HANDBOOK OF FOOD BIOENGINEERING
VOLUME 8

THERAPEUTIC FOODS



Edited by
Alina Maria Holban
Alexandru Mihai Grumezescu

Therapeutic Foods

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Handbook of Food Bioengineering, Volume 8

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Alina Maria Holban
Alexandru Mihai Grumezescu





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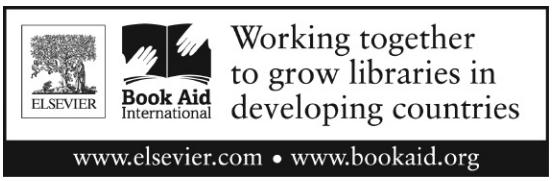
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Foreword

In the last 50 years an increasing number of modified and alternative foods have been developed using various tools of science, engineering, and biotechnology. The result is that today most of the available commercial food is somehow modified and improved, and made to look better, taste different, and be commercially attractive. These food products have entered in the domestic first and then the international markets, currently representing a great industry in most countries. Sometimes these products are considered as life-supporting alternatives, neither good nor bad, and sometimes they are just seen as luxury foods. In the context of a permanently growing population, changing climate, and strong anthropological influence, food resources became limited in large parts of the Earth. Obtaining a better and more resistant crop quickly and with improved nutritional value would represent the Holy Grail for the food industry. However, such a crop could pose negative effects on the environment and consumer health, as most of the current approaches involve the use of powerful and broad-spectrum pesticides, genetic engineered plants and animals, or bioelements with unknown and difficult-to-predict effects. Numerous questions have emerged with the introduction of engineered foods, many of them pertaining to their safe use for human consumption and ecosystems, long-term expectations, benefits, challenges associated with their use, and most important, their economic impact.

The progress made in the food industry by the development of applicative engineering and biotechnologies is impressive and many of the advances are oriented to solve the world food crisis in a constantly increasing population: from genetic engineering to improved preservatives and advanced materials for innovative food quality control and packaging. In the present era, innovative technologies and state-of-the-art research progress has allowed the development of a new and rapidly changing food industry, able to bottom-up all known and accepted facts in the traditional food management. The huge amount of available information, many times is difficult to validate, and the variety of approaches, which could seem overwhelming and lead to misunderstandings, is yet a valuable resource of manipulation for the population as a whole.

The series entitled *Handbook of Food Bioengineering* brings together a comprehensive collection of volumes to reveal the most current progress and perspectives in the field of food engineering. The editors have selected the most interesting and intriguing topics, and have dissected them in 20 thematic volumes, allowing readers to find the description of basic

processes and also the up-to-date innovations in the field. Although the series is mainly dedicated to the engineering, research, and biotechnological sectors, a wide audience could benefit from this impressive and updated information on the food industry. This is because of the overall style of the book, outstanding authors of the chapters, numerous illustrations, images, and well-structured chapters, which are easy to understand. Nonetheless, the most novel approaches and technologies could be of a great relevance for researchers and engineers working in the field of bioengineering.

Current approaches, regulations, safety issues, and the perspective of innovative applications are highlighted and thoroughly dissected in this series. This work comes as a useful tool to understand where we are and where we are heading to in the food industry, while being amazed by the great variety of approaches and innovations, which constantly changes the idea of the "food of the future."

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Series Preface

The food sector represents one of the most important industries in terms of extent, investment, and diversity. In a permanently changing society, dietary needs and preferences are widely variable. Along with offering a great technological support for innovative and appreciated products, the current food industry should also cover the basic needs of an ever-increasing population. In this context, engineering, research, and technology have been combined to offer sustainable solutions in the food industry for a healthy and satisfied population.

Massive progress is constantly being made in this dynamic field, but most of the recent information remains poorly revealed to the large population. This series emerged out of our need, and that of many others, to bring together the most relevant and innovative available approaches in the intriguing field of food bioengineering. In this work we present relevant aspects in a pertinent and easy-to-understand sequence, beginning with the basic aspects of food production and concluding with the most novel technologies and approaches for processing, preservation, and packaging. Hot topics, such as genetically modified foods, food additives, and foodborne diseases, are thoroughly dissected in dedicated volumes, which reveal the newest trends, current products, and applicable regulations.

While health and well-being are key drivers of the food industry, market forces strive for innovation throughout the complete food chain, including raw material/ingredient sourcing, food processing, quality control of finished products, and packaging. Scientists and industry stakeholders have already identified potential uses of new and highly investigated concepts, such as nanotechnology, in virtually every segment of the food industry, from agriculture (i.e., pesticide production and processing, fertilizer or vaccine delivery, animal and plant pathogen detection, and targeted genetic engineering) to food production and processing (i.e., encapsulation of flavor or odor enhancers, food textural or quality improvement, and new gelation- or viscosity-enhancing agents), food packaging (i.e., pathogen, physicochemical, and mechanical agents sensors; anticounterfeiting devices; UV protection; and the design of stronger, more impermeable polymer films), and nutrient supplements (i.e., nutraceuticals, higher stability and bioavailability of food bioactives, etc.).

The series entitled *Handbook of Food Bioengineering* comprises 20 thematic volumes; each volume presenting focused information on a particular topic discussed in 15 chapters each. The volumes and approached topics of this multivolume series are:

Volume 1: Food Biosynthesis

Volume 2: Food Bioconversion

Volume 3: Soft Chemistry and Food Fermentation

Volume 4: Ingredient Extraction by Physicochemical Methods in Food

Volume 5: Microbial Production of Food Ingredients and Additives

Volume 6: Genetically Engineered Foods

Volume 7: Natural and Artificial Flavoring Agents and Food Dyes

Volume 8: Therapeutic Foods

Volume 9: Food Packaging and Preservation

Volume 10: Microbial Contamination and Food Degradation

Volume 11: Diet, Microbiome, and Health

Volume 12: Impacts of Nanoscience on the Food Industry

Volume 13: Food Quality: Balancing Health and Disease

Volume 14: Advances in Biotechnology in the Food Industry

Volume 15: Foodborne Diseases

Volume 16: Food Control and Biosecurity

Volume 17: Alternative and Replacement Foods

Volume 18: Food Processing for Increased Quality and Consumption

Volume 19: Role of Material Science in Food Bioengineering

Volume 20: Biopolymers for Food Design

The series begins with a volume on *Food Biosynthesis*, which reveals the concept of food production through biological processes and also the main bioelements that could be involved in food production and processing. The second volume, *Food Bioconversion*, highlights aspects related to food modification in a biological manner. A key aspect of this volume is represented by waste bioconversion as a supportive approach in the current waste crisis and massive pollution of the planet Earth. In the third volume, *Soft Chemistry and Food Fermentation*, we

aim to discuss several aspects regarding not only to the varieties and impacts of fermentative processes, but also the range of chemical processes that mimic some biological processes in the context of the current and future biofood industry. Volume 4, *Ingredient Extraction by Physicochemical Methods in Food*, brings the readers into the world of ingredients and the methods that can be applied for their extraction and purification. Both traditional and most of the modern techniques can be found in dedicated chapters of this volume. On the other hand, in volume 5, *Microbial Production of Food Ingredients and Additives*, biological methods of ingredient production, emphasizing microbial processes, are revealed and discussed. In volume 6, *Genetically Engineered Foods*, the delicate subject of genetically engineered plants and animals to develop modified foods is thoroughly dissected. Further, in volume 7, *Natural and Artificial Flavoring Agents and Food Dyes*, another hot topic in food industry—flavoring and dyes—is scientifically commented and valuable examples of natural and artificial compounds are generously offered. Volume 8, *Therapeutic Foods*, reveals the most utilized and investigated foods with therapeutic values. Moreover, basic and future approaches for traditional and alternative medicine, utilizing medicinal foods, are presented here. In volume 9, *Food Packaging and Preservation*, the most recent, innovative, and interesting technologies and advances in food packaging, novel preservatives, and preservation methods are presented. On the other hand, important aspects in the field of *Microbial Contamination and Food Degradation* are shown in volume 10. Highly debated topics in modern society: *Diet*, *Microbiome*, *and Health* are significantly discussed in volume 11. Volume 12 highlights the *Impacts of Nanoscience on the Food Industry*, presenting the most recent advances in the field of applicative nanotechnology with great impacts on the food industry. Additionally, volume 13 entitled *Food Quality*: *Balancing Health and Disease* reveals the current knowledge and concerns regarding the influence of food quality on the overall health of population and potential food-related diseases. In volume 14, *Advances in Biotechnology in the Food Industry*, up-to-date information regarding the progress of biotechnology in the construction of the future food industry is revealed. Improved technologies, new concepts, and perspectives are highlighted in this work. The topic of *Foodborne Diseases* is also well documented within this series in volume 15. Moreover, *Food Control and Biosecurity* aspects, as well as current regulations and food safety concerns are discussed in the volume 16. In volume 17, *Alternative and Replacement Foods*, another broad-interest concept is reviewed. The use and research of traditional food alternatives currently gain increasing terrain and this quick emerging trend has a significant impact on the food industry. Another related hot topic, *Food Processing for Increased Quality and Consumption*, is considered in volume 18. The final two volumes rely on the massive progress made in material science and the great applicative impacts of this progress on the food industry. Volume 19, *Role of Material Science in Food Bioengineering*, offers a perspective and a scientific introduction in the science of engineered materials, with important applications in food research and technology. Finally, in volume 20, *Biopolymers for Food Design*, we discuss the advantages and challenges related to the development of improved and smart biopolymers for the food industry.

All 20 volumes of this comprehensive collection were carefully composed not only to offer basic knowledge for facilitating understanding of nonspecialist readers, but also to offer valuable information regarding the newest trends and advances in food engineering, which is useful for researchers and specialized readers. Each volume could be treated individually as a useful source of knowledge for a particular topic in the extensive field of food engineering or as a dedicated and explicit part of the whole series.

