let food be thy medicine and medicine be thy food". These products are classified as functional foods, dietary supplements and nutraceuticals "any material that may be considered a food or part of a food and that offers health and medical benefits including disease prevention and treatment". Perhaps the most descriptive term used to refer to this category is "nutraceuticals." Stephen DeFelice, founder and chairman of the Medicine Innovation Foundation in

Cranford, New Jersey invented this title (Biesalski 2001). The word “nutraceutical” blends the term “nutrient” with “pharmaceutical” (a prescription drug). Nutraceuticals may contain substances that are expressed as “natural” intention to treat or prevent disease but may not generally be recognized as safe (Ross 2000).

24.3 Multiple Nutrient Deficiencies and Public Health Considerations

The literature citing maternal health and birth outcomes have been dominating the importance of single macro-and micronutrients related studies. These studies evaluated each nutrient separately. The most nutrient intake interrelation studies tend not to find a link, particularly in industrialized populations in maternal dietary intake and birth outcomes studies (Cohen et al. 2001). In both developing and developed countries, there is a similar approach of public health authorities towards adopting a similar strategy which is most widely recommended for pregnant women. The utmost requirement during pregnancy is to take iron or iron/folate supplement on daily basis due to difficulties in achieving the recommended intake of these two micronutrients by diet alone (Peña-Rosas and Viteri 2009). In developing countries (low-socioeconomic status populations), undernutrition is most likely to exist in which diets are insufficient in high-quality, and nutrient-dense foods (e.g., animal-source foods) due to their cost. In such environments, women of childbearing age are often at risk of multiple nutrient deficiencies, so it is illogical to minimize the method of isolating a single nutrient. However, the logical approach towards improvement of overall diet quality, there has been inadequate testing or research on multiple micronutrient (Bhutta and Haider 2009; Allen 2005; Haider and Bhutta 2017).

Multi-nutrient supplementation RCTs (randomized controlled trials) produced mixed results and generally did not produce significant improvements over supplementation of iron-folic acid. Therefore, they were not implemented in the services of public health. The interactions between micronutrient and multi-nutrient supplements and certain micronutrient is of major concern that require specific explanation (Haider and Bhutta 2017).

Nonetheless, women receiving a multi-micronutrient supplement found a significant reduction in low birth weight in study of Shah and Ohlsson compared to those receiving an iron-folic acid supplement alone in a meta-analysis of the most recent studies, including predominantly very vulnerable populations (Shah and Ohlsson 2009). The fallout of Shah and Ohlsson studies also based on these finding that recommendation of supplementation of iron-folic acid to pregnant women is another step towards contributing a major change in public health policies (Shah and Ohlsson 2009). The determination of above mention benefits drive attention of Bhutta and Haider for further research to determine all maternal nutritional status levels and to ensure that perinatal outcomes are not negatively affected before multi-micronutrient supplementation becomes a universal policy (Bhutta and Haider 2009). They also recommended that optimal maternal nutrition can be achieved through various actions, including measures to reduce the burden of infections, provision of food supplements and reduction of food security in households, except for trace elements from maternal health (Bhutta and Haider 2009). Allen argued that the global focus on nutritional supplement systems is a “top-down” attitude to malnutrition and distracts from the search for a sustainable nutritional approach (Allen 2003). Researchers have reported an association between a high-quality or nutrient-rich diet and a reduced risk of adverse outcomes at birth in a limited number of observational food-based studies (Allen 2005; El-Bastawissi et al. 2007).

The animal food sources are considered best that’s why Allen emphasized to increase the consumption and production from those sources. This is another sustainable and cost-effective method of increasing the total value of the diet in the family, which, as shown by some models (Allen 2003). It is very important to understand and avoid the negative effect on fetus growth and development that is resulted from foodborne contamination alongside nutritional approaches that meet the nutritional needs of pregnant women. This may pose a potential threat to seafood, dairy products, poultry, meat, fruits and vegetables (Cox and Phelan 2009; Tan et al. 2009). In a review by Bhutta et al. (2008), nutritional approaches have been considered to have potential, but so far, it has not been sufficiently developed and tested (Bhutta et al. 2008).

24.4 Energy and Macronutrients

The daily energy requirement for stability of normal-weight women with relatively active lifestyle is increased if she is expecting child depending upon the pregnancy stage. A good balance of macronutrient is achieved by slightly increasing energy intakes within the dietary guidance recommendations. In addition, especially in obese and overweight women with increased risk of miscarriage, pre-eclampsia, gestational diabetes, as well as type 2 diabetes and obesity also resulted by excess calories and macronutrients intake during pregnancy may be just as damaging as their deficiency (Bruce 2014; Catalano and Demouzon 2015). Hence mother’s requirement during lactation for milk production is moderately increased. The Italian recommended dietary allowance in 2014 suggested a rise in calories as per requirements for a total of 76,530 kilocalories during the first trimester and additional requirement of 69 kilocalories per day. Similarly, 266 kilocalories per day and 496 kilocalories per day for the second trimester and the third trimester of pregnancy is required (Società di Nutrizione Umana 2014).

24.4.1 Protein

During maternal tissue and fetal development, protein synthesis demand increases and macronutrient such as protein needs more considerations during pregnancy, especially during the third trimester. Negative effect on health in term of weight and length at birth time is associated with unnecessary low protein intake while on the other hand, fetal development is also affected by high protein level (Kramer and Kakuma 2003). Protein Digestibility Corrected Amino Acid Score (PDCAAS) is the digestibility score for amino acid which is used to calculate the protein value (Schaafsma 2000). Animal product values are close to 1 typically together with all nine essential amino acids, while plant values range below 0.7. However, to improve the overall quality of protein, its better to take two or more vegetable foods with different amino acid composition (FAO 2013). Hence, increase in protein intake during pregnancy is another important step of International guidelines, especially during the second and third trimesters is important for maternal and fetal tissues and placenta and also ensure they will gain additional 21 grams (Trumbo et al. 2002). The increase of 1 gram per day recommended daily allowance (RDA) during the first trimester of pregnancy and 8 gram per day in the second trimester and in the same manner for 26 gram per day in the third trimester. If breast milk is already a significant proportion of the infant's diet, then, the normal consumption of protein during exclusive breastfeeding should be 21 gram per day in the first semester and 14 gram per day later (Società di Nutrizione Umana 2014).

24.4.2 Carbohydrates

Carbohydrates are the main source of energy for the body, and most particularly for the brain. Pregnant women need the carbohydrate energy to grow a healthy baby, as carbohydrate-derived glucose is the main fuel used to grow intrauterine (Clapp 2002). The recommended daily intake (DRI) during pregnancy for carbohydrates is 175 g/d. It is important for pregnant women to choose high-quality carbohydrates with a low glycemic index (GI), naturally found in whole foods such as whole grains, bananas, beans, peas, lentils, and low-fat dairy (Clapp 2002). Carbohydrates may also take the form of additional sugars such as table sugar, honey, syrup, cane sugar, agave, high-fructose corn syrup, and concentrate of fruit juice. All women, including pregnant women, must restrict their consumption of these sugars, as well as foods with high quantities of added sugar such as candy, cookies, and sugar-sweetened beverages (soda, juice, lemonade, sweetened teas, and other fruit-flavoured beverages). A Malaysian research by Loy et al. found that increased fruit and vegetable intake was associated with an increase in birth weight, length of birth and head circumference (Loy et al. 2011).

24.4.3 Fat

The role of quality of fats during pregnancy is extra eminent than their quantity, particularly for infant development and fetal growth. However, the main concern is to enhance the relative absorption of polyunsaturated fats by enough intake of docosahexaenoic acid (type of omega 3 fatty acid series) but not by raising the intake of total fats. It is of utmost importance for retina development and growth of the brain. A lot of factors affect breast milk content after delivery. These includes feed stage, feeding time and number of pregnancies, whereas lifestyles and maternal diet (dietary fat content, energy intake) are less relevant in severe malnutrition cases (Sauerwald et al. 2001). Indeed, the long-term intake of feed is reflected by release of deposits in the maternal compartment. Therefore, there is no need to change the overall intake of fat during pregnancy and lactation (Koletzko et al. 2007).

24.5 Micronutrients

Requirement for micronutrients as compared to macronutrients increases during pregnancy, and its intake in less amount (in case of low nutritional diet) can cause significant health issues for both the developing fetus and mother. Micronutrients played a fundamental role to support vitamins in their physiological activity (Trumbo et al. 2002; Koletzko et al. 2013).

24.5.1 Iron

The active involvement of iron, which is the functional part of myoglobin, haemoglobin and many other enzymes, plays an important role in oxygen transfer to tissues of the body. If iron deficiency occurs, anaemia may set in. Many enzymatic processes are carried out by the active involvement of iron. The deficiency affects 22% of women in European countries at childbearing age and 50% in developing areas. Children between the age of 6 and 36 months are more likely to have an iron deficiency (Stevens et al. 2013; Eussen et al. 2015). Meat, poultry, green leafy vegetables and legumes are the main dietary sources of iron. Iron may be present as a non-heme and its absorption is related to the composition of diet and individual's nutritional status. For example, polyphenols and phytates have the potential to resist non-heme iron absorption associated with ascorbic acid or fish and meat consumption. The human body can consume non-heme iron (2–13%) and heme iron (25%) (Società di Nutrizione Umana 2014).

When a woman becomes pregnant, she raises her iron needs until the third month of pregnancy. Iron controls a transition from the maternal compartment to the fetus through a complex transport mechanism consisting of release from the maternal liver followed by processing in the form of ferritin and then uptaken by placenta and smooth transfer to the fetus with the aid of specific protein, oxidation to Fe^{3+} and then storage in the form of ferritin and later transfer into fetal circulation system (Cetin et al. 2011). When women require higher concentrations of iron during gestation, insufficient intake can cause iron deficiency in pregnant mothers, which can have a negative impact on fetal development and growth. However, the risk of low birth weight, premature delivery and post-partum haemorrhages may be increased

(Allen 2000; Khambalia et al. 2016). Also, less iron intake during pregnancy may result in increased cardiovascular risk in adulthood, according to advanced studies (Alwan et al. 2015). Iron supplementation is prescribed to overcome birth problems during pregnancy (Siu 2015; Khambalia et al. 2016). Yet excessive iron consumption in the form of lipid peroxidation, gestational hypertension, oxidative stress, and impaired glucose metabolism also have negative effects (Krebs et al. 2015).

24.5.2 Iodine

Iodine as a functional constituent in thyroid hormones, plays a key role in the formation, growth and development of tissues and organs. Iodine is present in the body in an organic form that is bound to thyroglobulin. Its deficiency may cause an increase in the size of the thyroid gland (goitre) and an increase in the concentration of thyroid-stimulating hormone (Tetens 2014). Major dietary sources of iodine are fish and shellfish. These sea animals inturn, get the mineral from sea algae. The sea algae inturn get it from marine water. During rainfall and water evaporation, iodine is absorbed in the soil and absorbed by plants. Its average intake is less in population than that suggested by WHO. Iodine deficiency has effects mostly in the child population in Europe, and its intake is 85–88 g/day than that indicated by WHO (150 g/day) in all over the Italian area (Novaković et al. 2013; Pastorelli et al. 2015).

Iodine deficiency during pregnancy may cause risk of abortion, birth defects, neurological disorders and perinatal death. It is considered as the major cause of brain damage according to the WHO (Trumpff et al. 2015). Its requirement in general population can be fulfilled by supplementing diets with proper concentrations. Use of iodized salt in a meal can overcome this problem. Iodine is required during the pregnancy to produce a hormone named as fetal thyroid hormone, so women must increase the intake of iodine by 50% during the pregnancy (Bhutta et al. 2013). Fetus and the newborn baby are at higher risk of hypothyroidism even when the deficiency of iodine is mild or moderate according to the National observatory for the monitoring of iodoprophylaxis in Italy. Supplementation with iodine must be higher in the critical period from the second trimester of pregnancy to the third year after pregnancy (Istituto Superiore di Sanità 2016). According to EFSA, 200 g/day of iodine can avoid deficiency in critical period. During lactation, 200 grams per day iodine intake is recommended to sure the milk content of 100–150 grams per 100 mL (WHO 2016).

24.5.3 Calcium

Calcium is known to be the most abundant mineral found in the human body, constituting of 99% in the skeletal mass and also in the teeth. During the earliest age of life, calcium in the human body is at its peak in bone mass, as it helps in maintaining bone mass in adults and it doesn't stop there, calcium slows the physiological age-associated reduction of the measure of the bone mineral mass.

Genetic, hormonal factors and insufficient physical activity may aggravate calcium deficiency in humans. The metabolism of calcium is made possible by the presence of vitamin D, and lack of this vitamin can be as a result of calcium deficiency and when these happen, the outcome depletes mineralization of the bone matrix. Children with calcium deficiency may have rickets (Allen and Kerstetter 2005). Milk and its derivatives which contain about 50% of this mineral, with cereals and vegetables (each constituting 11%) are the major sources of calcium (Lombardi-Boccia et al. 2003). Calcium bioavailability for these food sources varies considerably, with milk and its derivatives and mineral water having the highest levels of bioavailability. Contrarily, fibre and phytate-rich vegetables have bioavailability levels that are quite low. The measure of calcium in the body is affected by the effectiveness of calcium absorption from foods and this is constant in adolescents and adults, there is however, a decrease in calcium absorption by 2% every 10 years in post-menopausal women (Allen and Kerstetter 2005).

Calcium is a critical mineral in the developmental stages of a fetus, and its demand is highly increased during pregnancy, which is usually an increase from a daily dosage of 50 mg/day at the halfway point, to 330 mg/day at the end and also during lactation which is due to the movement of calcium from the maternal skeleton to the fetus, a greater efficiency of intestinal absorption and an amplified increase in renal retention (Theobald 2005). Adequate calcium consumption is associated with high birth weight, effective blood pressure control and a diminished risk of premature delivery. The placental movement of calcium from the maternal compartment to the fetus is achieved as a result of the presence of active transport agents in the epithelium of the placenta. The amount of calcium circulated in the fetus is higher in the 20th week of pregnancy than those detected in the maternal plasma. Recommended daily calcium intake varies between countries to countries, and the daily dose for pregnant and breastfeeding women also varies between countries. The Italian RDA indicate calcium value of 1.2 gram per day in the gestational period, while the WHO recommends 1.5–2.0 gram per day from the 20th week until the end of pregnancy, particularly for women at risk of gestational hypertension. Risks of developing gestational hypertension and pre-eclampsia in pregnant women can be reduced by a low-dose calcium supplementation as recommended (Hofmeyr et al. 2014).

It should be noted, however, that hypercalcemia, i.e. calcium levels above normal range is associated with a high risk of developing Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) Syndrome. Interestingly, the measure of calcium secreted daily in breast milk varies considerably (150–300 mg/day), and this depends largely on the active transportation of calcium from the bones and reduced urinary secretions. The restoration of calcium stored in the maternal bones is achieved after the weaning and resumption of ovarian function (Olausson et al. 2012). Studies have shown that the secretion of calcium in milk gland does not depend on its dietary intake and supplementation. Hence, the daily calcium intake during lactation is nothing different from that of the healthy adult female population which is 1.0 g/day. Moreover, high risk of calcium deficiency during breastfeeding can be associated with women with a dietary calcium intake lesser than 300 mg/day and also adolescents who have high basal requirements of 1.2 g/day according to the Recommended Dietary Allowance (RDA) (Marangoni et al. 2016).

24.5.4 Vitamin D

Cholecalciferol (Vitamin D_3 from animal source) and ergocalciferol (Vitamin D_2 from plant source) are two main molecular species of Vitamin D. Dietary intake affect the circulating levels of Vitamin D. One of the hydroxylation processes that occurs during the metabolism of vitamin D is responsible for 25-hydroxy-vitamin D production. 1,25-hydroxyvitamin D is produced by hydroxylation process in the renal tubes and are regulated through the mechanism of feedback mechanism. The entirely process depends on phosphorus and calcium requirement (Spiro and Buttriss 2014). the exposure of sunlights (290–315 nm wavelength), cause the synthesis of vitamin D. Vitamin D is affected by factors of individual characters (sex, weight, phenotype) and environment (latitude, season, time of exposure with sunlight, degree of physical activity, pollution and use of supplements). Celiac disease, cystic fibrosis, liver and kidney disorders, ulcerative colitis and Crohn's disease are the problems associated with low synthesis of vitamin D in the epidermis layer because of ageing (Spiro and Buttriss 2014).

In Italy, people suffer from severe vitamin D deficiency mostly during the winter season (Freisling et al. 2010). Obese individuals are subjected to high intake of vitamin D because of greater depots of vitamin D in the adipose tissues (Cashman and Kiely 2014). Cod liver oil is a good source of vitamin D and food sources like fish (herring and salmon), high fat cheese, butter, eggs also contribute to a smaller portion of vitamin D. Metabolism of cytokine and immune system modulation are regulated by vitamin D_3 that is predominant from the maternal blood at the first stage of pregnancy. It also regulates hormone secretion and embryo implantation. Mothers and breastfed infants are usually affected by vitamin D deficiency during the winter season because natural sunlight is less (Lamberg-Allardt et al. 1986). According to recent studies, supplementation during pregnancy can overcome the risk of pre-eclampsia (De-Regil et al. 2016).

24.5.5 Folic Acid

Folic acids (folates, vitamin B9) play a vital role in several metabolic reactions occurring in the body like the biosynthesis of DNA and RNA, methylation of homocysteine to methionine, amino acids metabolism, amongst others. As a matter of fact, metabolized forms of folic acids serve as transport coenzymes that enhances the transfer of carbon units from one compound to another. As a result, folic acids are considered healthy and its deficiency may lead to megaloblastic anaemia, thrombocytopenia, leucopenia amongst other deficiency-related diseases (Hoey et al. 2013). Food sources of folic acids majorly includes; fruits (citrus fruits), green leafy vegetables, cereals and offal. The presence of anti-nutrients in these foods determines the bioavailability of folic acids, and this can invariably lead to a reduction of their absorption rates by the body. Folic acid requirements are gradually increased during the periconceptional stage, in relation to their use in creating cells and fetal tissues (Berti et al. 2012).

In a bid to curtailing the risks associated with neural tube defects in women of child-bearing age, maternal supplementation with folates is highly recommended (Cawley et al. 2016). Recent researches also deduced that the folic acid supplementation reduces the risk of congenital heart disease and support the proper development of the placenta during pregnancy (Fekete et al. 2012). Recommended dietary allowance of 50% is recommended for pregnant women while in non-pregnant women of childbearing age, 600 μg per day vs. 400 μg per day is recommended. In exceptional cases, the mother's supplement should begin at least 2 months before conception and can even reach 800 μg per day, the benefits of the higher ones are completely unclear (US Preventive Services Task Force 2009). The levels of folic acid in breast milk gradually increase from colostrum to mature milk and reach much higher levels than the levels measured in maternal plasma. The lack of a complementary relationship between the state of the mother and the content of breast milk indicates an active role of the mammary glands in the active transport and regulation of folic acid secretion only slightly affects food intake (O'Connor et al. 1997). In conclusion, the intake of folate-rich foods from nursing mothers should be increased by 25% to 500 g/day (EFSA NDA Panel 2014).

24.5.6 Polyunsaturated Fatty Acid

The major polyunsaturated fatty acid in the human brain and retinal rods are necessary for the development of brain and retina of the fetus during the pregnancy period. Docosahexaenoic acid (DHA) play a key role in psychomotor and neurodevelopment in first month of life when it is supplied in high amount through breast milk (Koletzko et al. 2011). It is beneficial for fetus and infant development. Omega 3 fatty acid has a great influence on maternal health and also has the capability to reduce the risk of immature birth. DHA also reduces the risk of overall infant health (Sallis et al. 2014; Mennitti et al. 2015). The human body has enzymatic ways to synthesize DHA through the use of alpha-linolenic acid (ALA), a metabolic precursor. There are clear evidences by experiments showing the conversion of ALA into longer chains fatty acid. In male, the conversion of alpha linolenic acid to DHA is low and almost impossible (Brenna et al. 2009). Because the human body has limited ability to produce polyunsaturated fatty acid with long-chain, so from the last few years, the concept of conversion of ALA to EPA and DHA is extended. ALA level is high in vegetable oil and minor component of plants while DHA and EPA are present in high amount in fatty fishes which are present in the cold sea. Terrestrial origin food has less contribution to the intake of long-chain fatty acids. Diets devoid of fish may leads to deficiency of DHA and EPA (Mozaffarian and Rimm 2006). The most severe cause of mortality in the united states is low consumption of omega 3 fatty acids that comes after high salt consumption according to a study (Danaei et al. 2009). About 80% of the population do not take the daily required amount of EPA and DHA as recommended according to international guidelines and this recommendations ranges from 250 to 500 mg per day (Pounis et al. 2014).

Due to the risks of contaminants in fishes, the European food safety authority says that there should be 1–4 consumptions of fish in the week, and this is to ensure a proper offspring development during pregnancy (EFSA Scientific Committee 2015). The consumption of 3–4 fish per week will not provide surplus aid according to the EFSA report. Balancing the amount of DHA and EPA reduce the problem of environmental pollution, and smallest fish such as anchovies, mackerel and sardines should have high preference (Mozaffarian and Rimm 2006). Now, one can get purified and safe formulations of long-chain fatty acids with algae also containing DHA. The order less property of fish oil can be easily integrated into EFSA and RDA products; 100–200 milligrams of DHA per day is recommended during lactation and pregnancy (Società di Nutrizione Umana 2014). If an individual consumes two fishes in 1 week, that will provide sufficient DHA in breast milk (Koletzko et al. 2007). Some specific individual groups, such as mothers who smoke during pregnancy and lactation, will require more DHA, and children from women who smoke will be less gestational at birth and will have lower levels of DHA compared to children born to non-smoking women. During postpartum smoking, the mother provides less DHA to the baby than breast milk (Agostoni et al. 2003, 2005).

24.6 Conclusion

Fetal growth and birth are influenced by maternal nutrition and this is considered a backbone for both. The public health importance and adverse birth outcomes in developing and low-income countries is a modifiable risk factor. To ensure maternal wellbeing and favourable outcomes of pregnancy, variation in consumption of a balanced diet from the preconception period is also important. Specific dietary intakes during pregnancy and lactation are often insufficient even in the most industrialized countries. Therefore, pregnant women should be advised to eat a balanced diet rich in high-quality carbohydrates, proteins, vitamins and other classes of food.