This series is primarily dedicated to scientists, academicians, engineers, industrial representatives, innovative technology representatives, medical doctors, and also to any nonspecialist reader willing to learn about the recent innovations and future perspectives in the dynamic field of food bioengineering.

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Preface for Volume 8: Therapeutic Foods

In the modern society, the concept of therapeutic foods has gained new insights among researchers, medical representatives, industry, and consumers.

Traditional uses of therapeutic foods were related to nutritional and therapeutic purposes in the form of dietary supplements. They were used for emergency feeding of malnourished children or to supplement the diets of patients having special nutritional needs. In recent years, the progress made in research and technology enabled the development of a true therapeutic food revolution. The concept of therapeutic foods was currently expanded to new avenues in medicine, that is, to prevent and even cure metabolic disorders or to promote a healthy lifestyle; and also in industry, that is, the development of green food industry. The concept of nutraceuticals and functional foods emerged in the last 20 years and the intense research made on this field has led to the development of numerous dietary supplements and food additives with health-benefiting properties.

This book aimed to bring together the most investigated and innovative approaches regarding the development of therapeutic foods to obtain valuable products with improved quality, ensuring health promotion and environment protection in the context of a sustainable food industry.

The volume contains 15 chapters prepared by outstanding authors from Serbia, USA, Pakistan, Greece, India, Czech Republic, Mexico, Bulgaria, and Peru.

The selected manuscripts are clearly illustrated and contain accessible information for a wide audience, especially food scientists, engineers, biotechnologists, biochemists, industrial companies and also for any reader interested in learning about the most interesting and recent advances in the field of therapeutic foods.

This volume includes an introduction into nutraceuticals (Chapter 1), *Nutraceuticals*: *Myths Versus Realities*, prepared by Sharif and Khalid. The chapter discusses the myths and realities about the use of nutraceuticals widely clearing prevalent ambiguities about their health benefits and the safe use before starting a new dietary regimen. A comprehensive deliberation regarding global market, trends, commonly used nutraceuticals, scientific evidence of reported health claims, mechanisms of action, nutraceuticals as diet replacement, comparison

between drug and nutraceutical, safety and quality indicators, labeling requirements, and regulatory bodies are debated in this work.

Chapter 2, entitled *The Impact of Functional Food and Nutraceuticals in Health*, prepared by Singh et al., explores the health benefits of the easily available nutraceuticals, which could act as adjunct lifesaving molecules for patients with chronic diseases, in a context of an established epidemiological relation between disease, lifestyle, and diet.

In Chapter 3, *Lipids as Nutraceuticals*: *A Shift in Paradigm*, Kumar et al. reveals potential advantages of the incorporation of lipid compounds into human diet as functional foods. This study comes as an explanation over the fact that dietary intake of some lipids has been shown to suppress inflammation, hypercholesterolemia, and dyslipidemia and are implicated in cellular signaling pathways, including programmed cell death and malignant transformation.

In Chapter 4, entitled *Plant Nutrition and Agronomic Management to Obtain Crops With Better Nutritional and Nutraceutical Quality*, Cabrera-De la Fuente et al. describe different growing systems, fertilizer management, the use of natural or synthetic elicitors, and the soil and climate conditions that lead to the production of crops with better nutritional and nutraceutical qualities.

In Chapter 5, *Mushrooms as Sources of Therapeutic Foods*, Soković et al. focus on recent literature regarding antioxidant, antimicrobial, antitumor, and antidiabetic properties of edible mushrooms. Molecular mechanisms involved in the health-beneficial effects of mushroom extracts and purified compounds are discussed.

Chapter 6, *Oregano*: *A Feed Additive With Functional Properties*, prepared by Giannenas et al., focuses on recent advances in the dietary use of oregano, which demonstrated notable beneficial effects on animal growth performance, feed efficiency, production traits, and product quality, as well as on modulation of immune system, intestinal architecture, and microbiota. Oregano could be regarded as a promising functional ingredient for an extensive use in animal nutrition.

In Chapter 7, *Potential of Chokeberry* (Aronia Melanocarpa *L.*) *as a Therapeutic Food*, Ćujić et al. reveal the health-beneficial effects of chokeberry, highlighting impact on decreasing oxidative damage markers, improvement in prooxidant–antioxidant balance after polyphenol-rich chokeberry juice consumption, and increase in polyunsaturated fatty acids and decrease in monounsaturated fatty acids in erythrocytes, suggesting a role in cell protection against oxidative stress.

In Chapter 8, *Beta Glucan as Therapeutic Food*, Vaclav et al. offer an up-to-date comprehensive review of the possible use of beta-glucan as a food additive, because beta-glucan has not only a prebiotic quality, but also immunostimulatory effects regardless of the route of administration, making it an ideal food additive.

In Chapter 9, *Nutritionally Enhanced Foods Incorporating Chía Seed*, Rendón-Villalobos et al. reveal the benefits of *chía*, one of the oldest crops cultivated for centuries for its high dietary fiber content, fatty acids, such as omega-3 and protein content. The seeds are considered energizing ingredients loaded with nutrients, and with a bland, neutral taste profile are suitable for both sweet and savory applications. Some attributes of the seeds refer, for example, to: low-glycemic index, help to maintain energy levels, and is also a great way to boost daily iron intake.

Chapter 10, *Perspective Therapeutic Effects of Immunomodulating Acidic Herbal Heteropolysaccharides and Their Complexes in Functional and Dietary Nutrition*, prepared by Georgiev et al., compiles information about the perspective therapeutic effects of herbal acidic heteropolysaccharides and their complexes, and emphasize on their role in functional and dietary nutrition.

In Chapter 11, *Soybeans*, *Flaxseeds*, *and Fish Oil in the Treatment of Renal Disease*, Ristić-Medić et al. reveal and discuss recent evidence regarding the impact of soybeans, flaxseeds, and fish oil supplementation in reducing the progression of chronic renal disease and even elimination of some clinical outcomes in hemodialysis patients.

Chapter 12, entitled *Maca*, *A Nutraceutical From the Andean Highlands*, prepared by Gonzales and Alarcón-Yaquetto, discusses the nutritional value and health benefits of hypocotyls of Maca. It seems that the therapeutic effects of Maca range from increase in sperm count and motility, improvement in embryo quality, reverse testosterone-induced prostate hyperplasia, and ovariectomy-induced osteoporosis, to enhanced learning and memory, delayed fatigue, and increase in the interferon gamma production, while reducing glycemia.

Chapter 13, *Phytochemical and Pharmacological Properties of Secondary Metabolites in Berries*, prepared by Jimenez-Garcia et al., summarizes the biological active compounds in berries in relation to the prevention and treatment of severe diseases, such as cancer. The study highlights the necessity to emphasize on the relation between plant crops and elicitors to increase their variety in bioactive compounds and synthesis capacity focused on nutrition and health.

In Chapter 14, *Functional Foods and Chemoprevention in Cancer*, Martínez Leo et al. describe current evidence related to the chemopreventive activity of functional foods and give an idea on the proposed multiple dietetic possibilities for cancer patients. Also, the potential to establish particular alimentary strategies that prevent and treat cancer in a more effective and personalized way is highlighted.

In Chapter 15, *Epigenetic Nutraceuticals in Cancer Treatment*, Supic et al. reveal new insights into the emerging field of nutritional epigenetics, which is a novel and complex scientific field focused on studying dietary bioactive components as epigenetic modulators

in chemoprevention and therapy of complex diseases, particularly cancer. The field of nutritional epigenetics seems to have very promising future in chemoprevention and treatment of cancer.

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SECTION I

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CHAPTER 1

Nutraceuticals: Myths Versus Realities

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1 Nutraceuticals

1.1 Preamble

The use of nutraceuticals and functional foods in daily life has increased many times over the last decade. Consequently, a wide variety of nutraceuticals claiming numerous health benefits are available in the global market. A huge number of these nutraceuticals are now used for self-medication purposes and are frequently available in pharmacies and supermarkets, fostered by extensive media advertising of their purported health benefits. There has been a boom in their sales as consumers increasingly seek to self-medicate, either with the hope that these products will treat ailments that were unsatisfactorily handled with prescribed drugs, or to avoid the adverse side effects of some prescribed drugs. Key factors for the expanding market of functional foods and nutraceuticals are increasing population, aging population, and consumer interest toward use of these products instead of medicines. With the global increase in life expectancy, the number of aged individuals is also escalating, and this population is typically prone to a large number of diseases. This will ultimately be a major factor promoting the use of nutraceuticals in future. For example, it has been predicted that arthritis will affect most of the population at some time during their lifetimes, triggering the demand for preventive and curative strategies.

Furthermore, the interest in nutraceuticals and supplements is increasing, powered through revolutionary studies that establish properties of nutraceuticals linked with public curiosity and client demand. Obesity is the most important concern worldwide, and the obese population is increasing in most developed and developing countries. In the United States, about 62% of the adult population comes under the category of overweight (according to body mass index estimates) and up to 50% of these are obese. According to the International Obesity Task Force, the number of obese in the many European countries has increased by 10%–50% in the past 10 years. Likewise, cardiovascular diseases are responsible for about 32% of the total deaths in the United States. Additionally, osteoporosis, arthritis, and cancer are also widespread.

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Although genetics is the most important factor in the progression of these ailments, most of them are preventable or could be minimized by way of a complete food regimen and managing weight. Moreover, people can optimize their well being through nutrient supplements. Another drive for food-based approaches is public awareness and education about the implications of nutritional sciences toward health improvement. Each year more and more journal articles are published, highlighting the link between healthy eating and wellness. Additionally, numerous books and popular articles related to healthy approaches are getting space on bookstore shelves. Besides print media, talk shows and health programs are aired globally focusing on various diseases along with prevention and cure strategies. This trend has further been triggered by advancements in telecommunication and web technologies and their increasing accessibility to the consumers. The Internet is rapidly becoming a major source of information for understanding etiology and remedy of various health ailments. All these technologies are increasing consumer interest in self-medication through various ways instead of seeking consultation from doctors (Lockwood, 2007).

1.2 Definitions

The term *nutraceuticals* is a blend of two words: *nutrition* and *pharmaceutical*. It was first used in 1989 by Stephen DeFelice, the founder of the Foundation for Innovation in Medicine. Nutraceutical substances fall in functional foods that provide medical benefits for disease prevention and treatment. These products ranges from supplements to genetically engineered and processed foods, such as soups, cereals, and beverages, to herbal products. Although currently there is no well-accepted definition for nutraceuticals, some common links exist among the definitions stated by various health practitioners and associations. According to the International Food Information Council, functional foods are "diet based substances that can offer a benefit for better health and also provide basic nutrition." The International Life Sciences Institute of North America has defined functional foods as "foods having bioactive dietary substances that provide benefits for human health beyond the provision of nutrition." Health Canada defines functional foods as "foods similar in physical structure to common foods, consumed as a part of regular diet and have various health benefits and also reduce the incidence of diseases" (Pathak, 2009). The *Nutrition Business Journal* classifies functional food as "foods which are fortified with isolates that reaches to functional levels, recovering performance and improving health, include enriched cereals, cereals bars, sport drinks, fortified foods, snacks, prepared meals, baby foods, and more." The American Dietetic Association in a position paper has defined functional foods by emphasizing that the term *functional* indicates those foods that have health value leading to certain health benefits for the consumer, for example, reduced risk of diseases. However, Wellness Canada states that nutraceuticals are products that are made from foods and then converted into capsules, tablets, powders, or in different medicinal forms that are no longer related with meals. Overall, a nutraceutical is proven to have physiological improvement and provide health benefits beyond basic nutrition (Wildman et al., 2016).

Table 1.1: Classification of nutraceuticals on the basis of origin.

Sources	Examples
Plants	Quercetin, lutein, luteolin, perillyl alcohol, gallic acid, indole-3-carbonol, glutathione, daidzein, pectin, genestein, allicin, calcium, potassium, zinc, selenium, lycopene, geraniol, lignin, capsaicin, cellulose, hemicellulose, carotene, ascorbic acid, tocotrienol, oryzanol, ionon, tocopherol, nordihydrocapsaicin, zeaxanthin, monounsaturated fatty acids
Animals	Conjugated linoleic acid (CLA), sphingolipids, eicosapentaenoic acid (EPA), docosahexenoic acid (DHA), choline, lecithin, coenzyme Q10, creatine
Microbial	*Saccharomyces boulardii*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *B. infantis*, L. *acidophilus* (NCFB 1748), *B. bifidum*, *Streptococcus salvarius* (subsp. Thermophilus)

Adapted from Wildman, R.E., Wildman, R., Wallace, T.C., 2016. Handbook of Nutraceuticals and Functional Foods, CRC Press, NY, USA.

1.3 Classification

Nutraceuticals are nonspecified natural therapies that are used to enhance wellness, prevent malignant processes, and control symptoms of various diseases (Radhika et al., 2011). These can be classified depending upon their origin, (Table 1.1) chemical nature (isoprenoid derivatives, phenolic substances, fatty acids and structural lipids, carbohydrates and derivatives), as well as sources. Furthermore, these can be grouped into the following categories.

1.3.1 Dietary supplements

A dietary supplement is a substance taken orally that contains a dietary ingredient meant to supplement the normal diet of an individual. The dietary material in these products could be minerals, vitamins, nutrients, herbs or different botanicals, bioactive agents, amino acids, or other resources related to enzymes and metabolites. These supplements could be isolates or concentrates present in capsules, smooth gels, tablets, pills, liquids, or powders and can include pro- and prebiotics, dietary fiber, omega-3 fatty acids, and antioxidants (Wildman et al., 2016).

1.3.2 Functional foods

Functional foods are designed to encourage the consumer to shift toward whole diet sources in their natural forms, instead of taking pills or capsules formed through a series of biochemical processes involving a large number of chemical agents that could harm the body. Functional foods can be fortified and enriched through a process called *nutrification*, which restores nutrients in a food to optimal levels or adds complementary nutrients such as calcium and vitamin D in milk and milk-based products. Common examples include cereals and grains such as oat and barley bran (known to prevent cardiovascular diseases and colon cancer), yogurts and other probiotics (to control intestinal flora), canola and other edible oils with low triglyceride levels (known to reduce cholesterol), omega-3 fatty acid–rich products like salmon (known to prevent heart diseases), and the plant stanols found in a variety of fruits, vegetables, and nuts (known to reduce cholesterol adsorption).

1.3.3 Medicinal foods

These include transgenic plants and other food products that may be loaded with medicines to prevent various health ailments (Ratnaparkhi et al., 2015).

1.4 Global Market

The worldwide market for dietary supplements and nutraceuticals has increased from US $117.3 billion in 2007 to US $176.7 billion in 2013 with a compound annual growth rate of 7.4%. The annual growth in the global market of nutraceuticals is about 8% and is expected to cross US $262.9 billion by 2020. According to a current report, the total market of dietary supplements and nutraceuticals in India is mounting at 21% per annum. It is presently valued at Indian Rs. 44 billion, but it could be worth more than Rs. 95 billion in 4 years. Although the nutraceuticals industry is at an initial stage in India, tremendous growth has been observed during recent years driven by the functional food and dietary supplement categories. The swift annual growth has been noted in dietary supplements (19.5%) and herbal products (11.6%). Nearly 66% of Americans and 47% of Japanese consume at least one type of nutraceutical annually, spending US $86 and 60 billion, respectively (Lockwood, 2007).

1.5 Commonly Used Nutraceuticals

Commonly used nutraceuticals along with dietary sources, extraction and manufacturing procedures, and analytical techniques used for their isolation are given in Table 1.2.

1.6 Mechanism of Action

Nutraceuticals follow the same mechanism of action as pharmaceuticals. These are available in forms, such as pills, capsules, and powders. After ingestion, active components are released in the body to perform their actions (Fig. 1.1).

The way by which nutraceuticals downregulate or upregulate any process in the body—by effecting certain enzymes, receptors, hormones, or genes—varies by the type, dose, and need for the nutraceutical in the body.

2 Natural, Nutraceuticals, and Pharmaceuticals

The common concept of the medical and pharmaceutical approaches to health involves looking for something that is required by the body to function properly. If the body is unable to produce something required for proper functioning, the normal metabolic process will be adversely affected. This “something” that is lacking in the body can be mimicked by certain chemicals manufactured in the laboratory and given to the patient. The chemical

Table 1.2: Commonly used nutraceuticals, their sources, and possible extraction techniques.

Nutraceuticals	Source	Manufacture/Extraction/ Fermentation	Analytical Techniques
Glucosamine	Bovine trachea, shellfish	Chitin acid hydrolysis, fish shell enzymatic hydrolysis	HPLC
Methylsulfonylmethane	Meat, milk	Distillate of peroxide oxidation ofDMSO	GC
Chondroitin	Cartilage, bovine trachea	Enzymatic hydrolysis of beef trachea	HPLC Agarose electrophoresis
Melatonin	Bovine pineal glands	Aqueous extraction of defatted beef pineal glands	RIA GC-MSR HPLC
Coenzyme Q10	Common foods	MeOH/NaOH digest of hearts/livers after H2S treatment, fermentation	HPLC
Carnitine	Skeletal and heart muscle	Chiral synthesis from butyrolactone	HPLC
Octacosanol/ policosanol	Sugarcane waste, wheat germ, rice bran	Ultrasound aqueous extraction	GC-MS
Acetyl-L-carnitine	Brain, liver, kidney	Synthesis from racemic carnitine	LC-MS
Docosahexaenoic acid/ eicosapentaenoic acid (DHA/EPA)	Fish, algae, plankton, seal blubber	Oil extraction, lipase-catalyzed enrichment, fish oil distillation, microalgal oil	HPLC GC
Creatine	Skeletal muscle	Synthesis	CE
Green tea extracts	*Camelia sinensis*	Aqueous extraction, synthesis of catechins	CE
Soy isoflavones	Soy and fermented soy products	Hydrolysis	HPLC
Lutein	Tomato, butternut squash	PET ether extraction of *Tagetes* spp	HPLC
Resveratrol	Red wine, *Polygonum cupsidatum* root, *Veratrum* sp., *Vaccinium macrocarpon*	Mid polarity solvent extraction	HPLC, CE, GC-MS, ELISA
Lycopene	Tomato, green algae	Tomato extraction, chemical synthesis	HPLC

Adapted from Lockwood, B., 2007. Nutraceuticals: A Guide for Healthcare Professionals, second ed. Pharmaceutical Press, London, UK.

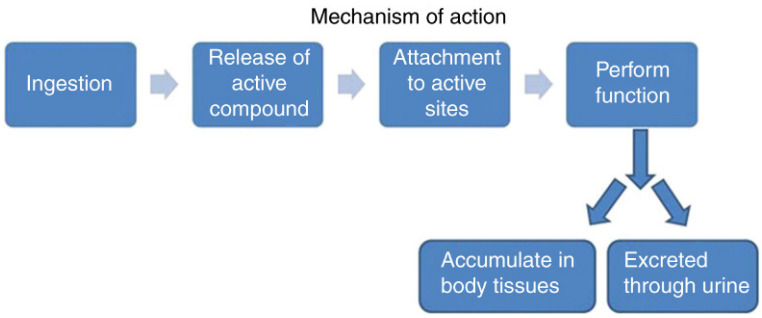


Figure 1.1: The Mechanism of Action for Nutraceuticals.

pathway previously disturbed due to lack of that specific component restores its function, and symptoms of deficiency and insufficiency of that particular component disappear. Drugs work by mimicking the action of natural substances normally present in the body. Sometimes these chemicals can also cause undesirable effects that are called "side effects." Many of these foreign chemicals cannot be broken down and metabolized in a proper way and recycled, so these disturb biochemical pathways and cause toxic side effects.