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Chapter 25
Bioavailability of Nutrients and Safety Measurements

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25.1 Introduction

Nutrition is the science of how living organisms consume and utilize food for their overall nourishment and sustenance. The role of nutrition in human health can be traced back to 400 BC with the quote of Hippocrates: 'Leave your drugs in the chemist's pot if you can heal the patient with food'. There are about 40 nutrients that are essential for growth and development of an individual. Among their many other roles in the human body, nutrients perform three basic functions – provide energy, build and maintain body cells, and regulate the body (Marcus 2013; Mahan and Raymond 2016; Titchenal et al. 2018). Important components of nutrients include carbohydrates, lipids, proteins, vitamins, minerals and water. These nutrients are the basic source of most of the foods and culinary chemicals. Certain non–nutrients substances such as dietary fiber and phytochemicals are useful in the prevention of diseases and possess therapeutic potentials. Certain foods may also contain microscopic organisms like bacteria that may either be beneficial or harmful and may find their way into our food supply. Nutraceuticals and functional foods exhibit beneficial effects on health and have physical and psychological roles in the diet. Most of the nutrients serve more than one function. Some serve as antioxidants and protect the body against oxidative stress, harmful free radicals from sunlight, and other environment hazards, while others help in protecting the bones, heart, and stomach, reducing blood pressure and protecting against cardiovascular diseases (Marcus 2013).

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25.2 Types of Nutrients

Nutrients can be classified as essential and non–essential; energy yielding and non–energy yielding; as well as macro and micronutrients. Essential nutrients are nutrients that the body cannot produce in sufficient quantities and must be obtained through the diet. These include carbohydrates, lipids and proteins, certain vitamins and minerals, and water. Non–essential nutrients on the other hand can be made by the body and are obtained usually from sources other than foods. Biotin and vitamin K produced by gastrointestinal bacteria, cholesterol produced by the liver, and vitamin D produced in the presence of sunlight in our body are some examples of non–essential nutrients. A person consuming a balanced diet is likely to have access to most of the essential and non–essential nutrients. Nutrients may also be classified as energy yielding (carbohydrates, protein and lipids), and non–energy yielding (vitamins, minerals and water). The energy–producing nutrients are metabolized by the body to release energy (Marcus 2013). In general, the body requires two types of nutrients: macronutrients and micronutrients. Macronutrients, like carbohydrates, fats and proteins are needed by the body in large quantities whereas, micronutrients, like vitamins and minerals, are needed by the body in smaller portions (Campbell 2017). If a person avoids foods to restrict calories on a regular basis, he/she runs the risk of nutrient deficiencies in the body (Marcus 2013).

25.2.1 Macronutrients

Macronutrients, the primary building blocks of our diet are found in almost every food item. During metabolism these are processed into chemical energy which are then converted into cellular energy for utilization by the body to perform bodily functions (Mahan and Raymond 2016; Titchenal et al. 2018). There are three main macronutrients: lipids, carbohydrates, and proteins.

25.2.1.1 Lipids

L*ipids are chemically defined* as water insoluble (lipophilic or hydrophobic) substances that are esters of fatty acids, phosphoric acid etc. They are found in eggs, shellfish, animal fat, soya products, olive oil, milk and coconut fat (Table 25.1). Together with carbohydrates, sterols and proteins, *lipids in the form of fats and oils* are the main constituents of living cells. Fats are glycerides of predominantly saturated fatty acids and solid at room temperature, whereas oils are glycerides of unsaturated fatty acids and exist in liquid form. These are abundantly found in butter, oils, meats, dairy products, nuts, seeds, and in many processed foods, and execute

Table 25.1 Types of dietary lipids, their patho–physiological functions, dietary sources, and deficiencies

Lipids	Patho–physiological functions	Dietary sources	References
Glycerolipids	Source of energy, membrane constituents, metabolic fuels and signaling molecules	Oils and fats of animal and plant origin	Donato et al. (2013), Messias et al. (2018)
Glycerophospholipids	Key components of cells' lipid bilayer, primary components of cellular membranes and binding sites for intra– and inter–cellular proteins, involved in metabolism and signaling and in eukaryotic cells, takes part in immune response system	Animal tissues (ganglion cells of the central nervous system), aquatic organisms	Donato et al. (2013)
Sphingolipids	Cellular signal, major components of cell membranes	Dairy products, Meat products and fish, vegetables, fruits, cereals	Yamada et al. (2017), Vesper et al. (1999), Norris and Blesso (2017)
Sterol lipids	Maintenance of membrane fluidity, biological roles as hormones and signaling molecules	Animal tissues, plants, fungi	Donato et al. (2013)
Prenol lipids	Protecting cellular membranes, stabilising cell proteins and supporting the body's immune system, vital role in cell metabolism, function as antioxidants and as precursors of vitamin A	Citrus, cranberry, bilberry, grapes, currants, raspberry, blackberry, tomato, arctic bramble, coffee, cloudberry and passion fruit; white bread, hop oil,	Messias et al. (2018), Fahy et al. (2005, 2011). SIDS (2005)
Saccharolipids	Components of membrane lipids	Microbes (gram negative bacteria), acylated form of glucose and sucrose in plants	Messias et al. (2018), Fahy et al. (2005, 2011), Ghangas and Steffens (1993)
Polyketides	Used as antimicrobial, antiparasitic, anticancer agents, messengers in cell–to–cell communication	animal, plant and microbial sources	Donato et al. (2013). Messias et al. (2018), Fahy et al. (2005, 2011)

(continued)

Table 25.1 (continued)

Lipids	Patho–physiological functions	Dietary sources	References
Fatty acids			
Saturated and *trans*–fatty acids	Increases bad cholesterol level in blood, heart diseases and stroke, atherosclerosis, hardening of the arteries, hyperlipidemia, hypercholesterolemia, coronary artery disease (CAD), peripheral artery disease (PAD), CVD, triglycerides in the bloodstream and promoting systemic inflammation, risk of hypertension, body weight/adiposity, diabetes, liver dysfunction	Animal tissues (red meat); dairy foods such as whole fat milk, butter, high–fat cheeses, ice cream products; fried foods; palm and coconut oils; some prepackaged foods, etc.	Destaillats et al. (2009), FAO (2010)
Monounsaturated fatty acids	Reduce risk of cancer, neurodegenerative, diseases, diabetes, early death, non–communicable diseases, body weight/ adiposity, CHD events; decrease bad cholesterol and increase good cholesterol	Animal flesh like red meat; whole milk products; nuts; high fat fruits (olives and avocados); Plant and vegetable oils (sunflower canola, avocado, macadamia, grape seed, groundnut sesame, and corn, almond, hemp oil); cashews, popcorn, whole grains (wheat, cereal, oat)	Martinez-Lacoba et al. (2018), FAO (2010)
Poly unsaturated fatty acids	Decrease risk of hypertension, arterial stiffness, improve cardiac function; prevent inflammation, heart disease, cancer, arthritis, depression, and Alzheimer; maintains optimum blood pressure, heart rate, triglycerides levels	Plants and vegetable oils (flax seeds, safflower oil, and unhydrogenated soybean oil); cold water fatty fish (salmon, mackerel, and sardines, tuna, cod, and anchovy); walnuts; and some types of vegetables	Sánchez-Villegas and Sánchez-Tainta (2018), Christie and Han (2012), FAO (2010)

three major biological functions in the body. They act as energy storehouses, serve as major structural components of cell membranes, and exercise a key role in cell signaling. In addition, they protect organs in fat–storing tissues, provide insulation to aid in temperature regulation, as well as manage other functions in the body (Mahan and Raymond 2016; Titchenal et al. 2018). There are three main types of lipids: glycerides, phospholipids, and sterols. Glycerides are fatty acid esters of glycerols. These are water insoluble and make up more than 95% of lipids in the diet and are commonly found in vegetable oil, butter, whole milk, cheese, cream cheese, and some meats. Phospholipids are water–soluble and make up about 2% of the dietary lipids. They are found both in plants and animals and are crucial for building the protective barrier around cell and organelle membranes. Sterols, the least common type of lipid, regulate biological processes and sustain the domain structure of cell membranes where they are considered as membrane reinforcers. Some important animal and plant sterols include cholesterol, ergosterol, stigmasterol and sitosterol (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Fatty Acids Fatty acid is a carboxylic acid with a long aliphatic chain that is either saturated or unsaturated at room temperature. Fatty acids differ from one another in carbon chain length and degree of saturation. The naturally occurring fatty acids have an unbranched chain of an even number (from 4 to 28) of carbon atoms. They usually have a *cis* configuration whereas *trans* ones are synthetic in origin. Vegetable oils are converted into semisolid fats by hydrogenation of the double bonds to overcome rancidity and to increase their shelf life (Mahan and Raymond 2016; Titchenal et al. 2018).

Fatty acids are required for the proper and normal function of all the body systems including circulatory, respiratory, nervous, integumentary, and immune system. The body is capable of synthesizing most of the fatty acids which are referred to as nonessential fatty acids. Fatty acids that are not synthesized by the body, and their requirement is fulfilled from diet are termed as essential fatty acids which play an important role in the life and death of cardiac cells, immune system function, and blood pressure regulation. Essential fatty acids also referred to as omega (ω) fatty acids are characterized as ω–3 and ω–6, ω–7, and ω–9 where 3, 6, 7, and 9 refers to the position of the first carbon–carbon double bond and the ω refers to the methyl end of the chain. ω–3 and ω–6 fatty acids are precursors to eicosanoid hormones which control other hormones to impart important body functions like the central nervous system and immune system. Eicosanoids derived from ω–6 fatty acids are known to increase blood pressure, immune response, and inflammation while those derived from ω–3 fatty acids are responsible for healthy heart. Docosahexaenoic acid (DHA), a ω–3 essential fatty acid plays an important role in synaptic transmission in the brain during fetal development (Gibney et al. 2009).

Phospholipids Phospholipids are diglycerides in which two fatty–acid molecules are attached to the glycerol backbone while the third hydroxyl function of glycerol backbone is attached to a phosphate moiety coupled with a nitrogen–containing group. This unique structure makes phospholipids water soluble. The phospholipids are thus amphiphilic lipids wherein the fatty–acid sides are hydrophobic (dislike water) while the phosphate group is hydrophilic (likes water) in nature. In blood and other body fluids, phospholipids form structures in which fat is enclosed and transported throughout the blood stream. In the body, amphiphilic nature of phospholipid binds together to form a double layer in cell membranes. This layer effectively not only protects the inside of the cell from the outside environment, but also allows transport of fat and water through the membrane. For example, lecithin (phosphatidylcholine), found in egg yolk, honey, and mustard, is a popular food emulsifier that does not allow the separation of fat and water. Another example is mayonnaise that demonstrates similar ability to blend vinegar and oil to create the stable, spreadable condiment. Phospholipids thus play an important role in making the appearance of sauces, creams and other foods fresher and appetizing (Mairrino et al. 1991; Gibney et al. 2009).

Phytosterols Phytosterols (sterols and stanol esters) are naturally occurring compounds that are found in plant cell membranes. Some examples of phytosterols are β–sitosterol, campesterol, stigmasterol, and ergosterol while the most abundant is sitosterol and campesterol. These are structurally similar to the cholesterol found in the human body. When consumed, phytosterols compete with cholesterol for absorption in the digestive system. As a result, cholesterol absorption is hindered and blood cholesterol levels reduced (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). Cholesterol has specific beneficial functions and is present in all body cells as it is an important substance in cell membrane structure. Approximately 25% of cholesterol in the body is localized in brain tissue. From cholesterol, body synthesizes vitamin D, glucocorticoids, and the sex hormones progesterone, testosterone, and estrogens. Stanols are also present in plants, but they form about 10% of total dietary phytosterols. Cycloartenol, stigmastanol, and campestanol are some examples of stanols (Gylling and Simonen 2015). Phytosterols perform several biological activities that promote the health of humans, animals, and micro–organisms by playing an important role in cholesterol absorption and lipoprotein metabolism, testosterone metabolism, alterations in cell membrane properties, induction of apoptosis in cancer cells, reduction of total and LDL cholesterol levels thereby reducing the risk of cardiovascular diseases, as well as prevention and treatment of colon, breast and prostate cancers (Ling and Jones 1995; Ogbe et al. 2015).

25.2.1.2 Carbohydrates

Carbohydrates are one of the most abundant substances found in green plants as a result of the photosynthesis process. They serve as energy source for humans and other living organisms. They are classified into four groups namely monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The smallest carbohydrates are monosaccharides such as glucose. When two molecules of monosaccharides are linked together, they form disaccharides. The three most common forms of monosaccharides are glucose, galactose, and fructose. Glucose is the most preferred source of energy for completion of cellular functions of the living organisms. Except during extreme starvation conditions, our brain completely depends on glucose as preferred source of energy. Another monosaccharide is galactose that differs from glucose only in the position of hydroxyl group on the fourth carbon atom. This small structural alteration causes galactose to be less stable than glucose and as a result the liver rapidly converts galactose to glucose which is then quickly utilized for energy production in cells. Fructose differs from glucose in its chemical structure, as the ring structure of fructose contains only five carbons. Unlike glucose, fructose is not an energy source for other cells in the body. Ribose is abundant in the nucleic acids (RNA and DNA), and some pentoses are components of fiber. Sugar alcohols like sorbitol, xylitol, and glycerol are industrially synthetic derivatives of monosaccharides. Due to their moderate sweetness and poor absorption sugar alcohols are often used by the diabetic patients in place of sucrose to sweeten foods. Due to their quick degradation by the bacteria in the mouth sugar alcohols do not cause tooth decay (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). The most common example of disaccharide is sucrose, lactose, and maltose composed of two monosaccharide units one of which is mainly glucose. Sucrose comprising of both glucose and fructose molecules is found in fruits and vegetables, and at high concentrations in sugar beet and sugarcane. Lactose, commonly known as milk sugar is prevalent in dairy products such as milk, yogurt, and cheese. Maltose on the other hand consists of two glucose molecules and is a common breakdown product of plant starches and is rarely found in foods as a disaccharide.

Oligosaccharides consist of three to six monosaccharide units but are not frequently found in natural sources. Polysaccharides are polymer of monosaccharides that may be branched or unbranched. Starch and cellulose are two main examples of polysaccharides which are considered as slow releasing carbohydrates. Starch is found in abundance in grains, legumes, and root vegetables, like potato. A plant starch amylose, is a small linear chain containing hundreds of glucose units, whereas, another plant starch amylopectin, is a large branched chain containing thousands of glucose units. These two starch molecules are found together in foods and are known as energy–storing molecules of plants. The smaller one (amylose) is less abundant. Eating raw foods containing starches provides very little energy as the digestive system takes longer to break them. Cooking results in degradation of larger starch molecule to small units which are easier to breakdown and easy to digest in the body. The starches that remain intact throughout digestion are called resistant starches. Bacteria in the gut can breakdown some of these and may benefit gastrointestinal health. Some of the isolated and modified starches are used widely in the food industry as food thickeners. Polysaccharides such as starch, cellulose and glycogen are large molecules comprising of many glucose units. Grains, milk, fruits, and starchy vegetables are the major food sources of carbohydrates whereas, non–starchy vegetables contain carbohydrates in lesser quantities. Carbohydrates are the perfect nutrients to meet the body's nutritional needs. Digestible carbohydrates in bulk are present in various foods, while indigestible carbohydrates provide a good amount of fiber with health benefits. Plant synthesizes carbohydrate (glucose) from carbon dioxide and water, by harnessing the solar energy. These glucose molecules are then utilized to make other larger 'slow–releasing' carbohydrates and smaller 'fast–releasing' carbohydrates (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). The simpler fast–releasing carbohydrates like monosaccharides and disaccharides stimulate the sweetness taste sensation, which is the most sensitive of all taste sensations. Some carbohydrates are much sweeter than the others. For example, fructose tops in the sweetness value.

25.2.1.3 Proteins and Amino Acids

Proteins are macromolecules composed of chains of subunits known as amino acids. Food sources of proteins include meats, dairy products, seafood, and a variety of different plant–based foods, most notably soy. Proteins provide structure to bones, muscles and skin, and play a role in conducting most of the chemical reactions that take place in the body (Mahan and Raymond 2016; Titchenal et al. 2018; Ribarova 2018). In each amino acid, amino group and a carboxylic acid group are attached to the structurally different cyclic/acyclic/alkyl/aryl moiety. Although structure of the twenty amino acids is unique, there are some chemical likenesses among them and are therefore, classified as non–polar, polar, acidic, basic, and neutral.

There are 20 different L–amino acids, and all are required to make different proteins present in the body. Eleven of these (*viz.* alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine) are called nonessential amino acids as the body can synthesize them. The remaining nine (*viz.* histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) are called essential amino acids as these cannot be synthesized in the body and must be obtained from the diet. The nutritional value of a protein depends on the type and quantity of amino acids contained in them. Thus, a protein structure is organized into four different structural levels such as primary, secondary, tertiary, and quaternary.

The first level is the one–dimensional sequence of amino acids that is held together by peptide bonds. They can be branched, coiled, fibrous, or globular, but their conformation is much more random and is not organized by their sequence of monomers. The second level of protein structure is dependent on the chemical interactions between amino acids, which cause the protein to fold into a specific shape, such as a helix (coiled spring shape) or sheet. The third level of protein structure is three–dimensional. As the different side chains of amino acids chemically interact, they either repel or attract each other, resulting in the folded structure. Thus, the specific sequence of amino acids in a protein directs the protein to fold into a specific, organized shape. The fourth level of structure is achieved when protein fragments called peptides combine to make one larger functional protein. The hemoglobin is an example of a protein that has quaternary structure. It is composed of four peptides that bond together to form a functional oxygen carrier. A protein's structure also influences its nutritional quality. Large fibrous protein structures are more difficult to digest than smaller proteins and some, such as keratin, are indigestible. Some fibrous proteins cannot be completely digested as all the amino acids are not absorbed and available for the body to utilize, thereby decreasing their nutritional value (Mahan and Raymond 2016; Titchenal et al. 2018; Ribarova 2018; Gibney et al. 2009; Lewis and Bayley 1995).

25.2.2 Micronutrients

Micronutrients are required by the human body in micro quantities and are considered essential for carrying out various functions. Sixteen minerals (Macro: Na, Cl, K, Ca, P, Mg, S; Trace: Fe, Zn, I, Se, Cu, Mn, F, Cr, Mo) and thirteen vitamins (A, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C, D, E, and K are the most significant essential micronutrients. Unlike macronutrients (carbohydrates, lipids, and proteins), micronutrients are not the sources of energy, but they assist in the process as cofactors and components of enzymes (i.e., coenzymes). Adequate intake of these micronutrients especially is necessary for healthy immune system and its normal functioning (Gibney et al. 2009; Campbell 2017).

25.2.2.1 Minerals

Mineral micronutrients are needed for regular metabolism and must be supplied with diet. While some minerals like calcium, phosphorus, magnesium and sodium are needed in considerable quantities, the others like copper, zinc, selenium, fluorine and chromium are needed in lesser quantities. Summary of different types, sources, and other valuable information of minerals is provided in Table 25.2.

Calcium Calcium is the most plentiful mineral in the body. About 99% of total calcium is located in the human skeleton while the remaining 1% is divided between teeth and soft tissues. Some amount is present in blood and other fluids in the human body. It plays a significant role in balancing body fluid, blood clotting, blood pressure regulation, enzyme activation, muscle contraction and nerve transmission (Marcus 2013; Gibney et al. 2009). It also helps in development and maintenance of bone and prevention of osteoporosis (WHO/FAO 2004a, b; Marcus 2013), reduces the absorption of dietary fat thereby lowering serum total cholesterol and low–density lipoprotein cholesterol concentrations (Vaskonen 2003). Calcium ion also plays a vital role in neuromuscular function, many enzyme–mediated processes, as well as providing rigidity to the skeleton by virtue of its phosphate salts (FAO/WHO 1998).

Table 25.2 Types of dietary minerals, their patho–physiological functions, dietary sources, and deficiencies

Minerals	Patho–physiological functions	Dietary sources	Deficiency	References
Selenium	Neutralizes free radicals and supports healthy immune system, thyroid hormone metabolism	Brazil nuts, mushrooms, seafood (especially oysters and tuna), Sunflower seeds, beans, meat, pork, beef, turkey, liver, poultry, eggs, oats, brown rice, ham, spinach, milk and yogurt	Keshan disease	Marcus (2013), Combet and Buckton (2019)
Sulphur	Component of many proteins, energy metabolism as part of the electron transport chain, slow down aging process, detoxifies the liver and cells, improves mental focus and clarity, improves quality of sleep, skin, and athletic performance, as a constituent of antioxidant	Cruciferous vegetables (broccoli, cauliflower, cabbage, kale, brussels sprouts, turnips, bok choy and kohlrabi), protein–rich foods (fish, poultry, meats, nuts and legumes), allium vegetables (garlic, onions, leeks and chives), eggs,	Reduced protein synthesis and endogenous antioxidants, like glutathione	Marcus (2013), Combet and Buckton (2019)
Zinc	Catalytic, structural and regulatory roles, energy metabolism, DNA and RNA synthesis, protein synthesis, expression of multiple genes, protection of mucosal cells, functioning of immune and reproductive systems	Meat, oyesters, beef, firm tofu, shellfish, legumes (lentils, chickpeas, and beans), nuts (pine nuts, peanuts, cashews and almonds), dairy products (milk and cheese), seeds (hemp, pumpkin, squash and sesame seeds), eggs, whole grains, potato	Growth retardation, sexual and skeletal immaturity, neuropsychiatric disturbances, dermatitis, alopecia, diarrhoea, susceptibility to infection and loss of appetite	Marcus (2013), Combet and Buckton (2019)
Calcium	Building bones, development of bones and teeth, transmission of nerve impulses, blood clotting, normal heart action, normal muscle activity	Milk, cheese, sardines, salmon, dark green, leafy vegetables like bok choy, broccoli and kale, fish, fortified beverages, soy products	Osteoporosis, osteomalacia, rickets, tetany, retarded growth, poor tooth and bone formation, balance, muscle contraction, nerve transmission	Marcus (2013), Combet and Buckton (2019)
Magnesium	Contributes to bone health and healthy immune function, skeletal development, gene regulation, nerve and muscle cell conduction	Green leafy vegetable, legumes, nuts, seeds, whole grains	Caused by a rare genetic abnormality, low dietary intake, hypocalcemia	Marcus (2013), Combet and Buckton (2019)

Potassium	Maintain healthy blood pressure level, contraction of muscles, maintain fluid balance, transmission of nerve impulses, osmosis, regular heart rhythm, cell metabolism	Oranges, bananas, dried fruits, vegetables, legumes, milk, cereals, meat	Hypokalemia, muscle weakness, confusion, abnormal heartbeat, low potassium intake	Marcus (2013), Combet and Buckton (2019)
Chloride	Hydrochloric acid in the stomach, chloride shift in erythrocyte plasma membranes, regulation of osmotic and electrolyte balances	Table salt or sea salt, processed foods and snacks, salted nuts, chips, sauces, canned food items, butter, salted meat, poultry, and fish, pickles, burgers, sandwiches, cheese, tomato ketchup, bacon and ham, tomatoes, celery, olives, lettuce and seaweed	Not diet–related only caused by clinical conditions	Marcus (2013), Combet and Buckton (2019)
Manganese	Catalytic co–factor for mitochondrial superoxide dismutase, arginase and pyruvate carboxylase, bone development, wound healing	Mussels, shellfish (clams, oysters, crayfish), whole grains, firm tofu, sweet potato, nuts and seeds (pine nuts, hazelnut, pecans, hemp seeds), leafy vegetables (lima beans, chickpeas, spinach, collards, peas, okra), and teas, pineapples	weight loss, dermatitis, growth retardation of hair and nails, decline of blood lipids, anemia, hormonal imbalance, low immunity, changes in digestion and appetite, infertility, weak bone, chronic fatigue syndrome	Marcus (2013), Combet and Buckton (2019)
Molybdenum	Co–factor for the iron– and flavin–containing enzymes that catalyse hydroxylation	Legumes (peas and lentils) beans (kidney beans, navy beans, and lima beans), nuts (almonds, cashews, chestnuts, and peanuts), soy products (soy milk, soybeans, and tofu), dairy products (cheese and yogurt), leafy vegetables, tomatoes, eggs, whole grains, liver	Deficiency difficult to induce	Marcus (2013), Combet and Buckton (2019)
Chromium	Insulin action, carbohydrate, lipid and nucleic acid metabolism	Meat, whole grain, fruits, vegetables, spices	Deficiency is rare	Marcus (2013), Combet and Buckton (2019)