The process used for the manufacturing of nutraceuticals is somewhat similar to that of pharmaceuticals, in a laboratory and factory setting. Additionally, their mode of delivery and ingestion is also the same as both are taken orally in form of pills and tablets. The main difference is that most nutraceuticals are prepared to fulfill the requirement of a particular chemical by extracting it from the already existing natural substances or dietary sources, while drugs can be synthetic. The aim behind the use of these dietary supplements and nutraceuticals is to produce the same effect as drugs without any undesirable side effects. Major examples of nutraceuticals include melatonin, pregnenalone, lutein, hyaluronic acid, L-cartinine, coenzyme Q10, and creatine. If a high dose of only a single chemical is taken, after utilization in the biological pathways, this can cause deficiency of other chemicals in the body. Sometimes nutraceuticals are also synthesized in laboratories and are not the same as those present in natural sources. In many cases, people have reported no or negligible improvements in their health status after taking synthetic vitamins even with the same dose level as natural or food-based supplements. Furthermore, some studies have shown further deteriorations in health due to side effects associated with the consumption of synthetic supplements.

Sometimes diet-based approaches are adopted for improved health and wellness. If anything in the body is not working properly or there is something within the body that is not produced properly, this might be corrected through a food regimen or supplied through dietary supplements. The natural way is to make a diet plan that can provide nutrition on one hand and the extra nutrients to fulfill the deficiency on the other. Pharmaceutical and nutraceutical approaches are not really appreciated by health professionals because these strategies are oriented to remove symptoms instead of correcting the reason for the malfunction. Sometimes sufferers take complete meals and supplements with a pharmaceutical- or nutraceutical-based approach. The proper way to optimum health is the use of whole foods as a first strategy to meet the needs of missing elements, followed by the use of food supplements and nutraceuticals, whereas pharmaceuticals should be used as a last alternative (Murdock, 2010).

3 Nutraceuticals: Myths Versus Realities

Currently, the scientific community is investigating the role of nutraceuticals in human nutrition, mainly focusing on functions that have a lot of scope and wide range of applications among consumers, health-care practitioners, food law regulators, food producers, and

distributors. Presently nutraceuticals are known to be necessary for optimal health, and the trend of using them without proper knowledge has increased in recent years. These products are thought to be as good as a balanced diet and even are replacing regular dietary products. However, lack of information about nutraceuticals creates a lot of uncertainty, apathy, and confusion among users. Numerous companies are producing nutraceuticals and playing with the health of millions of people who blindly believe in them. Many brands are producing synthetic products made from oxide minerals and other chemical substances that also accompany harmful substances, including preservatives, fillers, binders, coal tar, bitumen, gelatin, and waste products. There is concern about the effective utilization of these products by the human body as they may get lodged in body tissues, causing diseases or exacerbating existing ailments. In this context, commonly spread myths about the use of nutraceuticals and the facts based on scientific research are discussed below.

3.1 Role of Nutraceuticals in the Prevention and Treatment of Health Disorders

Nutraceuticals and dietary bioactive components are actually not medications, however, these are used to fill a nutritional gap in a balanced diet. These are helpful to fulfill daily nutritional needs that are difficult to meet with food alone, as in some physiological conditions the need for certain nutrients increases beyond normal limits. For example, before conceiving and during pregnancy the need for folate is increased to a great extent that cannot be met with diet alone, so it is supplied in the form of supplements. Likewise, Vitamin D supplements are used to meet the daily needs of individuals. In old age, calcium intake is suggested to prevent bone weakness and antioxidants to prevent aging marks on the skin. Supplements may have a role in preventing disease and promoting good health, but these don't have any role in the treatment of diseases. Therefore, the claim that supplements and nutraceuticals can treat such health disorders is completely false. There is a need to create awareness and educate the public about the proper use of supplements for real purposes. This can be achieved through awareness campaigns, public seminars, and print and electronic media without the influence of marketing agencies and manufacturing companies (Isidoro et al., 2016; Nikolić and Marković, 2015).

3.2 Supplements Can Correct Flaws in Diet

There is always an ambiguity in the minds of consumers that if they fail to maintain a balanced diet, then supplements and nutraceutical extracts can help them. Supplements are not the alternative to a healthy, well-balanced diet. If someone is dieting, or avoiding animal or milk products or any other food group, it will become challenging to get all desired nutrients. Globally a massive number of individuals are taking vitamin supplements as insurance for better nutrition to balance their diets. According to researchers, multivitamins don't have the same role in the human body as claimed by the manufacturers. Dietary

supplements may contain some micronutrients in higher amounts than recommended in dietary guidelines. In some cases, these high levels may result in risky intakes. The results of a research trial conducted involving 161,808 postmenopausal women under the Women's Health Initiative in 2009 were quite astonishing. The results showed that there was no reduction in death rate of women who were taking supplements as compared to control group. Furthermore, women taking the supplement were more prone to cardiovascular diseases or cancers of the lungs, breast, colon, endometrium, and rectum. The use of vitamin C, E, B_6, B_{12}, and folic acid did not seem to have any protective effect against cardiovascular diseases or cancer. In 2006, the US National Institutes of Health made a statement about lack of evidence for the recommended doses of multivitamins. In 2008, a Cochrane Collaboration review found that people in clinical trials who took supplementation of vitamin E, vitamin A, and beta-carotene had a higher death rate (Harvard Health Publication, 2009).

Supplements can be taken as an addition to a regular diet. When healthy adults who are getting most of their nutrients from a regular diet also start taking supplements, the risks of toxicity may increase. For example, too much vitamin A intake can increase the risk of osteoporosis; too much vitamin E can raise the risk of suffering from a stroke, and excessive iron can elevate risks of heart diseases. Excess amounts of some nutrients are stored in body fat and are not excreted, and these may become toxic after accumulating in the body. There is no need for multivitamins or individual supplements if the consumer is already taking plenty of fruits and vegetables and also consuming fortified cereal, juice, and yogurt or milk, which are essential components of a balanced diet. Even if the supplements are taken properly, these cannot provide all the benefits of normal healthy eating because consumption of fruits and vegetables also delivers many phytonutrients that have potential health benefits. According to researchers, about a million phytonutrients cannot be obtained merely using supplements rather than consuming natural foods and a balanced diet. These nutrients are available in antioxidant-rich foods, which not only support the immune system but also improve coordination between the cells.

3.3 All the Supplements Labeled "All Natural" are all Good

"All natural" does not mean that the supplement is 100% free from synthetic chemicals used during extraction and processing of nutraceuticals. These components can become part of the extracted substance. Likewise, the process of tablet or capsule manufacturing further contributes harmful substances in the final formulations. If a dietary supplement is made synthetically or has a mixture of natural and synthetic constituents, it will give some relief when consumed for the first time, but this relief will subsequently disappear and symptoms will reappear. The body recognized these vitamins as foreign agents (toxins) and the immune system starts to work in order to remove them from the body. The removal in most of the cases is via urinary excretion as the human system attempts to get rid of the major quantity of

these foreign chemicals. Research has shown that vitamin B_1, as synthetic thiamine (thiamine HCl) reduced fatigue initially but after the use of few days caused fatigue in the body by building up pyruvic acid. This can lead to a vicious cycle of belief that the body needs more thiamine that increases fatigue instead of reducing it. Vitamins present in natural dietary sources are enzymatically alive while synthetic ones are actually dead chemicals. According to a scientific study conducted on 30,000 Finnish subjects, consumption of synthetic vitamin A did not exhibit any antioxidant activity. A true antioxidant has a protective effect for the muscles of heart, the lungs, and internal arterial surfaces. In this experiment, the human subjects given synthetic beta-carotene had 8% higher numbers of fatal heart attacks, strokes, and lung cancer than those who received the placebo (sugar pills). It was noted that synthetic vitamin A did not impart any biological activity in tissues. Instead the synthetic vitamin further stressed the immune system in efforts to rid it from the body through the liver and kidneys. Hence synthetic supplements and vitamins are considered bad due to following reasons:

- These are made from oxide minerals and other harmful substances.
- These also include preservatives, binders, fillers, and other waste products that are considered harmful, as well as toxic.
- These are not easily digested by the body and so cannot be used appropriately.
- These are nonorganic in nature so are not digestible; any substance that is taken must be organic in nature so the body can break it down with higher efficiency in order to absorb its nutrients.
- These are manufactured by pharmaceutical companies and then sold to subsidiary health companies.

3.4 When a Nutrient is Introduced in Market, Stock it up

Supplements are freely available over the counter, so the general public is not necessarily aware of the sensitivity of their use. They should be aware of how vitamins or nutrients are advertised. Before starting the use of bioactive components that have many health claims that attract attention, the consumer should consult a doctor or dietician about the authenticity of the product. Likewise, more care should be taken to start a supplement if someone is suffering from disease where a higher dose could have bad effect on health. For example, people who are taking blood thinners or aspirin should be careful about using vitamin E and omega-3 supplements as these could further limit the ability of blood to clot and increase risk of bleeding. Ingesting these nutrients in whole foods is not harmful, but care must be taken to use supplement because a supplement could lead to excess.

It is also advised to know which nutrients are already present in the diet and which should be taken in supplemental forms. If a balanced diet is providing all the required nutrients in appropriate amounts, use of supplements may harm your body in the long run. In a study,

high levels of serum retinol were associated with high risk of prostate cancer. Conversely, other reports on nutraceuticals give conflicting results showing the beneficial effect of nutraceuticals in certain cases, such as improvement of endothelial vasodilation and rheumatoid arthritis and in LDL reduction. These confronting results of different studies create ambiguity in the minds of consumers about the use, side effects, and limitations of nutraceuticals that highlight the need for more refined and accurate scientific evidence for the use of nutraceuticals. Various areas are understudied, such as bioavailability, bioactivity of metabolites, safe dosages, dose response and toxicity of various bioactive compounds, and interaction of nutraceuticals with pharmaceuticals or between different nutraceuticals when taken together. Substances used as ingredients in the production of nutraceuticals may have quality and safety issues related to toxicity. Moreover, many nutraceuticals are not patented and thus are easily produced and supplied to the market, and so legislation is necessary to control the production and marketing of these nutraceuticals to avoid adverse effects associated with their use (De Silva and Lanerolle, 2011).

3.5 Nutraceuticals are as Good for Health as Diet

Nutraceuticals that are marketed to consumers often hold many functional and health claims. Bioactive substances including leucopene, phytosterols, omega-3 fatty acids, and flavonoids are claimed to be linked with the reduction of risk factors of cardiac diseases by reducing hypercholesterolemia and hypertension and decreasing free radicals or platelet-dependent thrombotic activity. Some are claimed to be responsible for anticarcinogenic activity with augmentations of microsomal detoxification systems and antioxidant defense. Scientific studies support that ingestion of bioactive compounds in the form of whole foods has significant benefits, including the protective effects of the Mediterranean diet and fish consumption in relation to reduced risks of cardiovascular diseases. In contrast, health claims attributable to most isolated biologically active ingredients when ingested, as nutraceuticals have not been supported in studies. A balanced diet has some additional health benefits and is more preventive as compared to isolated supplements because it is not the single component that has a role but different components in combination. For example, iron deficiency can be due to lack of iron or may be due to lack of vitamin C that is required for iron absorption. A diet source may contain both vitamin C and iron at the same time, but a supplement is only a salt of iron; hence, in that case the dietary source definitely gives better results (Espín et al., 2007).

3.6 In Vitro and In Vivo Studies are Enough to Believe Health Benefits of Certain Nutrients

The bioavailability, mechanism of action of the isolated bioactive components, and health claims about nutraceuticals are most often not supported by scientific evidence. The most

common claim about these products is the antioxidant activity of these compounds, which is mostly studied in in vitro trials without studying the antioxidant capacity of these products in human trials. Intake, metabolism, bioavailability, and distribution in various tissues of these polyphenols are important factors that must be established in connection to the biological effects of these nutraceuticals. Species like fish, yeast, mice, and flies have many genes that are identical to human genes, so these animals are considered "model organisms" and are generally used in research to study human genes and various human diseases. Research on rats has led to key advances in our ability to treat serious diseases and ailments prevalent in humans. For example, an experiment on mice resulted in successful treatments for acute promyelocyticleukaemia, or APL, which is a form of cancer that was previously largely not curable.

Thus, as mostly animal and in vitro studies are done, the claims and expected results in humans from intake of these products may differ from expected outcomes. In this context, clinical trials are emphasized to get more accurate results. The bioactive compound resveratrol, derived from grapes, is a good example in this regard. Human trials failed to prove the chemopreventive properties of this compound reported in various animal trials. Even megadoses of resveratrol were unsatisfactory to elicit systemic levels sufficient for chemopreventive efficacy in cancer patients. Another example is corticosteroids that are teratogenic in animals but not in humans, or thalidomide, which is not a teratogen in various animal species but is teratogenic in humans. One reason why animal experiments mostly do not replicate in human trials is that the animal assays are poorly planned, conducted, and analyzed. Another viable reason for this failure to copy the outcome of animal research in humans is that reviews and summaries of proof from animal research are insufficient.

Nutraceuticals are a very common example of innovation in biotechnological products with disproportionate and insufficient development of quality scientific evidence to back or counter their validity. But nutraceuticals may well have potential in the future if backed by solid verification and validation, especially for use under special conditions. Given the consumer market appeal of nutraceuticals, ample legislative safeguards are essential. Until further evidence can be found, health professionals must be alert to the possibility of opposed results of nutraceuticals, unsafe interactions with different medicines, and dietary imbalances because of overuse (Bracken, 2009; Vandamme, 2014). All these aforementioned myths are common among consumers, and we have discussed these examples to illustrate the nature of claims and the reality of the marketed products (Jafari, 2016).

3.6.1 Anthocyanins

Numerous scientific analyses have shown many health benefits associated with anthocyanins and anthocyanin-containing berries mainly due to their antioxidant capacity, monitored in in vitro studies and animal assays but not in human experimental studies. The isolated anthocyanin extracts or the berries have a large number of health benefits in relation to

various medical conditions including improvement of visual capacity, reduction of obesity, improvement of brain and cognitive function, and cancer and cardiovascular risk prevention. Berries and anthocyanins derived from berries have a lot of health-protecting benefits that are used as attractive fantasy to grab the attention of consumer by exaggerating the benefits during marketing and advertisement. The information about the composition and health claims of these nutraceuticals are provided mostly through Internet sources. The main dietary origin of nutraceuticals of this type is single-berry extract such as bilberry and wild blueberry, or a blend of more than two berries, such as blueberry, wild bilberry, elderberry, cranberry, strawberry, and raspberry extracts. On the label of these nutraceuticals the total amount of a specific compound is present as a milligram or percent value. In these tablets some other compounds are also present in a large amount and sometimes even more than the active compound, which is somehow misleading for the customer about the composition of the product. For example, nonspecified polyphenols can comprise up to 70% of the total composition of final products, but in the rare cases that marketers include the amount of these nonspecified polyphenols on labels, this ultimately misleads the customer. Also the dosage mentioned on the label of a tablet is not standardized and experimentally established but suggested on the basis of general observations and human need of antioxidants. Antioxidant capacity, which is a very important property of any nutraceutical, is also mentioned on the label but its validity is far less than reality because it is estimated from animal assays and in vitro antioxidant capacity by ORAC assays, which is different from the antioxidant potential in human body. Additionally, wellness claims linked with berry nutraceuticals include maintaining healthy blood glucose stages and decreasing the chance of cancers. Labeling makes clear that none of the statements have been tested and approved by the US Food and Drug Administration (FDA). Thus it is left to the consumer to research and acquire advice from well-being care authorities on the sort, phases, doses, and benefits that may be expected with the normal consumption of those products. Chemical alteration may occur in original bioactive compound during storage or digestion that may severely modify the bioavailability and bioactivity of the compounds. So the reported claims and general public perception about nutraceutical use is not always accurate due to this lack of standardization procedures (Espín et al., 2007).

4 Health Claims of Nutraceuticals

Health claims present on the labels of most nutraceuticals and dietary supplements are divided into the following three categories.

1. The nutrient content claim shows the presence of the nutrient and its percentage in a final product, and its content is written in comparison to the daily value of a certain nutrient.
2. Structure/function claims show the effect caused by a dietary supplement or nutraceutical on structure and functions of the human body.

3. Health claims explain the link between a food or bioactive ingredient and its ability to reduce disease risk.

5 Labeling Requirements

Labeling of nutraceuticals must include the following components:

- Identity statement (complete name and detail of the dietary supplement)
- Net quantity of all the contents present in a product (total amount of dietary supplement)
- Nutritional information
- Complete ingredient list
- Name, place, and complete address of business of the manufacturer, packer, or distributor

5.1 Nutritional Labeling

A Supplement Facts Panel is the nutrition label for a dietary supplement. The major differences that are present between a "Nutrition Facts Panel" and a "Supplement Facts Panel" are discussed further:

- A manufacturing body should list all the ingredients in dietary supplements without mentioning the daily needs or the recommended daily intakes in the Supplement Facts Panel in the label of dietary supplements. The manufacturing body is not allowed to list all ingredients present in the product in the Nutrition Facts Panel for foods.
- Complete sources for a dietary ingredient must be mentioned in the Supplement Facts Panel for nutraceuticals and dietary supplements, but there is no need to mention all detail on Nutrition Facts Panel for foods.
- It is not compulsory for the company to mention and describe the source of a dietary ingredient for dietary supplements if it is previously mentioned in the Supplement Facts Panel.
- It is compulsory for the company to include the specific plant part that is the source of the dietary ingredient in the Supplement Facts Panel for dietary supplements, but these are not allowed to mention this in Nutrition Facts Panel for foods.
- A company is not allowed to list zero amounts of any nutrient in the Supplement Facts Panel for nutraceuticals and dietary supplements, but the zero amounts should be mentioned in the Nutrition Facts Panel for food (Pathak, 2009).

5.2 Labeling for Quality

Quality labeling includes the following components:

- Scientific name
- Plant part(s) used

- Manufacturer's name and address
- Batch or lot number
- Date of manufacture
- Expiry date
- Ingredients
- Good manufacturing practices (GMP)
- United States Pharmacopeia standard (USP)

6 Safety and Quality Issues

6.1 Analytical

One important requirement to ensure quality of raw and formulated products is the provision of detailed monographs about the routine analysis of nutraceuticals. This is particularly important if the material can be analyzed by a range of methods giving different results, hence making comparisons between workers and products more difficult. One example of this situation is the analysis of carnitine, for which four different electrophoretic methods are available. Levels of carnitine in various products have been shown to be 19.3–25.3 g/100 g dependent on method, demonstrating the importance of establishing and using standard analytical techniques. A range of melatonin tablets has been tested against the USP General Tests and Assays for nutritional supplements, which outlines standard quantitative test parameters including disintegration, dissolution, hardness, weight variation, and friability for tablet formulations (Sarma et al., 2016).