(continued)

Table 25.2 (continued)

Minerals	Patho–physiological functions	Dietary sources	Deficiency	References
Phosphorus	Hydroxyapatite in calcified tissues, phospholipids in biological membranes, nucleotides and nucleic acid, maintain normal pH, storage and transfer of energy, activation of catalytic enzymes	Fish (tuna, salmon fillet, mackerel fillet), lean pork chops, firm tofu, dairy products (whole milk, low fat yogurt), lean chicken breast, scallops, lentils (white beans, chickpeas), seeds (squash, pumpkin, chia, hemp seeds), whole grains (oatmeal, brown rice, whole wheat pasta)	Hypo–phosphataemia, anorexia, anaemia, muscle weakness, bone pain, rickets and osteomalacia, general debility, increased infections, paraesthesia, ataxia, confusion	Marcus (2013), Combet and Buckton (2019)
Copper	Immune, nervous and cardiovascular systems, bone health, iron metabolism, haemoglobin synthesis, regulate mitochondria, gene expression	Seafoods (oyster, squid, lobster, crab), raw kale, mushrooms, sesame seeds, cashew nuts, beans, prunes, avocado, goat cheese, fermented soy foods, liver, spices, wheat bran	Homeostatic mechanisms	Marcus (2013), Combet and Buckton (2019)
Iron	Oxygen transport and storage, catalytic centre for metabolic functions, cell respiration and energy production, immune system, myelination and nerve development in the fetus	Fortified cereals, beef, shellfish (oysters, cuttlefish), dry fruits (apricots, peaches, prunes, figs), beans (white beans, soybeans, lentils, kidney beans, black beans, lima beans, chickpeas), vegetables (spinach, swiss chard, turnip, kale)	Anaemia, impairment of the immune response, adverse effect on psychomotor and mental development in children	Marcus (2013), Combet and Buckton (2019)
Iodine	Thyroid hormones, growth and mental development, antibiotic and anticancer	Seaweed (brown: kombu kelp, wakame; red: nori), cod fish, dairy products (milk, yogurt), iodized salts, shrimp, tuna, eggs, prunes plums, lima beans	Goitre, hypothyroidism, cretinism, trouble producing saliva and properly digesting food, swollen salivary glands and dry mouth, skin problems, including dry skin, poor concentration and difficulty retaining information, muscle pains and weakness, increased risk for fibrosis and fibromyalgia A higher risk for developmental problems in babies and children	Marcus (2013), Combet and Buckton (2019)

Fluoride	Fluorapatite in teeth and bones	Fluoride tends to get concentrated in tea, coffee, shellfish (blue crab, shrimp), grapes (seedless raisins, wine, grape juice), artificial sweeteners, sodas, potatoes, flavored popsicles, baby foods, broths, stews, and hot cereals made with tap water.	Risk of dental caries	Marcus (2013), Combet and Buckton (2019)
Sodium	Extracellular electrolyte regulation of osmotic and electrolyte balances, nerve conduction, muscle contraction, energy dependent cell transport systems, formation of mineral apatite of bone	Shrimp, ham, soups, cottage cheese, vegetable juices, salad dressing, pizza, sandwiches, canned vegetables, fishes, poultry, and meats, pickles, sauces	Not diet–related, trauma	Combet and Buckton (2019)

Magnesium Magnesium is required for essential cellular activities that support various physiological functions. It is involved in several enzymatic reactions including glycolysis, fat and protein metabolism, and adenosine triphosphate hydrolysis in which food is metabolized and new products are formed (Shils 1998). It also serves as a physiologic regulator of membrane stability and neuromuscular, cardiovascular, immune, and hormonal functions. Dietary restriction of magnesium and its deficiency results in reduced magnesium status, and impaired physiologic function and performance in untrained adults (Lukaski 2004). Green, leafy vegetables and unprocessed grains are the dietary sources of magnesium. Some water sources have also been reported to be good sources of magnesium (Lukaski 1995; Gibney et al. 2009).

Iron Iron is an essential element in living organisms as it plays a vital role in oxygen transport, DNA synthesis, and electron transport in various metabolic processes. Its deficiency and overload can lead to anameia, and other neurodegenerative diseases. The body requires iron for the synthesis of its oxygen transport proteins e.g. haemoglobin and myoglobin and for the formation of heme and other iron–containing enzymes involved in electron transfer and oxidation–reductions (Abbaspour et al. 2014). It is important for growth, healthy immune system and for energy production. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation (Malhotra 1998). There are two forms of iron: heme iron and non–heme iron. The heme iron is highly bioavailable and is found in animal products (red meat, fish, and poultry) and in some vegetable products (beans, nuts, and dried fruit), whole grains, fortified breakfast cereals, soybean flour and most dark green leafy vegetables (Arinola 2008). The heme iron content in food from animal source is about 40% of the total iron. Whereas, red meat provides more heme iron than white fish. Non–heme iron is less bioavailable and is found mostly in plant–based foods and makes up the remaining estimated 60% of iron found in animal products (Schönfeldt et al. 2016). Iron compounds recommended for food fortification include ferrous sulfate, ferrous fumerate, ferric pyrophosphate, and electrolyte iron powder.

Selenium Selenium is of fundamental importance to human health as it is required by the body in small amounts for proper functioning of the immune system. Its deficiency occurs when the body does not get enough mineral. The main functions of Se are in enzymatic activity, reducing the risk of coronary heart disease (CHD) through its antioxidant effect (WHO/FAO 2004a; Brown and Arthur 2001) and protecting against prostate cancer (Clark et al. 1998; Khokhar et al. 2012). It is associated with the protection of body tissues against oxidative stress and infection, and modulation of growth and development. Approximately 30, 15, 30, and 10 % of tissue selenium is located in liver, kidney, muscle, and blood plasma, respectively. The tissue selenium is present in proteins as seleno–analogues of sulfur amino acids. Other metabolically active forms of selenium include seleno trisulphides and acid–labile selenium compounds. The selenoenzyme thioredoxin reductase is involved in disposal of the products of oxidative metabolism (Howie et al. 1998). It contains two selenocysteine groups per molecule and is a major component of a redox system, having the capacity to scavenge toxic concentrations of peroxides and hydroperoxides which induce cell death and tissue atrophy (Mairrino et al. 1991). Another group of selenoproteins are the iodothyronine deiodinases essential for the conversion of thyrocin or tetraiodothyronine (T4) to its physiologically active form tri–iodothyronine (T3) (Arthur 1997). Keshan disease linked to coxasackie B virus and dietary deficiency of selenium was named after Keshan county of China. The primary symptoms of Keshan disease is myocardial necrosis leading to weakening of heart. Another major disease caused by Se is Kaschin–Beck is bone and joint disease referred to as osteoarthropathy which occurs in children of China and in Southeast Siberia (Ge and Yang 1993; Li et al. 1984) areas where the availability of soil selenium for crop growth is low (Khokhar et al. 2012). Acidic soils high in organic matter and iron oxide content appear to be responsible for fixing selenium in the forms that are poorly absorbed by staple crops for example cereal grains, typically have a selenium content of less than 0.01ug/g (Johnson et al. 1996). Best natural sources of Se include Brazil nuts, fish, whole grain cereals, pork, ham, chicken, cheese, and eggs, mushrooms, lentils, spinach, milk, banana etc.

Zinc Zinc is required for proper growth and maintenance of human body and working of the body's immune system. It plays a vital role in cell division, cell growth, wound healing, and breakdown of carbohydrates. It is distributed widely in plant and animal tissues and occurs in all living cells. It is required for the structure and activity of more than 300 enzymes from many species. The significance of zinc is reflected by the numerous functions and activities over which it exerts a regulatory role (Falchuk 1993; Lukaski 2004; Vallee and Falchuk 1993). It is required in the synthesis of nucleic acid and protein, cellular differentiation and replication, and glucose use and insulin secretion. It exerts regulatory actions in the production, storage, and secretion of hormones and regulating interactions between hormones and receptors and end–organ responsiveness. Adequate zinc is needed for the integration of many physiologic functions in the body such as immunity, reproduction, taste, skeletal development, behavior, and gastrointestinal function. Carbonic anhydrase, a zinc metalloprotein (Falchuk 1993; Vallee and Falchuk 1993) that regulates the elimination of carbon dioxide from cells, may play a role in facilitating performance. Natural food sources of zinc include oysters, red meat, poultry, beans, nuts, whole grains, pumpkin seed and sunflower seeds. Certain herbs like alfalfa, burdock root, cayenne, chamomile, chickweed, dandelion, eyebright, fennel seed, hops, milk thistle, mullein, nettle, parsley, rose hips, sage, sarsaparilla, skullcap, and wild yam are also considered as good source of zinc (Lukaski 2004; Gibney et al. 2009).

Chromium Chromium is an essential nutrient required for carbohydrate and fat metabolism. It has been found to potentiate the action of insulin at the cellular level (Lukaski 1999). The mechanism involves various enzymatic changes including insulin binding to its membrane receptor which results in an intermolecular phosphorylation cascade leading to increased insulin sensitivity (Vincent 2000). Physiologically active forms of chromium facilitate insulin action, which results in a decreased need for insulin (Anderson 1997; Mertz 1993). The perception that the general public may consume diets low in chromium (Anderson and Kozlovsky

1985) have prompted physically active individuals to consider chromium as a limiting nutrient for promotion of physical fitness and health. The effect of chromium supplementation on weight loss and glucose metabolism in mildly obese women has been examined (Grant et al. 1997). Although most diets may not provide adequate chromium, the use of chromium supplements does not provide impetus to body built up and physical performance (Althuis et al. 2002; Lukaski 1999, 2004). Besides the seafood (shellfish, mussels, oysters), other sources of chromium include vegetables such as broccoli, potato, green beans, whole grain products, fruits such as apples, bananas, grapes, as well as milk and milk products.

Copper Copper plays a significant role in various metabolic processes in the body and allows several critical enzymes to function properly. Copper is the third most abundant trace mineral in the body after iron and zinc and the total amount in the body ranges between 75 and 100 mg. It is present in every tissue of the body, but stored primarily in the liver, and to a lesser amount in the brain, heart, kidney, and muscles. It is a constituent of various enzymes and is necessary for the haematologic and neurologic systems (Tan et al. 2006) as well as for growth and formation of bone, and myelin sheaths in the nervous systems. It helps in the iron absorption (Chandra 1990), incorporation of iron in haemoglobin, absorption of iron from the gastrointestinal tract, and transfer of iron from tissues to the plasma (Malhotra 1998). Copper deficiency is associated with alterations in cholesterol metabolism, bone health, cardiovascular risk, immune function, and frequency of disease infections (Araya et al. 2007; Gupta and Gupta 2014), and decreased Fe levels in some human tissues (Gupta and Gupta 2014). Biliary obstruction increases the excretion of copper through the kidney and intestinal wall (Murray et al. 2000). Although, copper is essential for human health but its consumption in excess amount may be harmful. Some good sources of copper nutrient include cocoa, liver, kidney, oysters, peas, raisins, betel leaves, areca nuts and other nuts, molluscs and shellfish (Gupta and Gupta 2014). Further, soft water contains more copper than hard water and water from the tap contains more copper than reservoir water (Soetan and Oyewole 2009).

Manganese Manganese is involved in glycoprotein and proteoglycan synthesis and is a component of mitochondrial superoxide dismutase. As a co–factor in phosphohydrolases and phosphotransferases, Mn is involved in the synthesis of proteoglycans in cartilage (Murray et al. 2000), bone and cartilage development (Leach 1988; Saltman and Strause 1993); and wound healing through stimulatory and antioxidative activity (Marrotte et al. 2010), as a part of enzymes involved in urea formation, pyruvate metabolism and the galacto transferase of connective tissue biosynthesis (Nielsen 1998). It activates many important enzyme systems and is required for the synthesis of acid mucopolysaccharides, such as chondroitin sulphate, to form the matrices of bones and egg shells. Manganese deficiency may result in lameness, enlarged hock joints, and shortened legs in pigs; leg deformities with over–knuckling in cattle, perosis or slipped tendon in chicks, poults and ducklings; and nutritional chondrodystrophy in chick embryos. Beside these, skeletal deformities and defects in shell quality occur when the manganese intake is inadequate (Chandra 1990). The largest quantities of manganese are found in avocados, nuts and seeds, seaweed, and whole grains and in blueberries, egg yolks, legumes, dried peas, pineapples, and green leafy vegetables (Karak et al. 2017).

Molybdenum Molybdenum is an essential trace element for humans, plants and animals. In plants, it is taken up as MoO_4^{2-} and is involved in NO_3 reduction, protein synthesis and biological N_2 fixation. In humans, molybdenum has been reported to reduce the incidence and severity of dental caries (Burguera and Burguera 2007) and is beneficial to individuals with sulfite sensitivity, asthma and elevated urinary ratios. Mo supplementation in human diets has also shown anti–carcinogenic benefits (Miller et al. 1991; Penney 2004). Although Mo is essential, it might create adverse effects if exposure is excessive to humans through food and beverage (Penney 2004).

Potassium Potassium plays an essential role in the fluid balance in the body and regulates the heartbeat. Its imbalance with in body may result in irregular heartbeats, diarrhea, excessive sweating, vomiting and even death. If brain cells are affected, a potassium deficiency can cause a stroke (Marcus 2013) which can be prevented by taking sufficient amount of K in the food. It can also reduce high blood pressure (BP) when taken in desired amounts (Khokhar et al. 2012). It is found mainly in fish, fruits and vegetables like baked potato, banana, cantaloupe, lima beans, orange juice, poultry, meats, whole grains, tea and yogurt (Gibney et al. 2009; Marcus 2013).

Phosphorus Phosphorus functions for energy metabolism and in bone metabolism. About 80% of the body's phosphorus is found in bones, and is essential for cellular function in phosphorylation and dephosphorylation reactions. It also acts as a buffer in acid–base balance, and in cellular structure as part of the phospholipid membrane. Because of its essential role in energy production, severe hypophosphatemia can be a life–threatening event. Phosphorus deficiency symptoms include impaired cardiac function, reduced contractions of the diaphragm leading to a weakened respiratory state, confusion, reduced oxygen delivery to tissues, coma, and even death. It is present in animal products like meats and milk, dried beans etc. (Gibney et al. 2009; Mahan and Raymond 2016).

Sulfur Sulfur is associated as integral constituent of some amino acids and proteins in food. Sulfur helps these amino acids in maintaining their shapes so that they can perform their roles in the human body. It helps to make cells rigid, such as those that are found in the hair, nails and skin. Sulfur deficiency leads to extreme protein deprivation and can be avoided by intake of protein supplements (Gibney et al. 2009; Marcus 2013). Sulfur is found in dairy products, eggs, fish, meat, poultry and seafood, cabbage, eggs, dairy products, fish, garlic, legumes, meats, nuts, onions, raspberries, and wheat germ.

Fluorine Fluorine is generally found in drinking water in the form of fluoride. A small amount of fluoride is present in soil, plants, and animal tissues (Rinzler 2011). It is a highly protective trace mineral for emerging teeth and dental maintenance and stored in bones and teeth. It also protects bones from mineral loss, as in osteoporosis. Fluoride hardens dental enamel and thus reduces the risk of cavities. Daily intake of high amounts of fluoride has been associated with fluorosis, a discoloration and mottling of the teeth during development. Drinking water, mouthwash, toothpaste, poultry, processed cereals, seafood, and seaweed are important sources of fluoride (Gibney et al. 2009; Marcus 2013).

Chloride Chloride is a negatively charged electrolyte outside the cells where it associates with sodium. Chloride helps in keeping fluids balance as Na^+ is present outside the cells while K^+ remains inside the cell. Apart fluid balance, chloride is required for the maintenance of the acid–base balance in the body. It is essential for digestion of protein in stomach as it is part of hydrochloric acid that is required for digestion of protein. Its deficiency is caused by diarrhea, diuretics, excessive sweating or vomiting which may lead to apathy, appetite loss, growth deficiency in children or muscle cramps (Gibney et al. 2009; Marcus 2013). It is mainly present in table salt as sodium chloride, condiments, processed foods and naturally in various vegetables like celery, lettuce, olive, tomatoes and seaweed.

Iodine Iodine plays an essential role in thyroid hormones that regulate growth, reproduction, temperature and metabolism of cells in the human body. These hormones are also essential for protein synthesis, tissue growth, and reproduction. The best natural sources of iodine are seafood and plants grown near the ocean. Goiter (a swollen thyroid gland) is caused by iodine deficiency which reduced the production of thyroid hormones. This condition may cause injury to the thyroid gland, coupled with weariness and weight gain. Another severe deficiency known as cretinism causes mental and physical retardation. Diets that exclude iodized salt, fish, seafood or seaweed may be deficient in iodine. Use of non–iodized salts in commercial food production and cooking and the replacement of iodine–based salts with bromine salts in baking, iodine status has however, become a subject of public health concern (Gibney et al. 2009; Rinzler 2011; Marcus 2013).

25.2.2.2 Vitamins

Vitamins are required in appropriate amounts in our daily diet to carry out various important biochemical activities in the body. These are essential in our body for growth, development, disease prevention and to improve immune system. Vitamins are routinely taken along with the diet to sustain all the barriers of the immune system like skin, cell–mediated and humoral immune system responses (Aslam et al. 2017; Ibrahim and El–Sayed 2016). Some major vitamins (A, B, C, D, and E) have been reported to boost up the immune system by strengthening the activity of immune cells during any pathogen attack or intrusion of some toxic materials either inhaled from air or present in the foods (Aslam et al. 2017; Ravisankar et al. 2015). Vitamins are classified as fat–soluble (vitamin A, D, E, and K) and water–soluble (vitamins B complex and C). Different dietary sources, deficiencies, and physiological functions of different vitamins is tabulated in Table 25.3.

Table 25.3 Types of dietary vitamins, their patho–physiological functions, dietary sources, and deficiencies

Vitamin	Patho–physiological functions	Dietary sources	Deficiency	References
Vitamin A	Maintenance of healthy vision, healthy immune function, bone health, cell integrity	Vegetable oils (sunflower, safflower, wheat germ, corn, and soybean oils), nuts (peanuts, almonds, and hazelnuts/filberts), seeds (sunflower seeds, Sea buckthorn seeds), vegetables (broccoli, red sweet pepper, turnip green, mustard green and spinach), fruit/berry (Sea buckthorn, mamey sapote, avocado, mango, kiwi)	Night blindness, xerophthalmia, keratinization of the skin	Marcus (2013), Combet and Buckton (2019), Ravisankar et al. (2015)
Vitamin D (Calciferol)	Regulate calcium and phosphorus, bone health, healthy immune function, support cell growth, insulin secretion	Fatty fish flesh (like salmon), fish liver oil, beef liver, cheese, egg yolk, mushrooms	Rickets, osteomalacia	Marcus (2013), Combet and Buckton (2019), Ravisankar et al. (2015)
Vitamin E	Antioxidant, healthy heart immune function	Nuts, seeds, vegetable oils, green leafy vegetable and fortified cereals, Sea buckthorn seed oil	Deficiency is rare serious neurological dysfunction	Marcus (2013), Combet and Buckton (2019), Ravisankar et al. (2015), Titchenal et al. (2018)
Vitamin K_1	Blood clotting, cofactor for carboxylation	Green leafy vegetables (mainly spinach, cabbage, and kale), avocado, kiwi, grapes and plant oils	Excessive bleeding, bruising, under nails blood clotting, dark blood–containing stool	Schwalfenberg (2017), Halder et al. (2019), Chatron et al. (2019)
Vitamin K_2 (menaquinone–4: MK–4) and (menaquinone–7: MK–7)	Osteocalcin, calcium transport, prevents calcium deposition; as for MK–4 long chain form with longer half–life	Butter, eggs yolks, lard, and animal–based foods, Fermented foods, over–the–counter (OTC) supplements		Schwalfenberg (2017), Flore et al. (2013), Halder et al. (2019), Chatron et al. (2019)
Vitamin K_3 (menadione)	Inhibition of macrophage functions, mobilization of intracellular calcium	Synthetic analogue of vitamin K considered a pro–vitamin		Schwalfenberg (2017), Halder et al. (2019), Chatron et al. (2019), Chen et al. (2012)

(continued)

Table 25.3 (continued)

Vitamin	Patho–physiological functions	Dietary sources	Deficiency	References
Vitamin B_1 (Thiamin)	Coenzyme, assists in glucose metabolism, RNA, DNA, and ATP synthesis, nerve conduction	Pork, enriched and whole grains, fish, legumes	Beriberi, fatigue, confusion, movement impairment, swelling, heart failure, Wernicke–Korsakoff syndrome	Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_2 (Riboflavin)	Coenzyme, assists in glucose, fat and carbohydrate metabolism, electron carrier	Beef liver, enriched breakfast cereals, milk and dairy products, steak, mushrooms, almonds, eggs, green vegetables	Ariboflavinosis, dry scaly skin, mouth inflammation and sores, sore throat, itchy eyes, light sensitivity, seborrhoeic dermatitis	Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_3 (Niacin)	Coenzyme: assists in glucose, fat, and protein metabolism, electron carrier	Liver, meat, poultry, fish, peanuts, enriched grains, legumes	Pellagra, diarrhea, dermatitis, dementia	Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_5 (Pantothenic Acid)	Coenzyme, assists in glucose, fat, and protein metabolism, cholesterol and neurotransmitter synthesis	Sunflower seeds, fish, dairy products	Muscle numbness and pain, fatigue, irritability, neuromotor disorders, mental depression, gastrointestinal complaints and increased insulin sensitivity	Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_6 (Pyridoxine)	Coenzyme, assists in amino acid synthesis, glycogneolysis, neurotransmitter and hemoglobin synthesis	Meat, poultry, fish, legumes, nuts, whole–grain cereals	Muscle weakness, dermatitis, mouth sores, fatigue, confusion; disorders of amino acid metabolism, convulsions	Combet and Buckton (2019), Titchenal et al. (2018)

Vitamin B_7 (Biotin)	Co–enzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis	Liver, yeast, soy flour, cereals, egg yolks, fish, pork, nuts and seeds	Muscle weakness, dermatitis, fatigue, hair loss, impaired fat and carbohydrate metabolism	Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_9 (Folate/folic acid)	Brain and spinal cord development, heart health; coenzyme, amino acid RNA, DNA, and RBC synthesis	Leafy green vegetables, Enriched grains, orange juice	Diarrhea, sore mouth, confusion, anemia, neural–tube defects in babies	Marcus (2013), Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin B_{12} (Cobalamin)	Regulate metabolism, blood cell formation, mental function	Meat, poultry, fish, eggs, milk and milk products,	Muscle weakness, sore tongue, nerve damage, neural–tube defects, pernicious anaemia, loss of apetite, weight loss, neurological changes, poor memory	Marcus (2013), Combet and Buckton (2019), Titchenal et al. (2018)
Vitamin C (Ascorbic acid)	Antioxidant, healthy immune function, bone health	Orange juice, grape fruit juice, strawberries, sweet red pepper, tomato, Sea buckthorn leaves and fruits	Scurvy, bleeding gums, joint pain, poor wound healing, loss of dental cement, subcutaneous haemorrhage	Marcus (2013), Combet and Buckton (2019), Ravisankar et al. (2015), Titchenal et al. (2018)

Vitamin A Vitamin A is a group of unsaturated compounds that includes retinoids. β– retinol, retinal, retinoic acid and pro vitamin A like carotenoids (β–carotene) (Tanumihardjo 2002). It occurs naturally in food stuffs of animal sources like fish, beef, liver, eggs, and dairy products. Green leafy vegetables and fruits serve as plant sources for carotenoids that can be converted into retinol. Vitamin A plays a key role in enhancing eye sight, growth, reproduction, blood cells formation and improves body's immune response (Hinds et al. 1997; Akram et al. 2011; Mahan and Raymond 2016; Titchenal et al. 2018). Vitamin A not only supports the vision of eyes but also maintains the coverings and linings of the eyes and other tissues. As an antioxidant, vitamin A protects cellular membranes, helps in maintaining glutathione levels, and influences the amount and activity of enzymes that scavenges free radicals. It is released into the blood bound to a retinol–binding protein, which transports it to cells. According to WHO, the vitamin A deficiency affects 127 million preschool children worldwide which results in increased risks of mortality and morbidity from measles and diarrheal infections, night blindness, and anemia. Among women vitamin A deficiency is likely to be associated with high mortality related to pregnancy. So, its consumption in daily diet should be increased according to dietary reference intake's (DRI) recommended values (Gibney et al. 2009; Aslam et al. 2017; Titchenal et al. 2018; Akram et al. 2011).