6.2 Bioavailability

As with pharmaceuticals and other alternative medicines, it is essential that tablets and capsules disintegrate after ingestion and formulations release their components to make the active constituents bioavailable. The vast majority of products tested have been found to have satisfactory disintegration rates. In a study, out of 10 Japanese products containing coenzyme-Q10, all were acceptable, but one in four sterol products were found to be unacceptable. To date very few publications have appeared that contain disintegration values for nutraceuticals. The stability of active constituents and microbiological control has also rarely been reported. The range of formulations in use for delivery of nutraceuticals is increasing. For example, glucosamine is available as a gel, effervescent, liquid, and tablet. Other formulations of nutraceuticals now available include caplets, capsules, controlled-release preparations, and soft gels. No investigation into the levels of active constituents has yet studied liquid formulations, which would be expected to be affected by stability problems to a greater extent than solid formulations.

6.3 Contaminants and Adulterants

In nutraceuticals, possible contaminants include a large range of breakdown products, synthetic intermediates, cooccurring related constituents, fillers, and coating ingredients. According to general perception, the levels of contaminants present in concentrated forms are very low. However, in a study, 2 out of 33 named branded fish oil supplements were recalled from the UK market because of high levels of dioxins and polychlorinated biphenyls that could cause toxicity if accumulated in body. Likewise, 40% contaminants were detected in soy isoflavone tablets. Creatinine is a metabolite of creatine that is present in impure creatine supplements due to improper manufacturing procedures, while another by-product, dicyandiamide, could be present as a result of breakdown of the creatine. It has been speculated that creatine sold in liquid forms, particularly products for sporting enhancement, is more likely to break down within the product into creatinine. Creatinine and dicyandiamide in small amounts are not risky for health. However, these should not be present in the product as these are not useful. In a study, three commercial preparations of melatonin were analyzed by using high-performance liquid chromatography/UV and liquid chromatography electrospray ionization tandem mass spectrometry. The results showed approximately 0.5% contaminations in the formulations. A recent survey by the Cologne Doping Laboratory showed a risk of 25% contamination with forbidden substances (carnitine) for athletes. The 9/11 incident has stimulated wide-ranging interest into safety issues related to medicines, supplemental foods, and nutraceuticals in the areas of quality control and quality assurance and also in the covert introduction of microbiological and chemical toxins that are the agent of terror campaign or counterfeiting. Traceability is now being considered with the use of combination bar/chip coding, global positioning, and HACCP and nanotechnology marker assays (Van Breemen et al., 2008). In spite of all these efforts, in most cases no information is available about the identity and quantity of the contaminants in nutraceuticals due to lack of investigations and poor implementation of laws.

6.4 Dosage

It has been observed that some manufacturers advise the dosage levels in instruction labels far below those given during efficacy trials. For example, in a study, 5 out of 12 combinations in glucosamine, 3 out of 3 in chondroitin products, and 2 out of 4 glucosamine products were labeled with low recommended doses. The recommended doses of commercially available lutein in various packing ranges from 0.25 to 22.5 mg with a 90-fold difference. The main reason is that nutraceutical manufacturing companies are not as much developed and organized as the pharmaceutical industries as these do not have advanced analytical facilities due to poor implementation of drug and alternate medicine laws in various countries.

7 Global Regulations and Compliance

Globally the nutraceutical industry is not as organized as the pharmaceutical industry. Likewise, different countries regulate nutraceuticals in different ways. In case of the USA and Japan, governmental rules are not so strict especially if the product is not claiming any preventive or curing effects on health disorders. Harmonized rules among various countries are also lacking. Consequently, nutraceutical companies adopt the regulations and public preferences of each respective country to develop its marketing strategies, claims, and the ingredients that are listed on the label of products. Marketers also invest a lot in advertising their products at local as well as national levels and during the formulation of laws relating to their interests. Currently, legislative agencies and health sector organizations do not discourage the use of supplements, but rather encourage their use to the general public to improve consumer health status and to fulfill nutritional needs not fulfilled through regular diets due to food security issues (Bas, 2016). Global regulations for nutraceuticals and food supplements are briefly discussed below.

7.1 USA

The USA is the largest market of nutraceuticals, which are generally classified there as dietary supplements. A wide range of acts and regulations govern the market of dietary supplements. The Dietary Supplement Health and Education Act (DSHEA) have been in effect since 1994. Likewise, the Federal Food Drug and Cosmetic Act is one of the oldest laws in the USA that deals with all food products, food additives, drugs, and cosmetic items. International quality and safety systems are also being implemented by US manufacturers to gain consumer trust and for export purposes. Currently, any manufacturing company can make general claims about the structure or function of a nutraceutical, such as that pomegranate juice helps the heart and blood vessels. However, if the claim is that it prevents cardiovascular diseases, it must be approved by the FDA. Overall in the USA, manufacturers are held responsible for the implementation of laws and regulations, mainly GMP regulations that went into effect in 2007. The manufacturer is accountable for:

- Safety of supplements it makes or distributes
- False or misleading claims
- Claims that the product will diagnose, prevent, mitigate, treat, or cure a disease
- Notifying the FDA about the safety of the product
- Assurance of the purity, identity, strength, and composition of their dietary supplements

7.2 European Union

The European Union (EU) has a very strict range of regulations for nutraceuticals depending on the type of product to be marketed. The European Food Safety Authority (EFSA) regulates

these products and evaluates the claims made by manufacturers. It also maintains a public Register of Nutrition and Health Claims.

Nutraceutical manufacturers have to get certification and authorization from EFSA, which do the needful after analyzing and validating health claims. If any product is not already on this list of certified ingredients, it cannot be used unless it is approved by following detailed scientific testing by the company that manufactures it and a succeeding review by the EFSA. The EFSA also establishes minimum and maximum amounts of ingredients that are added to a supplement. Labels are not allowed to contain claims that this product will diagnose, prevent, mitigate, or cure a disease. Nutrition and health claims should necessarily be authorized at EU level. From 2008 to 2011, the EFSA inspected 2758 food-related health claims to check if they have any scientific basis or not. Only 10% of those claims were proved to be true.

7.3 China

China has a well-established registration process, but is costly and a bit lengthy. The main entities that are involved in policy making in the nutraceutical industry are the State Food and Drug Administration, which is responsible for registration of nutraceuticals; the Ministry of Health, which oversees the State Food and Drug Administration and approves newly introduced food ingredients; and the Administration of Quality Supervision Inspection and Quarantine, which controls all exports and imports.

7.4 Japan

A simple system of regulating foods with nutrient function claims assures the standard required for the maximum and minimum dose of all nutritional supplements in Japan. Foods for specified health uses (FOSHU) need approval before advertising the product and refer to the products that contain those components that have claimed health benefits. According to FOSHU, disease risk reduction claims are not allowed on products.

7.5 India and Pakistan

In India the Food Safety and Standards Act regulates the processing of nutraceuticals and their imports. The required manufacturing oversight in the case of nutraceuticals is weaker than that of pharmaceuticals (Singh and Sinha, 2012). Likewise, in Pakistan, alternative medicines and health products are regulated by the Drug Regulatory Authority of Pakistan under the Drug Regulatory Authority Act 2012.

8 Role of Nutraceuticals in Human Health

Herbal or plant extracts are effective sources of traditional and modern medicines. These are trendy with consumers due to reported health benefits of these nutraceuticals. Medically important plants provide a rich but insufficiently explored source of these bioactive

components that encompass many health benefits. These substances known as phytochemicals have numerous benefits:

- Substrates for a range of biochemical reactions
- Act as cofactors/inhibitors of many biochemical reactions taking place in the presence of enzymes
- Work as absorbing agents that bind with and remove unwanted toxins from the intestine
- Work as ligands that have negative influence on receptors in cells
- Scavengers of reactive oxygen species
- Improved stability and rate of absorption of numerous nutrients
- Improve the function of healthy microbiome in gastrointestinal tract (GIT)
- Inhibit the activity of harmful bacteria in GIT
- Substrates for healthy microbes in GIT

These phytochemicals that are present in certain foods can be extracted as nutraceuticals (Hasler, 2002). Compounds like polyphenols, phenyl propanoids, carotenoids, isoprenoids, terpenoids, anthocyanins, alkaloids, flavonoids, and phytoestrogens are very beneficial components present mostly in fruits and vegetables. The chemical compounds are effective against various health disorders, such as cardiovascular disease, depression, inflammatory bowel disease, the common cold, diabetes, cancer, arthritis, dyslipidemia, hypertension, and mental disturbances. A recent advancement in the field of nutraceuticals is the concept of *nutrigenomics*, which is the study of the effect of any dietary component on gene expression of a certain organism. Nutrigenomics is an important branch of nutrition that helps to understand the link between nutrients and genes in alleviating symptoms of different diseases (Mahabir and Pathak, 2016).

9 Conclusions

Nutraceuticals are the therapeutic components derived mainly from plant, animal, and marine sources. Globally consumers are using nutraceuticals for improved health and well-being. There is dire need of evidence-based scientific studies to explore the associated health claims of nutraceuticals. Likewise, there is need of harmonized laws, standards, and regulations to govern the ever-expanding market for these products. Consumers must have complete knowledge about the nutrient profile of nutraceuticals and dietary supplements to get maximum benefits and avoid possible side effects.

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CHAPTER 2

The Impact of Functional Food and Nutraceuticals in Health

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1 Introduction

Stephen DeFelice, chairman, Foundation for Innovation in Science coined the term nutraceutical in 1989 from "nutrition" and "pharmaceutical" (Avrelija Cencic, 2010). Nutraceutical refers to the naive or processed natural compounds exhibiting health benefits, therapeutic value, and marketed as nutrition supplements. Nutraceuticals can be derived from any living organisms—ranging from microbes, plants, to animals. They are of prime importance in therapy as they are safe for administration. Nutraceuticals chemically range from phytochemicals, vitamins, flavonoids, or any other natural antioxidants. Pharmaceuticals are symptom-based products that treat the disease or disorders but nutraceuticals are used to prevent the occurrence or progression of disease. Nutraceuticals exhibit wide-spectrum health benefits in contrast to pharmaceuticals, which are of very specific therapeutic value. Modern sedentary, stressed lifestyles without healthy food habits has provided an upthrust to the nutraceutical market.

1.1 History of Nutraceuticals

Nutraceuticals are not new to human civilization; instead, along with the development of humans as a society, we have also learned the health benefits of natural resources from experience, for example, ayurvedic medicines are mainly nutraceuticals, which trace back their origin around 5000 BCE and are still widely used.

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1.2 Components of Nutraceuticals

Nutraceutical components can be any natural compound with therapeutic and health benefits. They include dietary fibers, flavonoids, natural antioxidants, polyphenols, polyunsaturated fatty acids, vitamins, spices, and probiotics (Das et al., 2012).

1.3 Classification of Nutraceuticals

Several classifications of nutraceuticals have been proposed depending on various parameters. Depending on the establishment stage of nutraceuticals, Pandey and Saraf (2010), classify nutraceuticals as:

1. Potential nutraceuticals include those compounds that promise therapeutic value in few research/epidemiological studies but conform to large-scale clinical research is missing.
2. Established nutraceuticals include compounds that are well supported by clinical data to exhibit health benefits.

Depending on the source of nutraceuticals from which they are derived or isolated, they can be divided as:

1. Phytochemicals: derived from herbs or plants like flavonoids
2. Microbial derived nutrients: derived from microbes like vitamin A
3. Animal-based nutrients: derived from livestock

On the basis of chemical properties, nutraceuticals can be classified as:

1. Flavonoids
2. Vitamins
3. Polyunsaturated fatty acids
4. Prebiotics

Further nutraceuticals can be classified as:

1. Dietary supplements: These formulations have nutrients alone as a salt and are mixed with some buffers and preservatives.
2. Functional foods: Katherine Zeratsky (Zeratsky, RD, LD, www.mayoclinic.org) defines "functional food as food enriched with proponents who promote optimal health and help reduce the risk of disease, for example, oatmeal contains a soluble fiber which lowers cholesterol levels." In short, they are not just nutrients but foods enriched with nutrients of health value.

2 Disease Patterns Arising From a 21st-Century Lifestyle

Current lifestyles are stressful and sedentary with poor food habits and a disturbed biological clock. The prevalent pattern of lifestyle diseases (like diabetes, cardiovascular diseases) all over the world show positive correlations with occupations and lifestyles, for example, people

with sedentary lifestyles are more likely to be diabetic and people with more consumption of junk foods are more likely to bear cardiovascular diseases. A largely significant number of epidemiological and mechanistic research studies have established the relationship between ill health and lifestyles of the people. One of the surveys revealed that the people considered obesity and noncommunicable diseases as the biggest threat to the health of the UK population. Even though the survey was limited to the UK population, a number of reports are recognizing them as the biggest threats to the socioeconomic status of world. These reports indicate that people realize their lifestyles are going to be dangerous to themselves. Hence, people have included dietary supplements in their lifestyle as self-initiatives. Kessler et al. (2001) reported the tremendous increments in the consumption of vitamins, minerals, and herbal products. The United States has largest consumer market for health products but still the herbal/traditional market size is only 7% of its total consumer health retail market, but it's slowly growing at 4% CAGR rate over the review period of 2010–15 (Tian-Dan Liu and Fu-Kui, 2015) (Table 2.1).

3 Food as Medicine

3.1 Dietary Amino Acids and Their Effect on Brain Health

In all types of food amino acids are present. The human body requires amino acids for all types of functions, including battling against diseases and supporting growth. Some of the biomolecules present in the body are used to manufacture the few amino acids and the remaining ones are supplied by a well-balanced diet.

The neurotransmitters—chemical messengers across neurons in the brain—require amino acids for their synthesis. Amino acids, which have the property to cross the blood–brain barrier (BBB), initiate the manufacturing of most of the neurotransmitters. The deficiency of neruotransmitters can drastically affect people's capability to learn and can cause unpredictable consequences. Glutamine and asparagine are the two most well-known amino acids that act as neurotransmitters in the brain (Berger et al., 2009; Caspi et al., 2001; Kang et al., 2009; Leibowitz, 1990). For good nourishment, functioning, fast mental activity of brain and for smooth relation of brain with central nervous system it is necessary that gamma-amino butyric acid, glutamine, and glutamic acid are provided in the diet (Hayat, 2011; Woolsey and Gado, 2003). On treating alcohol-fed rats with supplementation of glycine, it altered the carbohydrate moieties of glycoproteins, enzymic, and nonenzymic antioxidant levels in the brain and the fatty acid composition. Glycine supplemention also normalized the lipid peroxidation in alcohol-fed rats (Senthilkumar and Nalini, 2004a,b,c).

By taking amino acids and vitamins in diet we can avoid minor seizures and epilepsy. They help to maintain good metabolism of the brain by improving brain function and by avoiding disorders and malfunctions. They remove the tensions and stress out of the brain. Amino acid supplementation to brain on no account has affects like tiredness, high blood

Table 2.1: Adjunct nutrients of standard anticancer therapy.

S. No.	Standard Anticancer Therapy	Adjunct Nutrients	Tumor Type	Side Effects of Standard Therapy	Significance
1.	Cisplatin	Selenium	—	Nephrotoxicity and DNA-damage, genotoxicity resistance	Protection against nephrotoxicity
2.	Cisplatin	Selenium, vitamin C	Ovary cancer	—	Reduced DNA-damage, prevents resistance
3.	Cisplatin	VitaminsC and E	Transgenic mice bearing lung carcinoma	—	Decreased rate of cancer growth
4.	Cisplatin	Vitamins C, E, β-carotene, retinoic acid	Melanoma cells	—	Inhibition of cell multiplication increased from 33% to 62%
5.	Tamoxifen	Vitamins C, E, β-carotene, retinoic acid	Melanoma cells	—	Inhibition of cell multiplication increased from 19% to 70%
6.	Dicarbizine	Vitamins C, E, β-carotene, retinoic acid	Melanoma cells	—	Inhibition of cell multiplication increased from 29% to 62%
7.	Irradiation	Vitamin A and β-carotene	Mice transplanted with adenocarcinoma	Reduces antioxidant levels	Increased survival rate compared to irradiation treatment
8.	Doxorubicin	Vitamin E	Hela cells	No effect on cell multiplication	80% reduction on cell multiplication when used in combination
9.	Doxorubicin	*N*-acetyl cysteine	Lung cancer in nude mice	—	Reduced lung metastasis
10.	Cyclophosphamide, ethotrexate, 5-fluorouracil	Vitamin C	Fibrosarcoma-bearing rats	Lipid abnormalities	Reduces level of total cholesterol, triglyceride, very low density lipoproteins, and low density cholesterol
11.	Sulindac	Green tea extract	Multiple intestinal neoplasia	—	Reduced number of intestinal tumors by 44.3% to 49%

pressure, insomnia, headaches, memory loss, or weight gain (Greenwood and Winocur, 2005; Pessoa, 2008; Wu et al., 2007).

For proper metabolism of the body and to produce hormones and enzymes we need a total of 22 amino acids for the body (Butterworth, 2003; Oh and Uribarri, 2006). Among major amino acids, 10 of them should be supplied by diets that are called as the essential amino acids

(Antonio et al., 2002). Essential amino acids are histidine, isoleucine, leucine, phenylalanine, lysine, tryptophan, methionine, threonine, and tyrosine whereas the nonessential amino acids are alanine, glutamine, taurine, arginine, cysteine, aspartic acid, cystine, glutaric acid, glycine, ornithine, proline, and serine.

Both the essential and nonessential amino acids are very important for the body and good health. The easy way to get all essential amino acids for the body is to eat animal proteins. Dairy products, eggs, and meat are the most commonly used food sources for getting essential amino acids. The people who depend on vegetarian food can't get necessary essential amino acids through their diet. Vegetarian food, such as seeds and beans, contain only part of the essential amino acids (Eckel et al., 2007).

3.2 The Role of Amino Acid in Brain Health

A meal with high carbohydrate levels increases the tryptophan levels in the brain, which leads to increase of the serotonin levels, which supports the normal sleep and happiness (Hairston et al., 2005). A protein-rich breakfast can raise the tyrosine levels in the blood and brain, which causes the neurons to synthesize dopamine and norepinephrine, which enhances the activity and alertness in the body. Several amino acids undergo a continuous process like incorporation, conversion, and elimination to correct and support transmission of nerve impulses. Essential amino acids feed the brains neurotransmitters, which are engaged in learning abilities and behavior.

Food that is rich in tyrosine and tryptophan can promote good brain chemistry and influence the mood. The indole side chain of tryptophan acts as the nucleus of the crucial neurotransmitter serotonin, which is manufactured from tryptophan. Moreover, tryptophan is vital in weight loss and reduces appetite, helps in removing stress and controlling hyperactivity in children. Tryptophan in combination with magnesium is one of the factors for heart artery spasm (Waider et al., 2011). Tryptophan is particularly rich in sea vegetables, bananas, peanuts, cabbage mushrooms, watercress, parsley, seeds, corn, spirulina, asparagus, beet greens, cucumbers, broccoli rabe, Swiss chard, carrots amaranth leaves, and chicory greens.

L-tyrosine is an amino acid precursor for excitatory neurotransmitters, such as epinephrine, norepinephrine, and dopamine. The function of these neurotransmitters is motivation, memory, alertness, and mood. L-tyrosine supplied through food can fight against the effects of stress and improves mental health. The availability of L-tyrosine in our body is positively correlated to the brain's efficiency in manufacturing important neurotransmitters. Low levels of L-tyrosine can lead to improper utilization of neurotransmitters, which lead to improper cognitive function (Hoffhines et al., 2006; Molnár et al., 2005). Lima beans, pumpkin seeds, peanuts, bananas, almonds, avocados, dairy products, fish, soy products, poultry, and cheese are food sources rich in tyrosine.