Vitamin B Complex Vitamin B complex consists of several different compounds that are grouped according to their functional distinctions (Hellmann and Mooney 2010). The members of B complex include thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9) and cobalamins (B_{12}) (Roje 2007). As discussed below, vitamin B complex (combined) and its individual constituents are used to promote the defensive role for prevention of diseases by boosting–up the immune system (Aslam et al. 2017).

Vitamin B_1 (Thiamin) Thiamin or thiomene plays an important function in glucose metabolism and acts as a cofactor for enzymes that break down glucose for energy production. It is used in the synthesis of neurotransmitters and is thus required for RNA, DNA, and ATP synthesis. The brain and heart are most affected by thiamin deficiency (beriberi) while other symptoms are fatigue, confusion, impaired movement, pain in the lower extremities, swelling, and heart failure. Another common thiamin deficiency known as Wernicke–Korsakoff syndrome causes beriberi like symptoms such as confusion, loss of coordination, vision changes, hallucinations, leading to coma and death. Whole grains, meat and fish are excellent natural sources of thiamin (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Vitamin B_2 (Riboflavin) Riboflavin is an essential component of flavoproteins, which are involved in various pathways of carbohydrate, lipid, and protein metabolism. Flavoproteins aid in the transfer of electrons in the electron transport chain. When riboflavin is taken in excess amounts as supplement, the excess is excreted through kidneys. Riboflavin deficiency, sometimes referred to as ariboflavinosis, is often accompanied by other dietary deficiencies associated with the majority of the

B vitamins. Riboflavin can be found in a variety of foods including milk. Due to photo–instability of riboflavin, milk must be stored in plastic containers or cardboard to help block the light from destroying the riboflavin in milk (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Vitamin B_3 (Niacin) Niacin also referred to as nicotinic acid or nicotinamide belongs to the group of pyridine carboxylic acid. It is a very important nutrient as every part of our body requires it for proper functioning. It is an essential component of the coenzymes nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), which is involved in the catabolism and anabolism of carbohydrates, lipids, and proteins. NADH is the predominant electron carrier and transfers electrons to the electron transport chain to make adenosine triphosphate (ATP). NADPH is required for the anabolic pathways of fatty–acid and in cholesterol synthesis. In contrast to other vitamins, niacin can be synthesized by humans from the amino acid tryptophan in an anabolic process requiring enzymes dependent on riboflavin, vitamin B_6, and iron. Niacin deficiency is commonly known as pellagra and the symptoms include skin rashes, rough skin, fatigue, decreased appetite, indigestion leading to diarrhea, dermatitis, dementia, and sometimes death. As a supplement, niacin may help lower cholesterol, ease arthritis, boost brain functioning, and treat respiratory and vascular disorders. Niacin is found in a variety of different foods such as yeast, meat, poultry, red fish, brown rice, peanuts, and peas (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Vitamin B_5 (Pantothenic Acid) Pantothenic acid or pentothenate is vital for healthy life. It is an essential nutrient that forms coenzyme A, which is the main carrier of carbon molecules in a cell. Acetyl–CoA is the carbon carrier of glucose, fatty acids, and amino acids into the citric acid cycle and is involved in the synthesis of lipids, cholesterol, and acetylcholine (a neurotransmitter). Signs and symptoms of its deficiency include fatigue, irritability, numbness, muscle pain, and cramps. Vitamin B_5 is generally used in combination with other B vitamins in the form of vitamin B complex formulation. Pantothenic acid is used as an ingredient for skin and hair care products; however, there is no good scientific evidence to suggest that pantothenic acid improves human skin or hair. Pantothenic Acid is widely distributed in all types of food, that is why a deficiency of this nutrient is very rare (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). Some important Vitamin B_5 sources are: mushrooms, legumes, lentils, avocadoes, milk, eggs, cabbage, potato, whole grain cereals, and yeast.

Vitamin B_6 (Pyridoxine) Vitamin B_6 contains 3 pyridine derivatives hence the name pyridoxine (Aslam et al. 2017; Shabbir et al. 2013). It acts as co–factor for certain enzymes that helps in carrying out their respective functions (Hellmann and Mooney 2010). Vitamin B_6 coenzyme is essential for the conversion of amino acid methionine into cysteine (Titchenal et al. 2018) and helps to control blood homocysteine level (Aslam et al. 2017; Shabbir et al. 2013). It guarantees a healthier skin, prevents hair loss, detoxify liver, cures anemia, and improves cognitive function.

A deficiency in vitamin B_6 can cause anemia, but it is of a different type than that caused by insufficient folate, cobalamin, or iron. Lower hemoglobin content results in less capacity for carrying oxygen and hence weak muscles, fatigue, and shortness of breath occurs. Other deficiency symptoms include dermatitis, mouth sores, and confusion (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). The body needs B_6 in order to absorb vitamin B_{12} and to make RBCs and cells of the immune system. It helps the body to make several neurotransmitters like serotonin (Aslam et al. 2017; Shabbir et al. 2013) which helps improve immune response to increase production of antibodies and increase communicative interactions between cytokines and chemokines (Kunisawa and Kiyono 2013). Its deficiency reduces the lymphocyte growth and proliferation, antibody formation and T–cell activity (Rail and Meydani 1993). Vitamin B_6 is found in a variety of foods and the richest sources include fish, beef liver and other organ meats, eggs, potatoes, green beans, cereals, whole grains, wheat germ, walnuts, and vegetables like cabbage, cauliflower, carrots, and fruits like banana.

Vitamin B_7 (Biotin) Vitamin B_7 also formerly known as vitamin H or coenzyme R is a water–soluble vitamin (NIH 2017). It is involved in a wide range of metabolic processes of fats, carbohydrates, and amino acids in both human and other organisms. Biotin is required as a coenzyme in the citric acid cycle and in lipid metabolism, and as an enzyme in the synthesis of glucose and some nonessential amino acids. A biotinidase enzyme is required to release biotin from protein so that it can be absorbed in the gut. Biotin deficiency is rare, but can be caused by eating large amounts of egg whites over an extended period of time. This is due to the fact that a protein in egg whites tightly binds to biotin making it unavailable for absorption. A rare genetic disease–causing malfunction of the biotinidase enzyme also results in biotin deficiency. Symptoms of biotin deficiency are similar to those of other B vitamins, but may also include hair loss when severe. Biotin can be found in small amounts in foods such as dairy products, eggs, fish, meat, seeds, nuts, and certain vegetables like cauliflower and fruits like banana (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Vitamin B_9 (Folic Acid or Folate) Vitamin B_9 is crucial for proper brain function and plays an important role in mental and emotional health. Vitamin B_9 deficiency can cause poor growth, gingivitis, tongue inflammation, shortness of breath, forgetfulness, and loss of appetite. Its deficiency in central nervous tissue may lead to depression, insomnia, fatigue, anxiety etc. (Huskisson et al. 2007). It can be taken orally or by injection in the form of folic acid and is used to treat anemia caused by folic acid deficiency. It plays a vital role in immunity enhancement as well as in the biosynthesis of nucleic acids, proteins (Stover 2004) in blood cells and nervous tissues (Huskisson et al. 2007). Vitamin B_9 deficiency also lowers production of CNS cells, red blood cells, white blood cells, and platelets in the bone marrow from dividing stem cells. Children whose mothers were folate–deficient during pregnancy have a higher risk of neural–tube birth defects. Folate is found naturally in a wide variety of food mainly in dark leafy vegetables, fruits, and animal products (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018) poultry, shellfish, salmon, tuna, whole grains, wheat germ, beans, soybeans, beets, dark leafy greens, orange juice, citrus fruits, cantaloupes, avocado, asparagus, milk, etc.

Vitamin B_{12} (Cobalamin) Cobalamin is a water–soluble vitamin that contains a cobalt metal ion. It is needed for nerve tissue health, brain function, and the production of red blood cells. It is necessary for metabolism of every cell of the human body, mainly influencing DNA synthesis, fatty acid, and amino acid metabolism for folate coenzyme function, and in hemoglobin synthesis. Vitamin B_{12} benefits CNS in many important ways. It maintains health of nerve cells, and helps to form the protective covering of nerve (myelin sheath) for smooth functioning of the nervous system. It is also involved in B–cell synthesis and T–cell multiplication of lymphocyte (WBC) to strengthen bones and immune system (Sakane et al. 1982). Vitamin B_{12} deficiency causes macrocytic anemia in children and adults. There is an increased risk for neural–tube defects in babies born to cobalamin–deficient mothers. Lower Vitamin B_{12} levels cause a reduction in RBC formation and prevent them from proper development. Other deficiency symptoms include sore mouth or tongue, weight loss, pale skin, diarrhea, and menstrual problems. B_{12} deficiency also renders people more susceptible to other infections (Gibney et al. 2009; Huskisson et al. 2007). Vitamin B_{12} cannot be synthesized naturally in humans and plants but present naturally in animal products such as fish, meat, poultry, eggs, and milk products.

Vitamin C (Ascorbic Acid) Commonly known as the L–ascorbic acid, vitamin C is essential in the diet for humans, although most other mammals can readily synthesize it. It is found in almost every type of body tissue but is present in higher concentrations in pituitary gland and central nervous system (Huskisson et al. 2007). It stimulates absorption of iron from the intestine and modulates transportation of iron for storage. It is involved in cellular growth and differentiation, collagen formation, and in the formation of catecholamine and carnitine (Kraemer et al. 2012). In the brain it aids in natural synthesis of vital neurotransmitters like dopamine and noradrenalin (Huskisson et al. 2007). Ability of vitamin C to easily donate electrons makes it a highly effective antioxidant. It neutralizes and removes reactive oxygen species (ROS) formed in immune cells which would lead to the destruction of immune cells (Maggini et al. 2007). Vitamin C actively takes part in warding off the infectious agents during an infection (Hume and Weyers 1973). It protects lipids both by scavenging free radicals and by aiding in the regeneration of vitamin E. It boosts up human immunity towards infections and cold illnesses by increasing phagocytosis, lymphocyte proliferation and neutrophil chemotaxis against exogenous pathogens. Vitamin C deficiency causes scurvy, the signs and symptoms include skin disorders, bleeding gums, painful joints, weakness, depression, and increased susceptibility to infections. It can be prevented by the intake of vitamin C rich fruits and vegetables. Higher intake of vitamin C reduces the risk of gout which is caused by elevated levels of uric acid characterized by recurrent attacks of tender, hot, and painful joints (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). Deficiency of ascorbic acid in the body leads to suppressed immune response, susceptibility to infections, weak collagen formation and in case of an injury wound healing process gets delayed (Wintergerst et al. 2006). The richest sources of vitamin C include: Indian gooseberry, citrus fruits such as limes, oranges, and lemons, tomato and tomato juice, kiwi fruit, strawberries, guava, bell peppers, papaya, and broccoli.

Vitamin D (Calciferol) Vitamin D is composed of two main groups: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) that have biological actions in human body (Kraemer et al. 2012). Ergocalciferol provides protection against inflammation of adipose tissues (Gao et al. 2013) and is mainly found in the bones and teeth and help in their maintenance (Gombart 2009). Cholecalciferol plays an important part in regulating human immune response (Mora et al. 2008). It regulates blood calcium levels in concert with parathyroid hormone and is transported via certain D_3–binding proteins to the specific tissues or cells where required (Haussler et al. 2013). Vitamin D_3 is naturally produced by the human cells in the presence of ultraviolet radiations from the sunlight whereas the D_2 is not synthesized in humans but is synthesized in the plants and can also be utilized (Bikle 2009). Lower concentrations of the vitamin D in body are the cause of increase in infection rates apart from rickets, and other bone and joint disorders. The lack of this vitamin has also led to the increased death rates due to cancers, heart diseases, tuberculosis (Hewison 2012) and other microbial infections (Gombart 2009). The intake of vitamin D results in prevention of cardiovascular diseases and prevents the growth of cancer cells, blocks inflammatory pathways, reverses atherosclerosis, increases insulin secretion, and blocks viral and bacterial infections (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018). The main sources of vitamin D from food includes fish, oyster, fish liver oils, egg yolk, cheese, mushroom but the common source of vitamin D present in nature is the early morning sunlight (Kraemer et al. 2012). Some food products like cow's milk, soy milk, orange juice, vegetable oils and certain cereals and oat meals that do not contain vitamin D are fortified with this vitamin.

Vitamin E The fat–soluble vitamin E is comprised of eight chemical forms including four tocopherols (α, β, γ, δ) and four tocotrienol. α–Tocopherol is the only form that is best derived from plants and is known for its role in maintaining health in the humans (Niki and Traber 2012). α–Tocopherol and other forms of vitamin E constituents are mainly responsible for protecting cell membranes against lipid destruction caused by free radicals, thus acting as antioxidant (Traber and Atkinson 2007). Vitamin C helps in protecting α–tocopherol from disrupting free radicals and therefore, to maintain vitamin E levels, vitamin C should be ingested as part of the diet. Vitamin E has many other important roles and functions in the body such as boosting the immune system by inactivating and inhibiting the free radicals (Maggini et al. 2007) by helping to fight off bacteria and viruses (Maslova et al. 2014). It also enhances the dilation of blood vessels and inhibiting the formation of blood clotting.

Vitamin E reduces the oxidation of low–density lipoproteins (LDLs), and therefore its supplementation protects against atherosclerosis and reduces the risk of developing cancer (Gibney et al. 2009; Ravisankar et al. 2015; Titchenal et al. 2018; WHO/FAO 2004a). When the diet is supplemented with vitamin E, it resulted in the mitogen stimulated increased T–lymphocyte multiplication, increased cytotoxic cell activity and maximize the action of macrophages against the intruders thus providing the strong basis of action against infections (Meydani et al. 2005; Pae et al. 2012). Increased vitamin E content also inhibits the activation of protein kinase C (PKC) enzyme which in turn leads to the reduced platelets, nitric oxide and also reduced superoxide accumulation in macrophage cells and neutrophils (Azzi et al. 2002). The deficiency of vitamin E in the body leads to the development of a disease called ataxia (Jiang 2014). The main sources of vitamin E are nuts (almonds, peanuts, hazelnuts), seeds, vegetable oils, wheat germ, green leafy vegetable and fortified cereals.

Vitamin K These are a group of fat–soluble vitamins having a common 2–methyl–1,4–naphthoquinone nucleus but differ in the structures of a side chain at the 3–position. They are synthesized by plants and bacteria. In plants the only important molecular form is phylloquinone (vitamin K_1) with phytyl side chain. Bacteria synthesize a group of compounds called menaquinones (vitamin K_2), which have side chains based on repeating unsaturated 5–carbon (prenyl) units and menadione (K_3) which is analog of 1,4–naphthoquinone with a methyl group in the 2–position. Vitamin K is critical for blood function, acting as coenzymes which play a crucial role in the production of many proteins that are involved in blood coagulation and calcium homeostasis. The original term vitamin 'K' comes from German word Koagulation (coagulation) meaning the ability to clot blood or prevent hemorrhage (Gibney et al. 2009; Ravisankar et al. 2015). Deficiency in vitamin K causes bleeding disorders. The role of vitamin K_2 in osteoporosis, vascular calcification, osteoarthritis, cancer, and cognition is studied by Schwalfenberg (2017) and its deficiency has been linked with vascular calcification and osteoporosis (Flore et al. 2013). However, vitamin K deficiencies are uncommon, but many drugs such as antacids, blood thinners, antibiotics, aspirin, and drugs for cancer, seizures, high cholesterol, and other conditions, can interfere with the effects of vitamin K (Chatron et al. 2019). It has also been observed that people with liver or pancreatic disease, celiac disease, or malabsorption conditions are at higher risk for vitamin K deficiency (Reddi et al. 1995; Kawashima et al. 1997). Signs and symptoms include nose bleeding, easy bruising, broken blood vessels, bleeding gums, and heavy menstrual bleeding in women. Vitamin K is found in highest concentrations in green vegetables such as broccoli, cabbage, kale, parsley, spinach, and lettuce and it can be synthesized via bacteria in the large intestine (Titchenal et al. 2018). Vitamin K_2 supplementation is quite safe and does not induce hypercoagulation (Asakura et al. 2001).

25.2.3 *Other Nutrients*

25.2.3.1 Dietary Fiber

Dietary fiber is categorized as either water–soluble or insoluble. While soluble fibers dissolve or swell in fluid, insoluble fibers do not dissolve or swell in fluids. These are found in plant (leaves, seeds, fruits, and stems) foods in ample amounts. Dietary fibers are polysaccharides that are highly branched and cross–linked and include pectin, gums, cellulose, hemicellulose, and lignin. Lignin, however, is not composed of carbohydrate units. Humans do not produce the enzymes that can break down dietary fiber. Unlike starch, dietary fiber does not break down into single sugars. It does not contain any calories, and hence does not supply energy. Instead, it passes through the gastrointestinal tract undigested, which supports healthy digestion. Other possible health benefits of dietary fiber include the prevention and management of adult–onset diabetes, certain cancers, coronary heart disease and digestive tract disorders. Soluble fibers like inulin, pectin, and guar gum are found in apples, barley, legumes, oats, prunes, rye, algae, and some root vegetables, such as onions and potatoes. They help provide texture and consistency to foods such as jellies (citrus pectin), salad dressings (guar gum) and ice cream (carrageenan). Soluble fibers help delay the absorption of glucose from the bloodstream and lead to improved sugar tolerance in diabetics; slow the absorption of cholesterol and help to lower blood cholesterol in coronary heart disease; and provide fullness and satisfaction when they take up fluid and swell during digestion, which may benefit weight management. Soluble fibers are more easily accessible to bacterial enzymes in the large intestine so they can be broken down to a greater extent than insoluble fibers, but even some breakdown of cellulose and other insoluble fibers occurs (Gibney et al. 2009; Marcus 2013; Titchenal et al. 2018).

Insoluble fibers include bran, flax, hemicellulose, lignans, nuts, seeds, avocados, the skin of fruits and vegetables like cauliflower, and whole grain foods. The seeds and skin of fruits and vegetables are particularly good sources. Cellulose is the most abundant fiber in plants, making up the cell walls and providing structure. Insoluble fibers help to increase bulk and soften the stool so it passes quickly through the large intestine. This feature makes it potentially beneficial in the prevention of digestive diseases, including colon cancer, diverticulosis and hemorrhoids. Insoluble fibers also assist in weight management in that they speed the transit time of food in the intestines. For this reason, they may also be protective against gastro intestinal diseases.

A third type of dietary fiber is resistant starch (RS) that resists digestion in the small intestine due to its fibrous outer coat. It can produce the benefits of both soluble fiber and insoluble fiber. While sugars and starches are absorbed as glucose through the small intestine and used for short–term energy or stored, resistant starch passes into the large intestine, where it acts like dietary fiber. Resistant starch may contain some calories, but it does not raise blood sugar. Legumes, whole grains, unripe bananas, plantains and raw potatoes contain resistant starch. Resistant starch is also manufactured by chemical processes. There is enough scientific evidence to support that diets high in fiber reduce the risk for obesity and diabetes, which are primary risk factors for cardiovascular disease (CVD) (US Department of Agriculture 2010).

25.2.3.2 Phytochemicals

Phytochemicals are the secondary metabolites of plants that function to protect the plant from environmental stresses including pollutants, ultraviolet light, and infestation by insect pests, fungi, and bacteria. Besides this, they provide various health benefits against oxidative stress, neurodegenerative disorders, cancer, diabetes, immune disease, and inflammation. These are found in small amounts in fruits and vegetables, grains, seeds, nuts, and legumes (Gibney et al. 2009; Lee and Min 2018; Titchenal et al. 2018). Plant–derived dietary phytochemicals from fruits and vegetables prevent or delay the onset of chronic diseases and perform other functions like mimicking hormones, altering absorption of cholesterol, and blocking the actions of certain enzymes. There has been a growing interest among consumers in plant–derived nutraceuticals and functional foods having scientifically proven health benefits (Howes 2018). Several clinical and epidemiological studies (*in vitro* and *in vivo*) have reported their relevance in maintaining health and preventing diseases (Howes and Simmonds 2014). Some important phytochemical nutrients like terpenoids, carotenoids, alkaloids, phenolics, phenolic acids, and flavonoids, organosulfur compounds and others exhibit significant health benefitting effects.

Terpenoids Plant–derived volatile essential oils are complex mixtures of phytochemicals like monoterpenes, sesquiterpenes, and diterpenes that are used for both culinary and medicinal purposes. Among the various volatile monoterpenes 1,8–cineole was found effective against respiratory disorders (Howes 2018). Similarly α–humulene has been found to be potent phytopharmaceutical in modulation of inflammatory pathways (Howes 2018; Guimarães et al. 2014). Diterpenoids are also reputed for their medicinal and phytopharmaceuticals properties. The steviol glycosides (diterpenes) found in *Stevia rebaudiana* leaves are good source of natural sweetener in the food industry and have potential health benefits. Triterpenoids are widely distributed in nature and occur as the C–30 aglycones or as esters or glycosides. Triterpenes from liquorice (*Glycyrrhiza* species) root include glycyrrhizin (a triterpene glycoside) and glycyrrhetinic acid, which have been investigated for their therapeutic potential to manage inflammatory lung diseases such as asthma. One of the major challenges with the use of natural product glycosides to manage inflammatory conditions, is that they may be hydrolyzed in the gut and the original biologically active constituent may not be adequately absorbed in this form (Howes 2018).

Carotenoids Carotenoids are lipid–soluble plant pigments found in photosynthetic plants, microbes, and in animal tissues. About 600 different carotenoids have been isolated and characterized in nature, and about 10% of these can be metabolized to vitamin A in a variety of animal species, including humans. Provitamin A carotenoids, such as α– and β–carotenes and cryptoxanthins, and non–provitamin A carotenoids, such as lutein, zeaxanthin, capsanthin, and carotenoids are lipid–soluble plant pigments found in photosynthetic plants and animal tissues, human blood have a variety of functions. Provitamin A carotenoids are an important source of dietary vitamin A that are found primarily in dark green leafy vegetables, such as spinach, yellow vegetables and in fruit, such as orange carrots, mango, and papaya. Their bioavailability is significantly more variable than that of preformed vitamin A (retinol) (Schönfeldt et al. 2016; Ravisankar et al. 2015).

Alkaloids Alkaloids are heterocyclic nitrogen containing natural products named due to their alkaline nature. Caffeine (1,3,7–trimethylxanthine) is the most consumed alkaloidal phytochemical in a large number of beverages in the world. Caffeine can be extracted from coffee, tea, cola, and cacao, or synthesised from uric acid. It is used in the preparation of beverages like coffee, tea, chocolate, cola drinks, as well as constituent in analgesic and diet pills. Caffeine acts as a stimulant of the central nervous system (CNS), heart muscles, and relaxes muscle structures including the coronary arteries and the bronchi. The beneficial effects of caffeine include prevention of cardiovascular and respiratory disease, inflammatory disorders, and as an agent that promotes kidney dieresis (Howes 2018; O'Keefe et al. 2018).

Phenolics, Phenolic Acids and Flavonoids Polyphenolics and flavonoids are the secondary metabolites present in plants having defensive mechanisms against oxidative stress, ultraviolet radiations, and other pathogens. Some of these are the most profuse antioxidants in the diet that modulates the activity of many enzymes and cell receptors. More than 4000 phenolics, stilbenes, phenolic acids, flavonoids, coumarins, tannins, lignans, and lignins have been recognized in plant foods like vegetables and fruits, grains, nuts, cereals, oilseeds, legumes, spices, chocolate, tea, and beverages (Scalbert et al. 2005). Among them, flavonoids are the most abundant polyphenols and are classified into flavonols, flavanones, flavones, flavanols, isoflavones, and anthocyanins (Afreen et al. 2018; Vauzour 2012; Yeh et al. 2017). Dietary fruits rich in polyphenolic compounds reduce the occurrence of cancer by inhibiting some enzymes that promote cell proliferation.

Some prominent phenolics with health benefitting effects include curcumins and resveratrol. Curcumin–I (1,7–bis–(4–hydroxy–3–methoxyphenyl)–1,6–heptadiene–3,5–dione) is a hydrophobic polyphenolic compound present in the rhizomes of *Curcuma longa* (turmeric). Among three curcuminoids (C–I, C–II, C–III), the more polar curcumin–III (demethoxylated curcumin, C–III) showed better activity than curcumin–I and II due to higher solubility and better bioavailability (Yadav et al. 2017). They exhibit significant antioxidative, anti–inflammatory, anti–angiogenic, pro–apoptotic, anti–cancer (Ghosh et al. 2015; Lu and Yen 2015; Pulido–Moran et al. 2016), anti–viral, anti–diabetes, and anti–neurodegenerative activities, as well as wound–healing properties. It is also used for the treatment of aging–related diseases like cardiovascular and neurodegenerative diseases, diabetes, atherosclerosis, rheumatoid arthritis, and hypertension. Another potent role of curcumin is as a nutraceutical to manage obesity and metabolic disorders (Howes 2018; Mobasheri et al. 2012). It has however limitations in therapeutic applications due to its low bioavailability and higher metabolic activity (Agrawal and Goel 2016; Cavaleri and Jia 2017). Resveratrol (3,5,40–trihydroxystilbene) the most common phenolics is found in grape (*Vitis vinifera*) juice, peanuts (*Arachis hypogaea*), pistachios (*Pistaciavera*) and blueberries (Perry and Howes 2011). It provides potent health benefits against cardiovascular disease, diabetes, cancer, and neurodegenerative diseases through its antioxidative and anti–inflammatory effects in circulatory system, potential of lowering cholesterol, and protecting against clots which can cause heart attacks and stroke (Howes 2018; Agrawal and Goel 2016; Cavaleri and Jia 2017).

Phenolic acids are categorized into two major classes: Hydroxybenzoic acid derivatives including protocatechuic acid, p–hydroxybenzoic acid, gallic acid, and hydroxy cinnamic acid derivatives like coumaric, caffeic, ferulic, chlorogenic, and sinapic acid. Gallic acid and caffeic acid help in preventing the cancer due to its potent antioxidant properties. Another potent antioxidant ferulic acid, present in oats, sweet corn, and tomatoes aids in reducing blood pressure and protect skin.

Flavonoids are a diverse group of phytochemicals found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. These are classified into flavonols (isorhamnetin, kaempferol, myricetin, quercetin), flavanols (catechin), gallocatechin (epicatechin gallate, epigallocatechin), flavones (apigenin, chrysin, luteolin, rutin), isoflavones (biochanin A), daidzin, formononetin, genistein, glycitein), flavononols (astilbin, engeletin, genistin, taxifolin), flavonones (eriodictyol, hesperidin, isosakuranetin, naringenin, naringin, taxifolin), anthocyanidins (cyanidin, delphinidin, epigenidin, malvidin, pelargonidin, peonidin, petunidin), and chalcones (butein, okanin) based on the structural variations in the number and positions of OH groups or the presence of ketonic group in the middle ring (Beecher 2003). Anthocyanins are another group of flavonoids that occur as glycosides and responsible for the blue, purple and red (as flavylium cation, 2–phenylbenzopyrilium) pigments in various fruits, vegetables and flowers. They depict anti–inflammatory, anti–hyperglycemic, anti–allergic, anti–microbial, and anti–cancer activities (Singh et al. 2017b; Woodward et al. 2009). Very recently, Cyanidin 3–O–glucoside has been reported to inhibit enzymes involved in CNS pathologies and enzymes like α–glucosidase and dipeptidyl peptidase–4 involved in type–II diabetes (Cásedas et al. 2019). Malvidin 3–O–glucoside, is reported for its anti–inflammatory actions in arthritis, as it decreases transcription of genes encoding inflammatory mediators and reduced inflammation in an animal model (Howes 2018; Decendit et al. 2013). The antioxidant and anti–inflammatory effects of the malvidin also contributed to the positive effects on health of moderate red wine consumption (Howes and Simmonds 2014; Howes 2018).

Quercetin is widely occurring plant flavonoid found in onions, apples, broccoli, grapes, blueberries, cherries, and *Ginkgo biloba*. It increases the survival rate of animals with diseases and is a potent antioxidant and has anti–inflammatory and anti–cancer activities (Kong et al. 2016). Rutin and quercetin present in Buckwheat (*Fagopyrum esculentum*), onion, berries, and apples provide protection against inflammation and cancer (Singh et al. 2009). Flavonol and kaempferol are widely distributed in common fruits such as apples and tomatoes, which defends the body against cancer, inflammation, and bacterial infections. The main flavonoid constituents of green tea flavanols (catechin, epicatechin, catechingallate, and epigallocatechingallate), flavonols (quercetin, kaempferol, and their glycosides), flavones (vitexin, isovitexin), and phenolic acids (gallic acid, chlorogenic acid), prevents the risk of cancer, CVD, and inflammations (Aneja et al. 2004). Catechins have also been known to improve the survival rate in animal disease models. Epidemiological studies suggest that chronic tea consumption may inhibit low–grade inflammation and benefit human health (Howes 2018; Wu and Schauss 2012; Vuong 2014), while monomeric and oligomeric flavan–3–ols from grape (*Vitis vinifera*) seeds reduce gene expression in leukocytes and improve vascular health in smokers when taken as a supplement in an intervention study (Howes 2018; Weseler et al. 2011). Soya (*Glycine max*) isoflavones such as genistein have potential anti–inflammatory benefits (Li et al. 2013) and healing metabolic disorders such as diabetes (Howes 2018). The other potential health benefits of flavonoids include antifungal (Friedman 2007), anti–bacterial (Cushnie and Lamb 2005), and anti–allergic activities (Kawai et al. 2007) and positive effect on the prevention of different types of cancer (ovarian, colon, lung, laryngeal, prostate, pancreatic, esophageal, breast, leukemia, renal cell carcinoma, and hepatocellular carcinoma) (Howes 2018; Agrawal and Goel 2016; Cavaleri and Jia 2017).

Organosulfur Compounds Several organosulfur compounds have been reported in garlic, onion, and several of cruciferous plants. Glucosinloates, the main nitrogen– and sulfur–containing compounds in cruciferous vegetables such as mustards and broccoli are known for their health benefits due to the presence of sulphur containing glucosinolates and other nutrients. They provide protection against cancer and cardiovascular disease. Glucosinolates remain intact unless brought in contact with the enzyme myrosinase by insects, food processing or chewing. Myrosinase release glucose and isothiocynate which inhibit mitosis and stimulate apoptosis in human tumor cells (Johnson 2002). Glucoraphanin (glucosinolates of sulforaphane) prevents cancer activity and is present in all cruciferous vegetables like broccoli, cabbage, and cauliflower (Singh et al. 2017a; Traka and Mithen 2009). Mustard oil helps in reducing the levels of glycosylated proteins, serum glucose, and lipid peroxidation, and stimulates glucose metabolism. Garlic and onion belonging to allium family have high amount of sulfur compounds like allicin, alliin, diallyl disulfides (in garlic), minerals, and vitamins, which help in preventing the numerous diseases like cancer, CVDs, obesity, and type 2 diabetes (Touloupakis and Ghanotakis 2010). Onion contains cepaenes and thiosulfinates, flavonoids (quercetin), vitamins, minerals (calcium, chromium), and dietary fiber which have antioxidant, anti–tumor, and immune enhancing properties. These compounds stimulate the action of amino acids to nervous system, reduce symptoms associated with diabetes mellitus, inhibit platelet aggregation (involved in thrombosis), and prevent inflammatory processes associated with asthma (Augusti 1990).

25.3 Nutrient Enhancers and Inhibitors

Interaction of nutrients with one another and with other dietary components at the site of absorption, affects their overall bioavailability. Addition of nutrient enhancers or inhibitors can therefore enhance, reduce or neutralize these activities. Nutrient enhancers increase the bioavaibility of nutrients by keeping a nutrient soluble and protect it from their interaction with the inhibitors. For example, carotenoids are fat–soluble, and addition of small quantities of fat or oil to the meal improves the bioavailability of carotenoids. Similarly, meat, fish, and poultry, while containing highly bioavailable iron, are also known to enhance the absorption of iron from other foods ingested at the same time. Although this meat factor has yet to be identified, it has been suggested that muscle protein may exert an influence. Nutrient inhibitors, on the other hand, reduce bioavailability by binding the nutrient in a form that is not recognized by uptake systems on the surface of intestinal cells, rendering the nutrient insoluble and, thus, unavailable for absorption. For example, phytic acid is abundant in certain plant foods (e.g. pulses, whole grain cereals, seeds, and nuts) and strongly binds minerals such as calcium, iron, and zinc in soluble or insoluble complexes, which are unavailable for absorption. The undesired phytic acid content of foods can be reduced by fermentation or by soaking and germination of pulses. The inhibitory effect of food constituents can also be used advantageously to provide health benefits. For example, phytosterols extracted from certain plant foods can be added in higher doses to a variety of other foods (e.g., spreads and fermented milk drinks) to reduce the absorption of cholesterol either from dietary sources or produced in the human body (Gibney et al. 2009; Gylling and Simonen 2015; Schönfeldt et al. 2016).

25.4 Nutrient Deficiency and Gut Health

Deficiency of micronutrients such as iron, zinc, vitamin A and folate is the main cause of hidden hunger across the world (Bailey et al. 2015) Nutrients–rich diet significantly modulates the metabolic activities of the gut microbiota (Le Chatelier et al. 2013) which uses micronutrients as substrates for the several physiological processes (Lozupone et al. 2012). Their deficiency may cause disruption in gut microbiota eubiosis balance leading to the development of colon cancer, allergy, cardiovascular diseases, obesity, and gastrointestinal disorder (Clemente et al. 2012). Acute deficiency of vitamin A has significant effect on microbiota composition and meta transcriptome, and enhances the population of *Bacteroides vulgates* (Hibberd et al. 2017) responsible for inflammatory bowel diseases, ulcerative colitis and Crohn's disease (CuÝv et al. 2017). Zinc deficiency is associated with reduction in gut microbiota diversity and production of short chain fatty acids (SCFAs) such as acetate and butyrate in the broiler chicken model (Reed et al. 2015). Likewise, deficiency of vitamin C and E leads to the depletion of microbiota of phyla

Bacteroidetes or *Firmicutes* while it promotes the growth of *Salmonella* and *Shigella* pathogens (Khan et al. 2012; Mach and Clark 2017).

Dietary proteins provide different amino acids which influences the host metabolisms and promote the gut microbiota growth and diversity, and undergo microbial fermentation to produce secondary metabolites such as bioamines, fatty acids and other nitrogen containing compounds (Lin et al. 2017). Fan et al. (2017) observed decrease in bacterial population in ileum, reduction of protein concentration and decrease in the concentration of metabolites such as SCFAs and bioamines. A consumption of diet low in Microbiota–accessible carbohydrates (MACs) or dietary fiber has been associated with reduction of gut microbiota diversity which results in development of colon cancer, diabetics, inflammatory and cardiovascular diseases (Holmes et al. 2011; Makki et al. 2018). A low fiber diet with abundance of *Bacteroides* levels, results in lower production of SCFAs (Chen et al. 2017). Some species of *Bacteroides* such as *B. fragilis* moreover cause diarrhea, inflammatory bowel diseases and gastrointestinal disorder (Wexler 2007). Recently, a study resulted in the richness of microbiota in the 15% crude protein diet along with the increase in the abundances of beneficial *Lactobacillaceae*, *Actinomycetaceae*, and *Micrococcaceae* (Chen et al. 2018).

25.5 Bioavailability of Nutrients

Bioavailability of desired nutrients to the cells from the gut plays a critical role for performing normal physiological functions (Fairweather–Tait 1997; Krebs 2001; Schönfeldt et al. 2016). Nutrient utilization is affected by a sequence of metabolic activities like uptake, solubilization, and enzyme secretion, enzymatic transformation, digestion, release, absorption and excretion. The bioavailability of macronutrients like carbohydrates, proteins, and fats, is generally higher (> 90%) than the micronutrients like vitamins, minerals, and bioactive phytochemicals such as flavonoids, carotenoids etc., and is dependent upon the amount of food digested, absorbed and/or utilized by the body. Thus, the supply of nutrients and non–nutrient bioactive compounds to the human body largely depends on both the amount in food and their bioavailability.

Bioavailability depends on many factors like age, gender, genetics, tissue uptake, nutritional status, physiological factors like gastric acid secretion, food matrix, and interactions between nutrients in the presence of enhancers and inhibitors in the diet (Davidsson and Tanumihardjo 2013; Yetley 2007). Some environmental factors like sunlight, wavelength of light in artificial environments, pH of the soil, fertilizer usage, degree of ripeness at harvest, and morphological source (stem, leaf, flower, or fruit), impact the character of nutrients and their bioavailability from plants. Food processing factors like thermal treatments, homogenization, lyophilization, cooking (boiling, frying, steaming), storage, mechanical treatments (grating, cutting, chopping, slicing, mashing, and juicing), also impact the bioavailability of nutrients from enzyme activation (Pressman et al. 2017). Heat/cooking, thermal and non–thermal processing, and cooking methods can result in variation in the bioavailabilities of nutrients present (Jordan 2014). Storage affects the content of polyphenols, in some fruits, vegetables, wines, and olive oils and refrigeration decreases their content (Gliszczynska-Swiglo and Tyrakowska 2003). Variability of intestinal factors like enzyme activity, transit time, colonic microflora, efflux transporters, and transporters influence bioavailability in individuals and nutritional milieu (Jordan 2014).

Dietary Fiber Dietary fibers mainly exist in unrefined cereals, legumes, nuts, fruits and vegetables. The effect of dietary fiber on nutrient bioavailability is linked to their physical properties in the gastrointestinal tract like cation exchange capacity, bile acid binding, water–holding capacity, viscosity, and ability to act as a substrate for fermentative microorganisms (Gallaher and Schneeman 2001). In general, dietary fiber alone does not have a major effect on the absorption of minerals (e.g. calcium or magnesium) (Heaney and Weaver 1995) or trace elements (Rossander et al. 1992; Sandström 1997), although α–cellulose may affect the utilization or endogenous losses of copper (Turnlund et al. 1985).

Lipids and Fatty Acids Lipids after their absorption in the intestine are broken down (metabolized) to fatty acids with varying length of carbon chain. Fatty acids with shorter chain length have lower melting points and therefore stays in liquid form (Mahan and Raymond 2016; Titchenal et al. 2018). Absorption of fats and fatty acids is affected by the water–holding capacity of dietary fibers such as pectins, psyllium, and various gums, which retain water within their matrix, forming highly viscous solutions in the small intestine. These can in turn alter gastric emptying time and slow down the rate of nutrient digestion and absorption (Gallaher and Schneeman 2001). Fats also increase the bioavailability of phytochemicals, like lycopene (in tomatoes) and β–carotene (in carrots) thus promote health and well–being. As a result, eating tomatoes with olive oil or salad dressing facilitates lycopene absorption (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Vitamins Bioavailability of vitamins in the body is largely affected by their chemical forms (Gibson 2007) that varies with age, sex, and physiological condition of an individual. Their quantities within the body are mostly regulated by the type of dietary intakes and eating habits. Vitamin absorption can be adversely affected by intake of tea, coffee (caffeine), smoking, alcohol, dietary fibers (phytates) etc. (Aslam et al. 2017). Sometimes increase or decrease of one vitamin also affects overall vitamin absorption. Some drugs like antibiotics, anti–inflammatory, histamine, anti–seizures, etc. decrease vitamin B complex, vitamin D and C levels in the body cells. Some diseases like 'celiac disease' and 'lactose intolerance' affect the digestive processes and decrease the vitamin absorption. Vitamin A activity in the diet is derived from retinoids like retinol, retinyl esters, and provitamin A carotenoids like β–carotene, α–carotene, and β–cryptoxanthin. Generally, the bioavailability of retinol is high (70–90%) but the dietary fat and intestinal infections can affect its absorption. The absorption of retinol gets reduced in individuals with diarrhea and intestinal disorders (Gibney et al. 2009; Ravisankar et al. 2015; Schönfeldt et al. 2016). Vitamin D helps absorb calcium from the small intestine into the bloodstream and helps to maintain normal blood calcium, which is essential to cardiac function. Calcium absorption in individuals is dependent on vitamin D status, and on the presence of binding substances like uronic acid, phytate and oxalate in the food (Khokhar et al. 2012; Allen 1982). Vitamin B_6 can be less available than other food/food products which is found in heat–processed milk products (e.g. pyridoxine β–D–glucoside) is also reported (Gregory 1997). In plants, especially mature grains, vitamin B_3 can be bound to sugar molecules which can significantly decrease its bioavailability (Gibney et al. 2009; Mahan and Raymond 2016; Titchenal et al. 2018).

Minerals Minerals are not efficiently absorbed and so their bioavailability can be very low. Plant based foods mostly contain factors, like oxalate and phytate, that bind to minerals and inhibit their absorption. Some minerals influence the absorption of others. For example, excess zinc in the diet, affects absorption of iron and copper. Conversely, certain vitamins enhance mineral absorption, like vitamin C boosts iron absorption, and vitamin D boosts calcium and magnesium absorption. Consumption of a high fiber diet interferes with mineral and nutrient bioavailability.

Iron is available in the heme or non–heme form. Although, 90% of the dietary iron is consumed in the non–heme form, but due to its low bioavailability, only about half of the iron is absorbed by the body. While absorption of heme iron is dependent on the iron status of the individual, absorption of non–heme iron depends not only on the individual's iron status, but also on the absorption modifiers consumed during the same meal. In general, higher the non–heme iron content of the diet, lower is the absorption efficiency (Yip and Dallman 2001; Gibney et al. 2009). Absorption is highest for water soluble iron compounds (e.g., ferrous sulfate, ferrous fumurate, ferric saccharate). The presence of soluble enhancers, such as ascorbic acid, and inhibitors, such as phytates, polyphenols, and calcium, consumed in the same meal showed a notable effect on the amount of non–heme iron absorbed. Heme iron is much less affected by the dietary factors and contributes more significantly to absorbable iron (Schönfeldt et al. 2016).

A number of dietary factors influence zinc uptake. Ligands, such as phytate, form insoluble complexes with zinc and prevent its absorption. Calcium increases binding of zinc by phytate (Flynn et al. 2009), and thus decreases zinc bioavailability and absorption (Spencer et al. 1984; Wood and Zheng 1995; Flynn et al. 2009). But high–fiber foods may also interfere with calcium absorption (Marcus 2013), the organic form of zinc (e.g. in oysters) is less affected by absorption modifiers than the inorganic forms (Solomons et al. 1979). High iron content in the diet also decreases zinc absorption. Earlier reports indicated that folic acid can also inhibit zinc retention and metabolism (Milne et al. 1984, 1990; Milne 1989; Flynn et al. 2009). Amino acids like histidine, methionine and cysteine facilitates zinc absorption (by removing zinc from the zinc–calcium–phytate complexes) (Mills 1985; Flynn et al. 2009).

Potassium present in intracellular fluid plays a key role in maintaining sodium–potassium balance for sustaining cell function. The small intestine is the primary site of potassium absorption, with approximately 90% of dietary potassium being absorbed by passive diffusion (Demigné et al. 2004). Less information is available on the health benifits and bioavailability of potassium from dietary sources (Bechgaard and Shephard 1981; Betlach et al. 1987; Stone et al. 2016). The potassium in the ionic form is water soluble and is easily lost during cooking and processing of foods. Sodium is readily absorbed from the intestine of healthy adults, carried to the kidneys, and subsequently returned to the blood to maintain appropriate levels (Mahan and Raymond 2016). Salting foods and discarding the liquid from the food materials induces sodium (Na^+) for potassium (K^+) exchange and reduces the potassium content of foods (Stone et al. 2016). It has been observed that higher consumption of cereal and processed foods, and lower consumption of fruits and vegetables has led to a diet lower in potassium and higher in sodium (Macdonald–Clarke et al. 2016).

Selenium compounds are efficiently absorbed by humans (Selenium–WHO 1987). For example, absorption of the selenite (inorganic form) is greater than 80% whereas that of selenomethionine (organic form of selenium) or as selenate may be greater than 90% (ReedáMangels 1992; Selenium–WHO 1987; Patterson et al. 1993). The overall availability of dietary selenium is not due to its absorption but its conversion within tissues to its metabolically active forms (e.g. its incorporation into GSHPx or 5′–deiodinase) (Contempre et al. 1996). The bioavailability of selenium in wheat, Brazil nuts, and beef kidney is 80–90%, but in tuna fish it is less available (20–60%) from selenite than selenium from other seafoods (e.g. shrimp, crab, and Baltic herring). The selenium in a variety of mushrooms has been reported to be of less available to rats (Khokhar et al. 2012).

Trace minerals like chromium, copper, and manganese are probably as important as iron, zinc and calcium. The bioavailability of these minerals from dietary sources is not much examined and is influenced by the same dietary factors that influence the bioavailability of iron, zinc and calcium (Campbell 2017; Gibney et al. 2009; Platel and Srinivasan 2016). While amino, keto, ascorbic acid and chelating substances like oxalate form chromium complexes and enhance chromium bioavailability, inhibitors like phytates, zinc and iron reduce chromium bioavailability. Copper (II) histidine chelate after complexation provides increased availability for copper (Lönnerdal 1996). Excessive zinc has been reported to impair bioavailability of copper at levels that exceed normal dietary intakes (Fairweather–Tait 1997; Platel and Srinivasan 2016).

In the small intestine, the elements of sodium chloride split into sodium cations and chloride anions. Chloride follows the sodium ion into intestinal cells passively, making chloride absorption quite efficient. When chloride exists as a potassium salt, it is also well absorbed. Other mineral salts, such as magnesium chloride, are not that efficiently absorbed but bioavailability still remains high. Although very soluble potassium is highly bioavailable, it is easily lost during cooking and processing of foods (Gibney et al. 2009; Titchenal et al. 2018). It has been reported that under normal conditions, the absorption of dietary iodine is >90% (Jahreis et al. 2001; Platel and Srinivasan 2016).

Proteins and Amino acids Although protein is a macronutrient that is easily absorbed by the human body, its bioavailability is directly linked to digestibility (absorption). The digestibility of proteins is influenced by total dietary energy intake and the quality of protein in terms of meeting metabolic demand. Heat treatment during processing leads to the formation of Maillard compounds and reduced lysine availability. Oxidization of sulfur containing amino acids reduced the bioavailability of tryptophan and threonine. High pH induces racemization of L–amino acid residues to D–isomers and formation of cross–linked amino acids, such as lysinoalanine, which also reduces bioavailability. Many foods contain other bioactive substances that may inhibit amino acid bioavailability. These inhibitors may be naturally occurring (e.g. tannins, phytates, trypsin inhibitors, glucosinolates, isothiocyanates), formed during processing (e.g. D–amino acids and lysinoalanine), or formed during genetic modification of crops (e.g. lectins in lentils) (lectins suppress growth at low levels and are toxic at high levels) (Lewis and Bayley 1995; Gibney et al. 2009; Schönfeldt et al. 2016; Ribarova 2018).

Carotenoids Carotenoid absorption is a comparatively slow process (Afreen et al. 2018; Leopoldini et al. 2011). Only six carotenoids and their metabolites have been reported to be metabolized in human tissues, particularly in the intestine. The bioavailability of carotenoids varies widely because of dependence on the structure of the carotenoid itself in food matrix. For example, astaxanthin from *Haematococcus pluvialis* (a green microalgae), owing to free carotenoid nature, has greater bioavailability than the polar β–carotene and lycopene from *Spirulina platensis*, and lutein from *Botryococcus braunii*. Esters of xanthophylls however have lower bioavailability. It has been suggested that their hydrolyzation occurs in small intestine for further absorption (Afreen et al. 2018; Dhankhar et al. 2012; Faraloni and Torzillo 2017). They are partially absorbed, which involves release from plant cells, and then form micelles with the help of fatty food and bile acids. Evidence from the literature indicates that thermal treatment and homogenization exerted positive effects on bioaccessibility of these compounds, however, dietary fiber produces a negative effect on their bioavailability (Afreen et al. 2018; Fernández–García et al. 2012). To make the carotenoids more bioavailable, the food industry has developed encapsulation of carotenoids present in fresh vegetables and fruits, employing them in crystal form or bound to protein complexes (Afreen et al. 2018; Ribeiro et al. 2010).

Phenolics, Phenolic Acids and Flavonoids Flavonoids are important dietary supplements found abundantly in green tea, red wine, and vegetables and fruits like onions, tomatoes, and apples (Giuliani et al. 2014; Rakers et al. 2014; Wang et al. 2011, 2018). Bioavailability of flavonoids is generally poor as flavonoids exist as glycosides. Flavonoid aglycones exhibit higher bioavailability and better absorption than glycosides by virtue of better membrane interactions. In contrast to *O*–glycosides, C–glycosides are more resistant to hydrolysis. Since absorption and excretion of *O*–methylated flavonoids are relatively slow, it facilitates better bioavailability (Surichan et al. 2012; Wang et al. 2018; Wei et al. 2014). Some reports suggested that isoflavone aglycones are more rapidly absorbed than their glucosides (Lim and Koffas

2010) whereas higher bioavailability of glucosides form of isoflavones is also reported (Lim and Koffas 2010). More interestingly, Richelle et al. (2002) and Zubik and Meydani (2003) did not observe any absorption difference when either aglycones or glucosides were administered. In another study, isoflavones glycosides were not found in human plasma after ingesting either 50 mg of isoflavone glycosides (daidzin or genistin) or 250 mL of soymilk (Lim and Koffas 2010).

The bioavailability of the most active green tea antioxidant, epigallocatechin gallate (EGCG) remains uncertain (Naumovski et al. 2015). Only a small fraction of tea catechins present in the intestinal tract after drinking tea can be absorbed, and therefore considered to be bioavailable. The pharmacokinetics study indicated that after oral administration of tea to rats, about 14% of (−) EGC, 31% of (−)–EC, and (−)–EGCG is available in the blood (Chen et al. 1997). Studies on the bioavailability of pure EGCG in humans are limited, as such studies were primarily focused on the pharmacokinetic properties of preparations where EGCG was imbedded in a polyphenolic complex, as part of green tea extracts (Lee et al. 2002; Naumovski et al. 2015).

Other Phytochemical Nutrients Several miscellaneous phytochemicals like resveratrol, curcumins and glucosinolates also exhibit nutraceutical properties. Resveratrol is a major contributor to the health benefits related with the consumption of grape products. *In vitro* studies confirmed the potential of resveratrol as a 'therapeutic agent' and yet animal and clinical trials revealed inconsistent and less promising results which might be due to low bioavailability (De Vries et al. 2018; Sahebkar et al. 2015). The low bioavailability of resveratrol results in low plasma concentrations which gives rise to limited systemic distribution and concentration that is not high enough in some active sites to elicit significant pharmacological effects. A study has revealed that when resveratrol is combined with some other phytochemical, the bioactivity get enhanced (Johnson et al. 2011).

Poor water solubility, low oral bioavailability, poor absorption in GIT, extensive pre–systemic metabolism, rapid systemic elimination, low intrinsic activities are major drawbacks for curcumin derived phytochemicals (Anand et al. 2007). Clinical trials have depicted that curcumin is safe at higher doses (12 g day^{-1}) in humans but unfortunately exhibit poor bioavailability. To improve the bioavailability further numerous approaches involving liposome, phytosome, nano–particles, nano–emulsions, cyclodextrin encapsulation and use of adjuvant like piperine, and the use of curcumin phospholipid complex (Anand et al. 2007) have been followed to enhance their activity and bioavailability (Mobasheri et al. 2012). In another study oral administration of liposome–encapsulated curcumin enhanced the bioavailability of curcumin. In another study, broccoli sprouts combined with glucoraphanin powder synergistically enhanced the bioavailability of sulforaphane (glucosinolate) and proved that regular intake of broccoli sprouts enriched with glucoraphanin powder reduces the risk of cancer compared with glucoraphanin powder or sprouts alone (Cramer et al. 2012).

25.6 Food Safety

Food safety is a concept that the food will not cause any harm when consumed according to its intended use (ISO 22000 2005; Shukla et al. 2014). One of its major areas of concern is to provide safe food to consumers in the growing competitive global markets. The responsibility of providing safe food lies with all the stakeholders of a food chain including farmers and growers, manufacturers and processors, food handlers and consumers (Gibney et al. 2009; Shukla et al. 2014).

The Hazard Analysis Critical Control Points (HACCP) is a program within the food industry designed to promote food safety and prevent contamination by identifying all areas in food production and retail where contamination could occur (Gibney et al. 2009; US FDA 2018). Increased production of processed food, rapid urbanization and changing lifestyles have led to a shift in dietary patterns as people are consuming more foods high in energy, fats, free sugars or salt, and people do not eat enough fruit, vegetables and dietary fibre such as whole grains (WHO 2018). In 1963, FAO and WHO jointly established the CAC with its member states from all round the world. CAC has instituted many specialized committees to formulate food standards based on scientific recommendations in all the fields of food safety and quality to ensure that food is safe for trade. The Food and Agriculture Organization (FAO) guideline (FAO/WHO 2003) on "Strengthening national food control systems" is focused on government agencies and food control authority self–assessment and capacity building. The assessment is based on six elements (food control management, food legislation, food inspection, official food control laboratories and food safety and quality information, education and communication) (FAO/WHO Codex Alimentarius 2018). The growing interest in nutrients and related substance such as fortificants, antioxidants, supplements etc. has prompted FAO and World Health Organization (WHO 2018) to develop guidelines for establishing safe upper levels of intake for nutrients and related substances (FAO/WHO 2006; Gibney et al. 2009; van der Meulen 2010).

In US, the Food and Drug Administration (FDA) closely monitors the ingredients, foods and beverages in food supply and protects public health by ensuring that foods and dietary supplements are properly labelled and are safe for consumption (Marcus 2013; USFDA 2018).

In Europe, European Food safety Authority (EFSA) is the major regulatory authority that serves under the European legislative and executive institutions along with 28 EU Member States. EFSA plays a crucial role in assembling and scrutinizing scientific data on risks correlated with the food chain to guarantee and communicate that European risk assessment is assessed by the extensive amount of appropriate, accurate, and timely accessible scientific data on food safety matters (EFSA 2018).

In India, Food Safety and Standards Authority of India (FSSAI), established as per the Food Safety and Standards Act, 2006 (FSS Act 2006), promotes and create awareness about food safety under the supervision of Ministry of Health and Family Welfare, Government of India as per the FSS Rules 2011, and Regulations 2011, 2017, and 2018. Over the years, FSSAI has developed various science–based standards for various food articles and regulate their production, storage, distribution, trade and import to facilitate safe and wholesome food (FSSAI 2019; Vats and Arora 2016).

25.7 Conclusion

Good nutritious, healthy, diversified, and wholesome diet not only satisfy our hunger and nourish our body but has a direct impact on overall lifelong health, and quality of life of an individual. Moreover, having quality eating habits along with adequate intake of nutrients is essential and can also reduce the risk of developing certain chronic and lifestyle–oriented diseases that could severely impact our health. Nutrients react differently once ingested and absorption can be influenced by a variety of factors, including quality of the food source; concentration of nutrient; chemical form; the matrix in which it is consumed; the composition of the whole meal, inhibitors, and enhancers; and the status of the host. Although bioavailability is only a partial measure of the benefit from a nutrient, this factor quantifies the amount entering the bloodstream. Understanding nutrient bioavailability helps optimize diets and set appropriate nutrient recommendations. In addition to nutrient content of foods, nutrient bioavailability should also be taken into consideration when nutrition–sensitive policies, nutrition interventions, and dietary guidelines are developed.

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Chapter 26
Genetic Engineering of Novel Products of Health Significance: Recombinant DNA Technology

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26.1 Introduction

Biotechnology is the advanced field of biological science which is associated with the maneuvering of biological systems to improve effectiveness and efficacy of living organisms for the welfare of humans (Taylor et al. 2008). It is used to insert normal genes into the human body for the treatment of genetic disorders. It is used to manipulate the genes in microorganisms in order to produce hormones, vaccines, drugs etc. It is applied in the synthesis of different food products using biological processes e.g. fermentation to produce bread and alcohol. As biotechnology started

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to draw attention from scientists and most people felt unsure about the new science, debates frequently centered on the possibility of the technology's ability in solving the problems facing human health and farm production (Levidow and Carr 1997). Unfortunately, the method and the speed by which biotechnology began to be applied in the society seemed to threaten many who were already working to solve the challenges of human health and agriculture (Brooks 2000). In recent era, the research on recombinant DNA technology is dominant in biological science. It changes the orientation of biological problems and their possible solutions such that scenarios involving genome alteration, formation of a new virus, chromosome or gene variant are now common place in laboratories (Dunne Jr et al. 2017; Liu et al. 2017). Recombinant DNA technology acts at the molecular level, where numerous biological barriers established from species vanish (Rosbash 2017). This is promising as all living cells have their unique DNA, which is the essential particle of life and is involved in transmission of hereditary information by a simple, yet universal genetic code (Vary 2017). Recombinant DNA technology has reformed natural science and has a growing influence on experimental medication, environmental benefits, food and crop production. It has made revolutionary alterations in crop production and for the conservation of biodiversity (Godfree et al. 2017). All living organisms are made up of basic bio-elements e.g. sulfur, nitrogen, oxygen, hydrogen, carbon, phosphorus and other elements in trace amounts (Karl and Grabowski 2017). Structures of living organisms are built up of proteins, which are responsible for basic cellular functions like fundamental metabolic pathways and produce secondary organic metabolites such as carbohydrates and lipids; the essential components of plants and animals (Onagi et al. 2017). The study of DNA, mRNA and protein (Central dogma of life) and pedigree analysis provides information about cause and treatment of genetic diseases at genetic level (Moreno-Moral et al. 2017). These approaches are being used in cases where particular genetic abnormality is unknown (Manolio et al. 2017). Recombinant DNA technology works by using different enzymes (Table 26.1). Humans are fully aware of biology as well as biotech-

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Table 26.1 Some enzymes used in recombinant DNA technology

Enzymes	Reaction	Primary use
Alkaline phosphatase	Dephosphorylation of 5′ end of nucleic acid	Prevent self-ligation by removing 5 phosphate group earlier to kinase labelling (Rücker et al. 2007)
BAL 31 nuclease	Degradation of DNA either from 5′ or 3′ end	Progressive DNA molecules shortening
DNA ligase	Catalyzes DNA molecules bonding	Unification of DNA molecules
DNA polymerase 1	Help to synthesize new strand of DNA from single parental strand	Double stranded cDNA synthesis; nick translation
DNase 1	Formation of single stranded nicks in DNA under suitable conditions	Nick translation; help in hypersensitive sites mapping
Exonulease III	Removes nucleotides from 3 ends of DNA	DNA sequencing; mapping of DNA protein interaction
λ exonuclease	Removal of DNA nucleotide from 5′ end	Play role in DNA sequencing
Polynucleotide kinase	Terminal phosphate group transfer from ATP to 5 hydroxyl group of DNA or RNA	^{32}P labeling of DNA or RNA
Reverse transcriptase	Use RNA templet to synthesize DNA	Synthesis of c DNA from messenger RNA
SI nuclease	Degrades single stranded DNA	Removal of hairpin in synthesis of c DNA
Terminal transferase	Nucleotides addition on 3′ end of DNA	Tail addition of homopolymer

nology; it is named in specific manner and is practicing successfully (Pamies et al. 2017). Scientists are working to alter growth or detection of defective trait or disease gene by using microorganisms, plants and animal cell, system or processes to make products for their use (Nazir et al. 2017). In modern times, several applications from recombinant DNA technology to tissue culture technology are involved in the production of different products and services (Bandyopadhyay et al. 2017). For having better crop variety and more predictable results, molecular improvements offer much better promising results (Perez et al. 2017). The scientists who are interested in improving disease resistance in any crop plant often face problems in the quest of adequate resistance in available germplasm (Montenegro de Wit 2017). There are many chances that the desired trait is virtually nonexistent in the species gene pool (Alexander and Woeste 2017). The genetic engineering presents the solution in the form of transferring a specific desire gene from a donor to the recipient in a more controlled manner because in this technology the donor has not been sexually compatible. Various ailments are being treated by different proteins (Table 26.2). The new technology allows us to approach directly to the DNA molecule for information (Rasekh et al. 2017). Sequence alignment of DNA and genetic engineering provides us information about working of detailed section of DNA (Bothmer et al. 2017). The word biotechnology represents alliance or relationship between environmental science and knowledge. Principally, biotechnology is a recently exposed scheme of

Table 26.2 A selected list of human proteins produced by recombinant DNA technology for treatment of human disorders (Chakraborty and Mungantiwar 2003)

Disorder	Recombinant protein
Anemia	Hemoglobin, Erythropoietin
Asthma	Interleukin 1 receptor
Atherosclerosis	Platelet Derived Growth Factor
Delivery	Relaxin (Bell et al. 1993)
Blood Clots	Tissue Plasminogen Activator, Urokinase
Burns	Epidermal Growth Factor (Hardwicke et al. 2008)
Cancer	Interferone, Tumor Necrosis Factor, Colony Stimulating Factor, Interleukins, Lymphotoxin, Macrophage Activating Factor
Emphysema	Apha 1 Antitrypsin
Diabetes	Insulin and Insulin Like Growth Factor
Female Infertility	Chorionic Gonadotropin
Free Radical Damage	Superoxide Dismutase
Growth Defects	Growth Hormone, Growth Hormone Releasing Factor, Somatomedin C
Heart Attacks	Prourokinase
Hemophilia A	Factor VIII
Hemophilia B	Factor IX
Hepatitis B	Hepatitis B Vaccine
Hypoalbuminemia	Serum Albumin
Immune Disorders	Interleukins, Beta Cell Growth Factor
Kidney Disorders	Erythropoietin
Nerve Damage	Nerve Growth Factor
Osteomalacia	Calcitonin
Pain	Endorphin and Encephalin
Rheumatic Disease	Adrenocorticotropic Hormone
Viral Infection	Interferons
Ulcers	Urogastrone

medication to age-old practices, e.g. grounding of wine and curd bread. These substantial processes are healthy planned aged and conservative biotechnology. A diversity of extremely responsive techniques can now be functional to the segregation and description of genes and the quantization of gene. An elementary perception is essential to these techniques; i.e. complementary base pairs form hydrogen bonds with every other- A with T and G with C. For instance, in DNA cloning,[1] a fastidious division of genetic material is unconcerned from its ordinary surroundings by means of one of numerous constraint endonucleases. For the purpose to obtain desire gene specific restriction endonucleases are used to cut DNA and producing sticky ends for proper insertion of gene into vector. The vector is also cut

[1] Cloning is a procedure in which identical copies of genes are produced. A gene can be cloned by polymerase chain reaction that produces a lesser number of copies with in laboratory and recombinant DNA technology that produces large quantity of genes in a living cell.

with similar endonucleases to produce sticky ends having complementary base pair. This is then ligated into one of more than only some vectors in which the DNA section can be enlarged and simulated in huge quantity (Rotenhoffen 2010). It is straightforward to segregate the cloned DNA, which preserve sequence and used as a probe in for numerous types of hybridization reactions to perceive additional, correlated, or adjoining pieces of DNA, or used to analyze gene products like messenger RNA. Treatment of the DNA to modify its organization, known as genetic engineering, is a key constituent in cloning (e.g. the building of chimeric molecules) and can be used to learn the purpose of a fastidious section of DNA and to investigate how genes are regulated. Chimeric DNA molecules are inserted into cells to create transgenic cells or into the fertilized oocytes to build transgenic animals (Al-Razem 2012). These approaches are used to learn genetic material instruction in a standard cellular situation and to modify cellular function. Techniques linking cloned DNA are adapted to place genes to suitable regions of chromosomes, to recognize the genes accountable for diseases, to learn how faulty genetic material causes disease, to make a diagnosis of inherited diseases, and increasingly to take care of genetic diseases and the treatment of heredity diseases that are difficult to treat (Saunders et al. 2012).

26.2 Historical Perspective

Most people think that biotechnology is a new discipline but in different ways, it involves many ancient practices like making yogurt and food by fermentation. Human have been using living organisms thousands of years ago for manufacturing different products. In history, Greek and Chinese and others remain involved in biotechnology about 2000 B.C Selective breeding technique used by humans from 1000 years ago for the production of desirable products. At the beginning of 1960s, prior to the introduction of gene cloning, genes studies frequently relied on accidental findings including bacteriophages ability to introduce bacterial genes into their genomes. For instance, incorporation of phage phi 80 strains was done in its genome with the help of lac operator and revealed that this DNA sequence has specific affinity to bind to the Lac repressor. The synthesis of numerous different investigational interpretations into recombinant DNA technology happened between 1972 and 1975, in the course of the hard work of some research groups functioning mainly on bacteriophage lambda (λ).

26.3 Recombinant DNA

Manipulation of DNA for use in medicine, research, or biotechnology is called genetic engineering or recombinant DNA technology. There are three possible ways to get a gene of interest. They are: isolation from chromosomes, chemical synthesis and making it from mRNA. After the discovery of DNA model by Watson and Crick, many scientists who worked on DNA were able to understand its potential in the living body. On the basis of such discoveries a new technique of recombinant DNA technology was established which molecular biology would advance in the next era. It is acquired DNA that is obtained by combining DNA from two dissimilar sources or DNA having a foreign piece of DNA. Its examples are a mouse and human DNA, bacterial and human DNA, bacterial and viral DNA, DNA from two different humans. The purpose of recombinant DNA is to synthesize products for benefit of life (Sanchez-Garcia et al. 2016). Recombinant DNA is produced synthetically from two or more DNA pieces integrated into a single fragment. Splicing of gene and genetic manipulation/modification, recombinant DNA technology and genetic engineering are statements that are engaged to express gene management of an organism. The advancements of these novel techniques have discovered a huge quantity of biochemically distinct proteins of therapeutic importance and formed a massive attraction for pharmaceutical companies. The biochemically acquired medicines are being employed in the management of various disorders. Recombinant DNA technology also plays a significant role in forensics, DNA profiling in relationship investigation and in parenthood assessment.

26.4 Recombinant DNA Formation and Application

Restriction enzymes discovery in the 1970's led a revolution in recombinant DNA technology. Along with the discovery of self-replicating plasmid, the possibility of gene isolation, purification and insertion was created. Recombinant DNA is completed by three dissimilar methods such as transformation, phage introduction mostly lambda phage and non-bacterial transformation i.e. gene gun or microinjection (Van den Eede et al. 2004). The primary stair in transformation is to choose a section of DNA to be inserted into a suitable vector which is either a bacteriophage or plasmid (Casadaban et al. 1980). The recombinant DNA technology not only recombine fragment of DNA in cloning for coding of a specific gene but also manipulate gene to change their sequence of regulation. The coding region could be placed under control of a promoter and be introduced into an expression system which may be a virus or bacterium for the observation of regulation alteration. Coding regions of recombinant DNA produces proteins having unique properties. The subsequent stair of DNA is to incise required section of DNA with a restriction enzyme which produces sticky ends on vector and also on the gene of interest (endonuclease or exonuclease) and afterward ligate the DNA (gene of interest inserted into the vector at a suitable position with DNA Ligase. The insert has a selectable indicator, which permits for identification of recombinant molecules. Ampicillins and tetracycline antibiotic indicator pBR322 and pSC101 are frequently used in a host cell lacking a vector in which the new gene inserted dies when exposed to an assured antibiotic, and the host with the vector then exist. The vector is inserted into a host cell, in a procedure termed transformation. One instance of a probable host cell is *E. coli*. The host cells have to be particularly equipped to obtain the unfamiliar DNA. Selectable markers are capable of color change, antibiotic resistance or any other characteristic, which can differentiate transformed hosts from untransformed hosts. Dissimilar vectors contain dissimilar properties to make them appropriate to special applications. The properties comprise regular cloning sites, mass, and elevated duplicate figure (Ando et al. 1999).

For transfer of a gene of interest, plasmids are usually used as the vector and their isolation methods differ according to the host organism. Based on gene phenotypic effect and their location in chromosome, genes are further divided into subgroups like simple, complex, operons, regulons gene and multiple regulons (Humayun et al. 2017). Gene of interest obtained by using altered restriction enzymes produce blunt end which can be attached with vector without the addition of homo-polymer tail but the blunt end does not permit cloned fragment recovery after propagation. The DNA restriction site linkers without cohesive ends (cDNA, Synthetic DNA, sheared DNA) are also available commercially (Qin et al. 2017). Sometimes more than one plasmid is injected into an individual cell for the indirect hidden plasmid selection (Khurana et al. 2017). In non-bacterial transformation, a bacterium such as *E. coli* is not used. In microinjection, the DNA is injected directly into the nucleus of the cell being transformed (Lotti et al. 2017).

26.4.1 Agriculture

The development of genetically modified (GM) foods, pesticides, fungicides, biotic and abiotic resistant crops, and fruits for the enhancement of quality food production to meet the demand of a rapidly increasing population, can be done using artificial conditions (Christou and Twyman 2004; Kelemu et al. 2003). Golden rice which is an enriched source of vitamin A, the production of medically important agricultural products like human growth hormone production and edible vaccines are also examples of GM crops (Dunwell 2002; Lemaux 2008; Raskin et al. 2002). Gene recombination is as important as a mutation in the production of genetic variation in higher plants. The recombination of genetic variability is largely determined by the breeding system (Lande 1980; Wiens 1984). A whole series of mechanisms promoting cross-fertilization and genetic systems for self-incompatibility have evolved which have been derived from flexible cross-fertilized systems (Barrett 2002). It, therefore, becomes necessary for a taxonomist to have full knowledge of the breeding behavior of the population (Daniell et al. 2015). Tissue (cell suspension) culture technique and micro-propagation technique are also used to obtain desired secondary metabolites in the plant (Wilson et al. 1971). High quality crops are produced by cross-fertilization of different varieties, inserting a new gene from other suitable varieties or from a wild relative (Gilbert and Tekauz 2000). In developing countries, genetically modified crops produced are mainly cotton and rice. Such advancement in plant genetic recombination led to a revolution in agriculture in 1970s in Pakistan (Ashraf 2010; Stone and Glover 2017). Biotechnologists are working to evaluate the genetic diversity of different species of plants and animals for selection, management, and protection of the germ plasm (Nisar et al. 2011). Analysis of genetic diversity in the genome of a species is done by using different molecular markers e.g. proteins and DNA which are commonly used as markers due to their stability under different environmental conditions. These markers are more informative and accurate as compared to phenotypic markers. In recent times, a number of them are being used to find genetic diversity at DNA level. These markers show the degree of relationship in individuals of same species without environmental influence (Yadav et al. 2007). Recombinant plants are established to meet the food demand in future. According to the International Service for Acquisition of Agri-Biotech Applications (ISAAA), currently, more than ten million farmers of 25 different countries are benefiting from genetically modified crops.

26.4.2 Nanobiotechnology

Nanotechnology is a new approach in science that involves materials having capacity to manipulate physical as well as chemical properties of a substance at molecular level. In nanobiotechnology, atomic or molecular machines can be made by incorporating them with biological systems so it is also considered as a combination of biotechnology and nanotechnology (Emerich and Thanos 2003). Nanotechnology is the development of materials or devices with sizes ranging between 1 and 100 nanometers. It plays an important role along with biotechnology for development of useful tools to study life. Nanotechnology is helpful in drug targeting of diseased tissues (Dong and Feng 2004). Nanoproducts can be bioaccumulated in more concentration than formal drugs at affected sites. Nanoparticles increase vascular permeability in tumor tissues through better transmission. It is also helpful in drug delivery to brain cells by overcoming the blood-brain barrier (Maeda et al. 2000).

Nano-biotechnology is also applicable in clinical science like disease diagnosis, drug delivery and molecular imaging. In diagnosis, semiconductor nanocrystals that can emit more light than typical organic compounds are used to help in diagnosis of damaged tissues. Nano gold particles are decorated with DNA short segments which form the basis to detect the presence of a given genetic sequence. If required sequence is present in the sample, it binds to complementary DNA segment and forms a dense web of visible gold balls. This technology helps for the detection of pathogenic organisms. Another new technology for diagnosing disease is the synthesis of protein chip that can be treated with chemical group or small protein component which binds with specific proteins that contains a certain biochemical structure (Mitra et al. 2006).

By using nanotechnology, we can develop drugs for diseases that cannot be targeted by conventional pharmaceuticals. Nanomedicines will be helpful to cure diseases in near future and also enhance normal human physiology (Fakruddin et al. 2012).

26.4.3 Pharmacology

Recombinant technology is a current technique utilized for the production of therapeutic agents. Drugs produced by recombinant technology are recognized as genetically engineered drugs, recombinant DNA expressed products, biopharmaceuticals, and biologics (Baeshen et al. 2016). Recombinant pharmacology products are factor VIII, tumor necrosis factor, superoxide dismutase, enzymes, insulin, coagulation modulators, growth hormone, granulocyte colony stimulating factors, GM-CSF, anti-rheumatoid drugs, erythropoietin, transforming growth factors, platelet-derived growth factors, fibroblast, interleukins, and interferon (Kishore and Krishan 2009). Biotechnologists also seek to identify cancer-causing genes known as oncogenes. Tumor suppressor genes get special attention to make proteins by encoding these genes to make drugs that inhibit cancer cell growth (Zhu et al. 2015).

26.4.4 Medicine

Recombinant DNA technology places genetics in the mainstream of medicine (Olson 2017). A genetic testing will provide us great information from common to rare diseases of humans (Qiao et al. 2017). This technology is used to identify and allocate faulty gene loci (Jin et al. 2017). Genetic probes are used to diagnose the disease in its early stage as well as to determine its probability in future generations (Kelley 2017). The knowledge of disease is advanced due to the discovery of methods for diagnosing gene defects (Wright et al. 2017). Recombinant DNA technology also helps humankind in the treatment of many diseases, which could be difficult or impossible to treat. Some common methods are *in vivo* and *ex vivo* (Santangelo et al. 2017). Gene therapy, antiviral therapy, recombinant vaccination, synthesizing clotting factors, fluorescent fishes, glowing plants, development of diagnostic proteins etc.; are different examples. Salmonella-specific biotinylated DNA probe has been developed for fast routine recognition of Salmonella. Stem cell technology is also utilized in enhancing the growth (regeneration) of damaged organs. Many recombinant proteins that are synthesized by manipulating DNA are now being used for the treatment of diseases. Protein engineering has been utilized to develop second-generation variants with better pharmacokinetics, structure, strength, and bioavailability. For instance, in the neutral solutions used for treatment, insulin is frequently assembled as zinc-containing hexamers. Absorption is however limited by this self-association. By creating single amino acid replacements, nowadays, molecular biologists are capable to make insulin that is fundamentally monomeric at therapeutic concentrations. This insulin has been discovered to not only be able conserve their biological action but more effectively absorbed by 2–3 times. Few years ago, scientists have recognized that all therapeutics will not be effective for all patients due to their different immune system responses. Presently however, new research and technologies have been made to enhance the identification of human differences and development of specific treatment for specific individual termed as personalized medicine. The idea behind personalised medicine is that some medicines may cause allergic reactions to some individuals while being beneficial to others. The Pharmacogenetic approach focuses on patient genes and the different drug responses they are capable of evoking. The purpose of personalized medicine is to detect and manage diseases via conventional medicine based on mechanism of action to solve a clinical problem in complex diseases like cardiovascular disorder and cancer by considering both genetic makeup of individual patient and environmental factors.

System biology helps in mechanism based disease development, preventive medicine and treatment monitoring. It's also useful in the detection of changes in molecular profile at early stages of disease development.

26.4.5 Role of Biotechnology in Animal Sciences

Animal biotechnology deals with genetically engineering animals through the application of molecular biology techniques, which ultimately prove helpful for pharmaceutical, agricultural and industrial improvement. It is also used to synthesize a number of proteins that are beneficial for improvement of growth and treatment of animals as well as humans. Biotechnology offers an opportunity to genetically engineer poultry, goat, and other livestock animals for the production of pharmaceutical products and as model organisms for the study and treatment of human diseases. For example, cataract surgery, dialysis technique, polio vaccine etc.; were first performed on animals for experimental purposes. Nowadays, scientists are interested in the use of farm animals as a source of organ transplantation for treatment of organ failure. Mouse are used for experimental purposes due to small size, short generation time and its genetic studies make it model animal for research.

Animal scientists focus on application of recent techniques for gene transfer in animals that are not interbreeding normally for assistance in reproduction. Such technique is known as cloning. Cloned animals can be used for research purposes. They are also helpful for understanding cellular and molecular mechanisms as well as helpful in the treatment of diseases. Transgenic animals are produced by inserting desired gene of interest in them. Gene of interest injected in cell is done using different techniques like retroviruses-mediated, pronuclear micro-propagation; sperm mediated transfer and embryonic stem cell methods. In Canada, scientists developed Enviro-pig by using techniques of genetic engineering that produce phytase enzyme in its saliva that is more environmental friendly due to reduced levels of phosphorous released in urine and faeces than natural pigs. Phytase break phosphate in pig food and reduce amount of phosphorus up to 30% (Streiffer and Rubel 2007).

26.4.6 Recombinant DNA Technology in Disease Diagnosis

Recombinant DNA procedure is now greatly used to identify and treat human pathology by detecting a molecular defect in humans. It also helps to understand and detect genetic diseases, which were previously considered as non-treatable diseases. Other diseases like infectious diseases and neoplasia or due to mutation or deletion of nucleotides at one or more sites can also be diagnosed, which will be helpful in medicine. In the twentieth-century, recombinant DNA technology is a rapidly growing field, especially in disease diagnosis. Due to development of these techniques, the physician is able to diagnose the disease with more accuracy. Scientists working in DNA technology are struggling for improvement of procedures so that it is accessible, affordable and acceptable to the community.

26.4.7 Recombinant DNA Pharmaceuticals

Recently, molecular medicine formulation synthesized by using different molecular genetics methods are helpful for the investigation of disease, defective genes identification and for replacement of abnormal gene functioning with normal ones. The study of disease-causing genes responsible for disease and its expression in the normal individual has been possible by the advancement of recombinant DNA and by adopting advanced cloning techniques. The term gene cloning is commonly used for different methods suitable and most effective to utilize DNA in a test tube and also to return it within an expression system where it acts efficiently. The tools that are commonly used for the purpose of gene cloning include suitable vectors, genes incorporation, and the enzymes synthesis. The biological, and methodological obstacle created by cell factories for the production or rDNA pharmaceuticals is the main challenge in the development of protein-based molecular medicine. Recombinant DNA technologies might have an ability to exhaust conventional cell factories, thus new production systems need to be deeply explored and incorporated into the production pipeline. More thoughtful comprehension to overcome problems of host cell physiology and cell responses during protein production and biological stress would necessarily require more suitable tools and techniques (either at genetic, metabolic or system levels) to boost high-yield and production of high–valuable proteins. Apart from the expected integration of unusual mammalian hosts such as transgenic animals or plants, microbial cells prove as exceptionally strong and suitable hosts, and gaining knowledge about the biological aspects of protein production would hopefully boost the performance of such hosts. In view of this, not only commonly used bacterial and yeasts cell but unconventional strains or species are under observation as promising cell factories for synthesizing recombinant drugs. Their incorporation into productive processes for human pharmaceuticals would hopefully push the trend of marketed products and prove to be helpful for overcoming the demands of pharmacological companies.

26.4.8 Genetically Engineered Recombinant Human Insulin

Human insulin has been prepared in microorganisms in such a method that its organization is entirely similar to that of the usual molecule. This decreases the likelihood of effects ensuing from antibody manufacturing. In numerous studies regarding pharmacological and chemical aspects of insulin, recombinant DNA human insulin available in the market has demonstrated identical characteristics as insulin secreted from the human pancreas. At the start, the main trouble met was the contagion of the last brand in the cells of the host, escalating the threat of infectivity in the fermentation broth. This risk was eliminated by the prologue of decontamination procedures. This decreases the requirement for multifaceted and valuable purification techniques.

26.4.9 Human Monoclonal Antibodies

These are specific immunoglobulins that show a wide range of biological activities. These are used in diagnostic, antigen-binding sites of antibodies which have the potential for development of bioactive peptides due to their specific ligand binding activity. Hybridoma technology that uses fusion of myeloma and B-cells, helps in monoclonal antibody production. Recombinant DNA technology results in chimeric and humanized antibodies development with high efficacy in cancer. Antibodies are classified as member of isotype, these are IgA, IgD, IgE, IgM. In humans, the most prevalent is the IgG type. It is expected in the near future that due to the production of humanized antibodies, it will aid the prevention of untreated diseases.

26.4.10 Sex Identification by DNA Analysis

DNA study can also be utilized to recognize the gender of a person. The hereditary diversity among the genders is the custody of a Y chromosome by men, so recognition of deoxyribonucleic acid (DNA) detailed for the Y chromosome would facilitate men and women to be illustrious. DNA study can also be utilized to recognize the gender of an unborn baby. Judging if a fetus is a male or female would be generally late if anatomical differences are awaited to be established and the gender recognized by scanning; however, under several conditions, an earlier sign of gender is needed. An instance is when the family background of the people point to an uncertainty that an unborn child might suffer from an inherited disease and the parents desire to make a premature choice regarding whether to carry on with the pregnancy. The 3rd function of DNA-based gender recognition and the one that has been responsible for a lot of the improvement in this era is in the investigation of archaeological samples. Men and women skeletons can be differentiated if key bones such as the pelvis or the skull are intact; however, with incomplete remnants or those of kids, there is not sufficient sex-specific anatomical differentiation recognition to be made. If primordial DNA is conserved in the skeleton, a DNA-based technique can inform the archaeologists if they are dealing with a man or a woman (Shivanand and Noopur 2010).

26.4.11 DNA Analysis in the Identification of Crime Suspects

Persons after committing a crime, try not to leave any materials that could be used to trace them using their DNA. This trace can be done using amplification of that DNA by the polymerase chain reaction (PCR). These types of practices were not possible in the past. But, now due to DNA analysis, scientists are able to identify the exact person that is involved in a particular crime. This also enables us to do justice in complicated cases by using the material of criminals. The study of the human genome shows that genome is similar at different points but the arrangement of nucleotide is not same, i.e. it shows polymorphism. The area of DNA that show polymorphism is used to differentiate criminals. The markers that study genome mapping is single nucleotide polymorphism (SNPs), short tandem repeats (STRs) and restriction fragment length polymorphisms (RFLPs). Out of these, SNP is commonly used to identify the intergenic regions (Jobling and Gill 2004); (Balding and Donnelly 1996).

26.5 Advantages of Recombinant Technology

The advantages includes that it provides significant capacity. There is no need for ordinary or natural factors (Ma Julian et al. 2005). A tailor prepared invention is straight forwardly restricted. Consumption is unrestrained. It is inexpensive. It is opposed to ordinary inhibitors (Kohno et al. 2000). Production of proteins having therapeutic effects like hormones, enzymes, production of monoclonal antibodies, production of insecticide and pesticide crops, golden rice and bioremediation are some common products (Vashishth and Tehri 2015).

26.6 Disadvantages of Recombinant Technology

Recombinant technology products has developed into a commercialized and large foundation of earnings for business persons. But somehow, this product affects the natural immune system of the body. It can obliterate normal bio-network that relies on natural sequence (Bawa and Anilakumar 2013). It can cause alteration that could encompass destructive belongings. It is a chief global apprehension. Some are used to make organic weapons such as botulism & anthrax to target humans with detailed genotype. Another concern relates to the creation of superhuman beings (Maghari and Ardekani 2011).

26.7 Conclusion

It is now extensively acknowledged that biotechnology is categorically valuable from agriculture to medicine production, diagnosis, and treatment of diseases, gene manipulation, and bioremediation for environmental pollutant removal for the benefit of populations. The outgrowth of biotechnology is also advantageous for well-being, farming, food fabrication, creation of engineering enzymes, and appropriate organization of the atmosphere. Numerous pharmaceutical products are produced from this expertise such as insulin, growth hormone, interferons, erythropoietin, and hepatitis B vaccine. It is indeed a whole new frontier to solving man's problems.

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Chapter 27
Good Manufacturing Practices and Safety Issues in Functional Food Industries

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27.1 Introduction

Functional foods are a critical kind of food that requires close monitoring as they directly affect people's health. Every step of their processing, from preparation to distribution needs careful and advanced planning (WHO 2008). During processing, foods may be contaminated if not well looked after (Pal and Mahendra 2015). Good manufacturing practices ensures that the food being processed is safe and of high quality. Good manufacturing practices are a series of coordinated activities that ensure that products are safe and coming out as intended (Martinović et al. 2016). They are an enterprise that should endure time and call for dedication and financial investments because customers/clients would want to have products that have

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undergone GMP to ensure safety and quality (Campbell and Tyler 2012). The following are a few important reasons that warrant adherence to GMP.

Regulatory Compliance Good manufacturing practices are not mandatory but adhering to their guidelines help manufacturers be in almost a sure line to be in compliance with regulations. These regulatory requirements could be at state, city, district, regional or country levels and the regulations themselves, as opposed to GMP are usually not optional (Mtewa and Mtewa 2017).

Competitiveness The implementation of GMP is one way that also helps manufacturers to remain competitive enough in the line of their respective businesses. Since it ensures a smooth application of sound quality and safety management systems, the safety and quality of functional food products from the facility is more easily and conveniently achieved. These products eventually satisfy customers and keep the product's demand high (Pal and Mahendra 2015).

Pre-qualification and Certification GMP certification and implementation in the functional food industry may help the facility and the product get pre-qualified for a particular market or approvals in some states. It may also help in getting pre-qualifications for some more advanced certificates such as quality management and food safety management certifications. Certification may be voluntary or mandatory depending on the area of focus or legislative requirements. Some certifications may give you an edge by just decorating your business profile. There are clients, who are well organized and mindful of the benefits of GMP and demand specific certifications if they are to consider any supplier for potential contracts (Campbell and Tyler 2012).

27.2 Personnel Commitment and Responsibilities

GMP demands full commitment from all players in a functional foods processing unit, from top management to the last level of the organogram. Top management commitment is the most critical as they are the ones that shape the direction of the processing unit envisioning market, competitors and regulatory demands. Top management has a responsibility to enforce adherence right from policy formulation to motivators upon GMP related performance such as rewards and punitive actions. There is a need for clear demarcation of duties and responsibilities for each and every staff position to avoid conflicts that would end up into negligence of GMP which has costly consequences (Mtewa and Mtewa 2017).

27.3 Facilities, Establishments and Designs

GMP becomes easy when it begins right from the designing stage of the facilities. In this case, the designing should envision how GMP will be implemented at the time when the building plan is being laid out (Patel and Chotai 2011). Most processors find it difficult to implement required structural modifications years after the facility was constructed because it is reasonably expensive apart from posing a risk of developing material faults in the walls of the building. For the systems to be flexible, the structural design of the facility itself has to allow critical changes that can take place any time without affecting the entire facility at the lowest cost possible. Space as to be enough for orderly packing of materials and placement of equipment. This ensures that there is no chance of mixing up unrelated materials, like raw peanuts and processed peanut butter, which could lead to the contamination of the finished product (Signore and Jacobs 2005). The construction should be done in a way to separate defined areas to prevent contamination. For example, a raw material storage room has to have everything required for the purpose and should be constructed separately from the finished product storage room, also with its own defined characteristics. No rooms or sections of the building should be constructed without purpose. Washrooms need to be located in strategic locations with less chance of causing cross-contamination in the building and septic tanks be laid out away incase in future they overflow for some reasons (Mtewa and Mtewa 2017). In addition to the layout designing, the premises surrounding the processing unit should be designed in a way that will keep the place safe from all potential product contaminants, accessible, attractive and re-assuring to customers and inspectors. This could be as basic as minimizing the presence of earth surface with concrete, bricks or bitumen and keeping surrounding grass surfaces minimal and always short.

Apart from general designing, the layout for functional food processing units need to make sure that the facility has all food contact surfaces made of appropriate materials that cannot pose a risk of contaminating the product (Vu et al. 2014). The most commonly used surfaces are stainless steel and aluminum. Wooden material should not be used inside the food processing unit due of the following reasons: (1) Wood can easily absorb atmospheric moisture or flowing surface water during wet cleaning sessions. This water and air is a good breeding environment for pathogens which can find their way into the product. (2) The wooden material can easily be cut off accidentally or otherwise and the small prices can find their way into the product.

Construct enough hand-washing facilities for all factory entrance points, break areas, rest rooms and manufacturing areas, where there is no restriction of wet cleaning. Water systems flow back potential must always be avoided to avoid contamination and spillage of the source by exposed or used water. A facility should be free from any unnecessary openings to avoid entry of pests and insects. Cracks and holes need to be sealed on ceilings, doors, windows, floors and walls. Sometimes, insects and pests find their way through the space that is usually found under the doors, this is common on loading dock doors. It should be made sure that loading dock doors and any other doors are flush to the floor when closed (Signore and Jacobs 2005). For key holes, it is recommendable to use coverable holes rather than the open ones which are common on the market (US FDA 2017).

27.4 Control Systems

27.4.1 Traceability Activities

Traceability is a process that ensures clear linkages amongst raw materials, individuals, tools and procedures in real time. It is one of the best methods to verify functionality of a quality assurance system and good manufacturing procedures. This requires that each and every record that the processing facility is using should be easily accessible within the premises. These include test results, validation documents, customer queries, complaints and regulatory certifications among others (Eudralex 2012).

Any missing links should raise serious questions about compliance and adherence to systems and this obviously has negative implications to customers or clients who are quality minded. Your quality system should indicate how you do traceability in your facility including people responsible and frequencies. A calendar for traceability, reports and records from previous exercises should always be in place for reviews (Patel and Chotai 2011).

27.4.2 Food Hazard Control

Food is one of the important materials living things need for survival. Ideally, food is supposed have good quality and safe for consumption. Food may sometimes be contaminated with hazards that when consumed may affect consumers health (Pal and Mahendra 2015). Achievement of safe food supply is one of the activities involved in public health programs aimed at protecting the public through prevention, minimisation and elimination of risks associated with food hazards. Several studies have reported cases of harm arising from unsafe food and efforts are therefore implemented in the food industry to ensure food safety (Martinović et al. 2016). A hazard is a biological, chemical or physical agent with potential of causing harm, illness or injury (Pal et al. 2016).

27.4.2.1 Biological Hazards

Biological hazards mostly comprise of microorganisms such as harmful bacteria, yeasts, molds, protozoa, viruses and parasites. There are several sources of biological hazards and these include raw materials and personnel contamination (Pal et al. 2016). Biological hazards are the highly dangerous and widely controlled hazard and many guidelines have been developed for the control of biological hazards as compared to physical and chemical hazards (Keener 2001). Microorganisms have both beneficial and harmful effects. For example, some foodstuffs such as cheese, sour cream, yogurt and other fermented dairy products are made from reactions of certain bacteria, yeasts and molds. Hence, these are added intentionally in the food processing. Apart from food production, some microorganisms also play important roles in our daily life. Therefore, we are surrounded by both beneficial and harmful microorganisms and in hazard control, experts (food processors, public health officials and regulators) the intention is to get rid of those that harm people called pathogenic microorganisms (Cornell University 2018).

Some microorganisms (bacteria, protozoa and viruses) are too small to be seen with naked eyes and you need a microscope to see them. Other microorganisms (yeasts, molds and parasites) can be seen with naked eyes. Microorganisms' survival and growth depends on adequate food, water, temperature and air (others need no air or minimal air). Depriving them any of these may stop their growth and multiplication, lead to hibernation or death. Hence, to get rid of them, one needs to disturb the availability of those essential elements and this is sometimes achieved by preservation methods like drying or smoking (Gutierrez 2003).

Microorganisms' metabolic processes can produce by-products and these increase as they grow in size and number. Some by-products have beneficial effects on human beings as it leads to the production of food for them, while others are undesirable for human being because they lead to spoilage and wastage of the food, sometimes even affecting their health. For example, the growth of yeast produces dough, carbon dioxide, acids and flavors and the dough rising leads to production of bread that we eat, and in this case, the process may be desirable. However, when the process takes place in another kind of food like fruit juice, the same by-products leads to spoiled food and the process may be undesirable. In some cases, the by-products produced in other microorganisms like pathogens may be toxins that can cause diseases. Table 27.1 below shows the commonly found biological hazards.

During the processing of foods, the amounts and types of microorganisms can be increased, held constant, reduced or destroyed. Even though processing can be used to destroy harmful microorganisms, many safe microorganisms can survive the treatment and continue to live. For example, milk is pasteurized, or heat-treated, to destroy pathogens. After pasteurization, milk is safe to drink even though non-pathogenic microorganisms survive.

Table 27.1 Types of biological hazards in foods and food products

Microorganism	Examples	Hazardous effect
Bacteria	*Clostridium botulinum* (spore former)	Central nervous system (CNS), shortness of breath, blurred vision, loss of motor capabilities and death.
	Listeria monocytogenes (non-spore former)	Infection, severe listeriosis, septicemia, meningitis, encephalitis and still-births.
	Salmonella spp. (non-spore former)	Infection and death
Virus	Hepatitis A virus	Fever, abdominal discomfort and jaundice.
	Norwalk virus	Nausea, vomiting, diarrhea, abdominal pain (gastroenteritis), headache and low-grade fever.
Parasites	*Giardia lamblia*	Diarrhoea, abdominal cramps, fatigue, nausea, flatulence (intestinal gas) and weight loss.
	Entamoeba histolytica	Dysentery (severe, bloody diarrhoea).
	Ascaris lumbricoides	Intestinal and lung infection.
	Diphyllobothrium latum	Abdominal pain, cramping, flatulence and diarrhoea.

27.4.2.2 Chemical Hazards

These are chemical compounds that can cause illness or injury upon exposure for a short or long period of time. Chemicals are used in our daily lives and serve their purpose with minimal or no negative effect when used as recommended. Chemicals occur naturally but they can also be introduced into the environment intentionally or unintentionally. Different chemicals have different physicochemical properties, which also give them different toxicological profiles. Because of this difference, acceptable exposure amounts have been established for each chemical such that certain amounts are allowed to be present in products, beyond which they increase the risk of causing harm. For example, chemicals can be helpful and are purposefully used with some foods, such as pesticides on fruits and vegetables. Chemical risk occurs when chemicals are used without control or when the recommended treatment rates are exceeded. Apart from depending on intrinsic property of a compound, chemicals risk or toxicity may also depend on other factors such as period of exposure and concentration (Castro and Hollyer 2014).

Chemical hazards are generally categorised into three groups (Table 27.2). The first group is described as naturally occurring chemicals (including allergens). These are mostly chemicals originating from plants, animals or microorganisms such as toxins from bacteria. The second group is intentionally added chemicals. These are mostly chemicals that are added to enhance the growth of the raw materials for producing food, during manufacturing process and during storage of food. There are set procedures and precautions for their use to ensure that safety of the consumers is not compromised. As already mentioned, limits of exposure have been set for every chemical and this is checked by regulators and within manufacturing entities to ensure adherence to the limits. The third group belongs to unintentionally added chemicals. These compounds get into the food unintentionally and it is

Table 27.2 Summary of the three (3) groups of chemical hazards exposure of foods

Chemical class	Examples of sources	Hazardous effect
Naturally occurring chemicals	Certain fish species (e.g., tuna, mahi-mahi)	Toxin production from fish spoilage
	Nuts, seafood	Allergic reaction in sensitive people.
	Corn	Toxin (like aflatoxin) formation from mould growth on corn and similar foodstuffs.
	Molluscan shellfish	Toxins like domoic acid from some food the fish eats.
Intentionally-added chemicals	FD&C Yellow No. 5	Allergic-type reactions in some people.
	Sodium nitrite (preservative)	Toxic in high concentrations.
	Vitamin A (supplement)	Toxic in high concentrations.
	Sulfiting agents (preservative)	Allergic-type reaction in sensitive people.
Unintentionally added chemicals	Agricultural chemicals like pesticides and herbicides	Toxic at high levels and long-term exposure.
	Cleaning chemicals like acids and caustics	Chemical burns at high levels.
	Maintenance chemicals like lubricants and paint	Toxic.

challenging to eliminate them entirely. But it is possible to limit their amounts in the food. These include antibiotics in animal feeds and sanitizers and inks in foodstuffs that may arise from packaging materials.

27.4.2.3 Physical Hazards

Physical hazards are materials that can cause physical harm such as choking, cuts, dental complaints such as broken teeth, oral injury or laceration and/or bleeding, trauma to the esophagus, abdomen or other associated organs of the alimentary canal, and adverse events. These may also have other adverse events and surgery sometimes to remove them. Examples include glass, metal fragments, wood fragments and many more. These are materials that are normally not supposed to be present in food. Physical hazards are one of the widely reported hazards in food because most of them can be easily seen using eyes and their effects are usually immediately after exposure (Keener 2001).

27.5 Hazard Analysis Critical Control Point (HACCP)

Hazard Analysis Critical Control Point (HACCP) is a systematic and preventive approach to food safety. HACCP has been in use since 1960s by most countries around the world (CFI 2018). HACCP is not only involved in the inspection of finished food products; it is also involved in the finding, correction and prevention of the aforementioned hazards in production processes (Schedule 2012).

In HACCP guidelines, a hazard is a material or condition in food that can cause illness or injury. So food stuffs that have undesirable conditions or contaminants such as insects, hair, filth, spoilage, economic fraud and violations of regulatory food standards not directly related to safety are not a concern. In addition, spoiled food may not look, smell or taste good, if it is not concerning people's safety then that is not covered by HACCP. This becomes a HACCP concern if the food is spoiled by pathogens or contaminated by toxic microbial by-products that have potential to affect consumers. HACCP system development is guided by seven principles outline below.

27.5.1 HACCP Principles

There are seven principles of HACCP and these are followed by every country that adopts it. The first principle states that every process requires hazard analysis. This requires there should be a plan to identify all potential food safety hazards as well as their control measures. As a matter of illustration, for every cooking process, one of the potential hazards is pathogen survival that may arise from inadequate cooking time or temperature. The second principle states that it is important to identify critical control points in every production process; points for prevention, elimination or reduction of hazards. Based on our earlier example, cooking process could be one of the critical control points as it requires some measures to prevent pathogen survival. The third principle requires establishment of critical limits for every critical control point. These are acceptable limits of hazard that cannot compromise the safety of food. In the cooking process example, critical limits could include specific time and temperature to which the cooked food should be exposed. The fourth principles require the establishment of procedures to be followed when monitoring the critical control points. The procedures should be as detailed as possible to ensure safe operation of the process. In the cooking example, this could include the use of a thermometer to check the temperature periodically. The fifth principle involves the establishment of corrective measures. These are actions that must be taken to restore a process to its track when there is a deviation from the critical limits. For example, in the cooking illustration, when the required time or temperature is not reached, corrective measures could be either further cooking or destroying the product. The sixth principle requires the establishment of procedures for verification of an activity or condition. This involves application of methods, procedures, tests, sampling, monitoring and evaluations of a process to determine whether a control measure at a critical control point are running according to planned company's written HACCP program. The seventh principle is record keeping. A company is supposed to keep records to show that there is effective application of the critical control points and for official verification by a regulatory body or internal control team (CFIA 2018; US FDA 2017).

HACCP may be mandatory in some sectors of the industry and may be just recommended for others. Development, implementation and maintaining a HACCP system is industry's responsibility because food manufacturers have the most control over the products they manufacture, so they have the greatest impact on the safety of their products (WHO 2008).

27.5.2 Strategies for Controlling Hazards

Several strategies have been developed for identification and control of hazards. A successful HACCP program or system requires concerted efforts in many sectors of the manufacturing process such as quality control (QC) procedures, sanitation and hygiene programs and good manufacturing practices (GMPs) (Keener 2001). Table 27.3 summarizes some ways of controlling hazards.

27.5.3 Requirements for Effective Implementation of HACCP System

Effective hazard control requires collaborative efforts by research and development (R&D), manufacturing, sanitation, quality assurance and purchasing personnel, vendors, suppliers, regulators and the company's quality control and assurance. Furthermore, companies need to collaborate with growers, intermediate handlers and transporters so that they can be supplying ingredients and raw materials of good quality (Keener 2001).

Table 27.3 Ways of controlling hazards on functional foods

Contaminant	Transmission pathways	Control
Biological contaminants	Food or water that is contaminated by faecal material shed by infected hosts.	Good personal hygiene practices by food handlers, proper disposal of human faeces, elimination of insufficiently treated sewage to fertilize crops, and proper sewage treatment.
Physical contaminants	Manufacturing environment, raw materials and ingredients, plant equipment, contractors and employees.	On-line visual inspection, in-line metal detection, the use of magnets, on-line automated vision systems, X-ray technology, and screens, filters and sieves.
Chemical contaminants	Raw materials, ingredients and personnel practices such as packaging materials.	Working with growers, intermediate handlers and transporters maintain chemical integrity of ingredients and raw materials.

27.6 Sanitation and Hygiene

For a HACCP system to work effectively it requires solid base of hygienic measures, conditions and requirements. This can be achieved by implementing an active and well monitored and evaluated permanent food hygiene program (WHO 2008). For a facility to ensure achievement of food hygiene requirements and measures, it need have robust management and supervision system, well designed and constructed facilities with in-built hygiene structures and arrangements, supplier control system and methods, well designed and constructed production equipment, have maintenance, cleaning and sanitation equipment and protocol, pest control, waste management system and tools, personal hygiene of its workers, having workers who are healthy, with good behaviour, control for visitors, chemical control, measures for preventing cross-contamination and use of packaging materials that prevent food from contamination as well as materials that have minimal contamination in themselves. Of particular interest is the quality of incoming materials. It is possible to control the hygiene of an entity, but it could be challenging to control hygienic tendencies for another company that supplies raw materials. Hence every company needs guidelines on the handling of incoming raw materials (WHO 2008).

It is important to make sure that every employee who gets in contact with raw materials, processing utensils and machinery and packing materials goes for medical check-up regularly. Carefully, without raising workplace discrimination concerns, communicable infections should be targeted in the check up and those that are found infected should be put on treatment and immediately removed from the food contact tasks until there is evidence of recovery. This helps to protect a huge number of product consumers out there from potential infections. Certificates of fitness to undertake food contact tasks should always be available with clear dates and results from an authentic health facility.

27.6.1 Raw Materials Handling

All incoming materials into a manufacturing entity should have quality safety standards that must be met by a supplier. These materials include raw materials, ingredients and cleaning, disinfection and packaging materials. Any materials with lower than established standards, contaminated with hazards must be rejected (EudraLex 2012). Every supplier should be evaluated and approved prior to materials supply if possible to know if they meet the quality standards and fit for use. Raw materials and ingredients require inspection, sorting and testing if necessary. Materials also need storage and stock movement controls (WHO 2008).

27.7 Training for Processing and Other Personnel

Training is a fundamental requirement for every personnel employed by food processing entity. Training requirements depend on nature of the food handled and hazards involved. Training should include personnel's roles and responsibility and the skills required. Apart from structured training, refresher training is also required for the personnel to keep abreast of new developments in the industry. For every training, there must be routine review and update where necessary (US FDA 2017). There is a need for a clear training calendar which must be respected. Evidence for adherence to training calendar are training certificates and/or signed attendance forms. Training can be tailored to suit specific tasks that are crucial in a functional foods processing unit such as how to thoroughly clean hands. All employees concerned with any part of the product must undergo relevant training or short courses every specified period of the year.

27.8 Pest Control and Environmental Monitoring Program

Ever facility that is manufacturing functional foods must have clear programs on how pests will be prevented from entering and contaminating warehouses and processing units. Well identifiable bait traps should be placed at strategic locations and this must be reflected in a document plan as well. The levels of contamination of the environment must also be monitored regularly. These include airborne microbials and insects. All controlling chemicals must not pose a risk to the products. These tasks may be sub-contracted to experts to ensure that they are doing the right things.

27.9 The Food Safety Team

Every functional food processing unit must have a team dedicated to the safety of the product. This team should be empowered to make critical decisions that will protect the products from being unsafe (Lund 1994). The team functions well when head by the quality assurance manager who must report directly at the top management. The food safety team should ensure to hold regular relevant meetings to address previous observations and plan for the coming production days. The food safety team should be able to evaluate all matters to do with safety of the product which includes personnel health, quality of the food, job descriptions and limits of key production personnel, pest control among others. All members of the team must undergo regular trainings and their membership and meeting minutes to be recorded and documented.

27.10 Internal and External Systems Audits

Systems audits are basically a series of inspections and probes that are regularly conducted on a processing unit to ascertain its levels of compliance to acclaimed systems in use (Mtewa and Mtewa 2017). If a manufacturer indicates that they have quality assurance systems in place, these systems have to be assessed and recommendations for improvement made. Internal audits are conducted by trained members of the facility while external ones are conducted by other inspectorates from outside the facility. These must be well planned across the year and be sure to cover all aspects of systems at the facility (Primus Lab. 2015). They should be designed in a way that aims at auditing systems as opposed to auditing individuals. Audits provoke the traceability of records and documents that the facility indicates to have and must be found for every item sought (WHO 2008).

27.11 Product Specifications and Conformance

All functional foods that are being manufactured should have clear indicators to acceptability or rejection. These can be set within the facility but usually, they are derived from national or international guidelines and specifications (US FDA 2017). For functional foods, the WHO sets its own standards although regional bodies of the European Union, African Union, SADC or other blocks set their own customized standards for their won markets (Mtewa and Mtewa 2017). Specifications are basically values that mark a range of test results that will be accepted by a market or a regulator, with, at most times, an average value as a target. For instance, one paste product may be accepted at vitamin D_3 level of 500 μg/g as a target with a minimum allowable value of 450 μg/g and a maximum allowable value of 550 μg/g. During production, performance of the products should always be watched by a team of trained personnel so that appropriate interventions should be timely made.

27.11.1 Laboratory Tests and Validation

Laboratory tests should be carried out only by qualified and trained staff. Minimum standards of personnel that can conduct the tests should be clearly stated and matching what is actually on the ground. Validated test protocols should be in place (Vu et al. 2014). Validation of test results can be done using standards as well as by collaborating with a second and a third independent laboratory that should produce statistically comparable results (Catsambas et al. 2002). All test results must be entered as raw data into a laboratory book or computer data sheet without a possibility of a non-verifiable cancellation. If in a book, they should be entered in ink, with dates, signatures and details of the technician who conducted the tests. Similar details should be entered in a computer data sheet which should be programmed in a way to protect every singular detail of the results entered.

27.11.2 Handling of Non-conforming Products

Non-conforming products are those that are outside the acceptable ranges and should not be sold at any cost. Depending on severity of the deviation, a decision can be made to either accept the product with minimal reworking, major reworking or completely rejecting the product and dispose off. Every detail of the non-conformance should be recorded and documented. At all times, the root causes should be researched, traced and identified to avid recurrence of the non conformance. Corrective and preventive actions should then be taken on the product and/or any other item that was or caused the product to be out of conformance.

27.12 Equipment and Maintenance

27.12.1 Equipment Management

Functional food processing facilities need to have a master list of all pieces of equipment and tools available. Equipment should always be in good functioning state. If one malfunctions, it should immediately be logged in and reported to those in authority for decisions on checks, service and/or immediate replacement (Mtewa and Mtewa 2017). The malfunctioning tool should be taken away from the work station and appropriately stored for remediation. All measuring, monitoring and testing equipment critical for ensuring quality should be calibrated according to procedures that should be available on file. The calibration procedure should include a requirement for a log of all tools and equipment that must be calibrated and a schedule for calibration for each which should indicate date of calibration and/or servicing, calibrating and/or servicing body or individuals, date that the calibration and/or servicing will no longer be valid and the date set for the next calibration and/or servicing (Lund 1994). Bodies and individuals that calibrate and service your tools and sets of equipment should be approved professionals. Certificates form calibration and servicing should be kept chronologically with copies kept by appropriate personnel for easy traceability.

For all major sets of equipment, the facility should have a written maintenance program in place. This needs to be in two parts; preventive and reparation maintenance plans (Schedule 2012). In the preventive maintenance program, the functional food facility should set deliberate schedules over which machinery and equipment shall routinely be inspected and checked for functionality and faults, no matter the status quo. This helps to identify any potential deviations from perfection.

This is a good proactive way of avoiding machinery or tool malfunctions. Reparative maintenance on the other hand is the one that is conducted reactively as a response to a malfunction or a fault.

All maintenance programs need to have details such as particular technicians who need to undertake a specific task on a set of equipment or machinery. Well signed records and documents on maintenance should always be stored. If there is need for any modification on machinery, this has to be stated together with dates, personnel involved as well as reasons that compelled the modifications (Schedule 2012).

27.13 Cleaning Procedures

The maintenance of the facility and equipment goes well with an effective cleaning program. Similarly, there's a need for a detailed plan for ach and very room, tool and equipment in the facility. Evidence needs to be available on the safety and effectiveness of all the chemicals and detergents being used in cleaning (Mtewa and Mtewa 2017). Authentic material safety data sheets (MSDS) provide the best safety profiles of cleaning materials and any other chemical in the facility.

All cleaning materials being used in the facility need to be resistant to corrosion and non-toxic (Lund 1994). If metals are used in the processing facility, they should be stainless to avoid contaminating functional foods with scrap-offs from metallic surfaces. If paint is used for decoration or some reason, care must be taken as well to avid peel offs from finding their way into the products. It is generally recommended to avoid paint in functional food processing units.

27.14 Customer and Manufacturer Issues

Customers constitute an important section of product manufacturing. It is important to have a point of contact between customers and manufacturers. This ensures a good interface for interaction through which feedback from customers can be received, thereby informing on how the products are fairing on the market.

27.14.1 Product Information and Customer Awareness

Customers need to be made aware of any important details that functional foods being manufactured have. These include any potential risks such as allergens, manufacturing and expiry dates, storage conditions, manufacturers name and full address and instructions for use and storage, all in a legible manner and in a language that is understood by the market base (ASEAN 2013). If some information is missing or falsified, there is a high risk that patients might be harmed by consuming the product which is an offense that can cost the manufacturer a lot of money and reputation.

27.14.2 Customer Complaints Handling and Recall Procedures

Every complaint that a customer lodges against functional food products, the manufacturer needs to take seriously and trace the root causes of such complaints right from the supply chain of all materials used during the processing of that particular product batch to distribution (WHO 2008). No complaint should be ignored. Clear procedures of what to do upon receipt of complaints should be in place at all times. Customers must be advised what to do with any remaining purchases they made, and bad product from the market should be recalled and destroyed, with evidence (Campbell and Tyler 2012). Feedback on any complaint to customers must be documented and kept safely.

27.15 Advantages of GMP and Food Safety Systems

As earlier indicated, good manufacturing procedures help the facility as well as the products retain and gain more ground with it market base. In this case, customers are more trusting in a facility that is clear in what it is doing with well elaborate systems in place ensuring that the functional foods they are manufacturing are safe and of high quality. In addition to that, inspectorates, regulators and certifiers have more confidence in a product that has been produced in a facility that has good manufacturing practices in place. In general, GMP assures confidence and trust from the market which facilitate the survival of the business.

27.16 Future Prospects in Functional Foods Manufacturing

Good manufacturing practices provide a platform that ensures that the products being manufactured continuously improve in terms of quality and safety. This encompasses all areas in the facility from executive management through cleaning to engineering and the supply chain of all materials in use in the processing facility (Lund 1994). With the changing world dynamics, GMP is always improving from observations and research. One challenge that GMP is faced with is lack of awareness to country/village manufacturers of functional foods. The practice are either not known or understood which make village processing units fail to compete on bigger markets. To make it even better, school curricula need to adopt it so that much of the practices can be inculcated in the learners at an early stage.

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Correction to: Aloe Species as Valuable Sources of Functional Bioactives

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In the original version of this book, the initial of one of the co-author's name was incorrectly published as M. Deepak which should have been published as S. Deepak. The author's correct name has now been updated in this revised version of the book.

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