One of the investigations with mice has proved that histidine analog AG/01 is very potent against the toxicity of free radicals and has showed a decreased amount of brain damage. This indicates that histidine effected brain cells directly and protected the neurons. Histidine also plays a vital role in human central nervous system as neuromodulator and neurotransmitter (Mrozek et al., 2003). The major food sources of histidine are wheat, rice, rye, meat, eggs, fish, dairy products, and poultry.

Threonine is the amino acid that acts as the protein balancer in the human body. Some of the functions of threonine include healthy liver function, stabilize blood sugar levels, and form tooth enamel (Libao-Mercado et al., 2006).

Isoleucine is not manufactured in the human body so it must be supplied through diet. Deficiency of isoleucine leads to disorders, such as irritability, confusion, headaches, dizziness, depression, and maple syrup urine disease, which are genetically altered metabolism (Nelson and Cox, 2005). Cheese, dairy products, turkey, chicken, lamb, nuts, lentils, peanuts, soy proteins, and meat are some of the foods that are rich in isoleucine.

Among the 30% to 50% of NH_2 groups, which the brain utilizes for the synthesis of glutamic acid, are obtained from leucine. Valine, isoleucine, and leucine are the branched chain amino acids (BCAAs), which mostly skip the first-pass metabolism; among the three leucines is the first amino acid that crosses the BBB faster than the other two (Cota et al., 2006).

Anxiety-induced disorders are caused by serotonin receptors in the intestinal tract due to their hyperstimulation. Lysine amino acid inhibits the hyperactivation of serotonin receptors (Smriga et al., 2004). In the gastrointestinal tract lysine directly reacts with serotonin receptors and has an anxiolytic action and therefore reduces the anxiety. Navy beans, potatoes, soybeans, lentils, yeasts, beans, chickpeas, kidney beans, carfish, milk, eggs, and beef are good sources of lysine (Razak et al., 2016; Razak and Viswanath, 2015a,b,c).

Valine in its volume and shape roughly looks like threonine and it exhibits some stimulatory effects. Valine has important functions like growth and repair of tissues, muscle metabolism, and nitrogen balance in the body. Valine is rich in some foods, such as mushrooms, sesame seeds, soy, peanuts, lentils, meat, fish, and cheese. Alanine removes toxins released during muscle breakdown so that the liver can metabolize them and remove them from the body. The cholesterol levels in the body are also checked by alanine (Zagórski and Sehested, 1998).

In the human body, the main functions of aspargine are to support the nervous system to maintain equilibrium, regulate metabolism, and they also act as detoxifiers. Aspartic acid has its effect on cellular energy so it is used to fight fatigue and depression. Sometimes aspartic acid plays a crucial role in manufacturing of other amino acids (D'Aniello, 2007).

Cysteine has multiple functions, such as scavenging free radicals (as an antioxidant) and detoxifiers in the body. It is essential for healthy hair, nails, and skin (Bulaj et al., 1998).

Cystine is one of the amino acids that support the growth of glutathione, which is a crucial detoxifier and cystine is used in cosmetics to sustain youthful and glowing skin (Aslaksena et al., 2006).

Glutamine is the most available amino acid in the blood. Glutamine also has many functions, such as boosting the immune system, good digestion, and proper brain function. Many studies have stated that glutamine plays a major role in the suppression of hunger (Brosnan, 2011). Glutathione is the only amino acid that is found in every cell and is effectively involved in every system in the body. Some of the functions of the glutathione are that it is used in certain types of cancer, increases sperm count in males, decreases the blood pressure, enhances brain function, protects the cell from oxidative tension, and has antiaging characteristics (Couto et al., 2013).

Glucose, which is needed by the human body for energy, is sometimes supplied by glycine. The main role of glycine in the human body is to improve digestive health, to enhance proper cellular function and growth. The major part of the collagen, which is involved in healing and elasticity, is made up of glycine (Snyder et al., 2005).

Proline supports the regeneration of the skin and it helps in formation of new cells. Proline is also present in cartilage and collagen. It is very useful to reduce wrinkles and sagging. The important function of proline is to maintain joints and muscles flexible (Pavlov et al., 2010). Serine is basically found in the cell membranes (phosphotidyl serine) and it is very essential for immune health and muscle formation. For proper function of the brain and for formation of chemicals that enhance mental stability and mood serine is very essential (Mothet et al., 2000).

Taurine is a digestion improver, detoxifier, and free amino acid that travels through the blood stream. It is plentiful in muscle, retina, brain, and other organs. It has multiple functions, such as immune function, retinal and neurological development, and conjugation of bile acids, regulation of cellular calcium levels, athletic performance, and brain function.

Glutamate is amino acid that gives out a specific taste known as umami; when it is present in blood it functions as signal for ingestion of protein. In recent reports, it was stated that old age was associated with low levels of glutamate in brain striatum when evaluated with magnetic resonance spectroscopy (Zahr et al., 2013). Zahr et al. (2013) also reported that any change in the brain glutamate levels will lead to behavioral changes found in usual aging, high-systolic blood pressure, and poorer performance on complex vasomotor tasks. Arginine is nonessential amino acid produced in our body with the help of other amino acids but infants cannot synthesize arginine so it is considered as the essential amino acid (Wu et al., 2004a,b). Some of the foods that are rich in arginine include chicken, meat, yogurt, pork, beef, turkey, wheat, peanuts, sesame seed, cashews, popcorn, brown rice, and walnuts. In the human body, arginine is present in large quantities in protamines and histones.

Arginine is responsible for the formation of ornithine and urea as it involves the urea cycle. Ornithine is also involved in the formation of polyamine. Arginine also has a role in the formation of creatine, which enhances muscle mass and strength. Arginine plays a vital role in the formation of citrulline and nitric oxide with the aid of the enzyme nitric oxide synthase. This nitric oxide has significant roles, such as causing vasodilation by smoothing muscles of blood vessels and the suppression of platelet aggregation. Selenocysteine is the same amino acid, having a similar structure like cysteine. We cannot find selenocysteine free amino acids in the cells to be used. Food sources of selenocysteine are rice, nuts, corn, oats, wheat, Brazil nuts, soy beans, chicken, cheese, fish, seafood, and turkey. In some foods it occurs in the form of selenate and selenomethionine.

3.3 Amino Acid in Synthesis of Neurotransmitters

The amino acids, such as tyrosine, glycine, GABA, glutamine, tryptophan, and taurine are involved in the synthesis of neurotransmitters (Kodirov et al., 2006). The brain needs a healthy diet for proper function. The nerve chemicals that dominate our brain are depended on our diet. Production of neurotransmitters in the brain is very essential so supplementation of amino acids in daily life is very crucial. Hence, taking of amino acids in food will definitely boost brain health and function.

3.4 Role of Fruits in Boosting Memory

Citrus fruits are well known for their high content of antioxidants, potassium, folate, and vitamin C, which are very powerful and reduce the risk of heart stroke, coronary disease, oxidative stress, and inflammation. Citrus fruits help blood vessels to flow continuously so that sufficient oxygen is supplied to brain (Arvanitakis et al., 2004). Lycopene, which is present in tomatoes, is more potent than the vitamin E and carotene that protect the brain and heart from disorders. Researchers have found that heating the tomatoes will help the human body to absorb lycopene (Bauer, 2007). Some of the inflammatory damage to brain function can be reduced by flavonoids present in apples. Apples have antioxidants and quercetin, a photochemical that is good for the skin. The blockage of the artieries can be avoided by taking pomegranates as they have more antioxidants and polyphenols (Beck, 2008).

The cellular damage of brain and body can be avoided by eating berries that possess powerful antioxidants. Compared to normal berries, blueberries contain high amounts of antioxidants and they are very helpful for brain and heart health (Null, 2008).

3.5 Fruits for Treating and Preventing Brain Diseases

Many reports prove that the presence of hydrogen peroxides is responsible for the oxidative damage during the pathogenesis of Alzheimer's disease. Most of the antioxidants and

polyphenols are very effective neuroprotective agents and are potential fighters against hydrogen peroxide. Studies show that vegetable and fruit juices play a crucial role in delaying the pathogenesis of Alzheimer's disease (Dai et al., 2006).

The aging of the brain is due to the constant concession to fight against different types of stress. The inability to fight, defends, and balance against cellular generation of reactive oxygen species leads to oxidative stress. The reactive oxygen species damages the proteins, lipids, carbohydrates, and nucleic acids. In oxidative stress, the brain is the main organ that is damaged more (Lau et al., 2005).

Vegetables and fruits that are rich in polyphenosl are very beneficial in treating brain and heart diseases. Cranberries, strawberries, and spinach are very rich in antioxidant ability; this is proved by measuring the modified oxygen radical absorbance capacity assay (Wu et al., 2007).

3.6 Grape Juice for Healthier Brain

Resveratrol, apolyphenol, which is present in grape juice, has many functions, such as the suppression of degenerative nerve disease, Alzheimer's disease, viral infections, and cancer (Shankar et al., 2007). Some of the studies have stated that the main function of resveratrol is to protect the genome of the organisms through antioxidants property (Gatz and Wiesmuller, 2007). It has also other functions, such as lowering the calorie content in the brain, heart, and muscles. These dietary interventions suppress the gene expression associated with the heart, which results in prevention of muscle aging and heart failure. The polyphenols present in the grape skin can help avoid Alzheimer's disease. Drinking grape juice 3 times a week will help to enhance the cognitive function (Barger et al., 2008).

3.7 Therapeutic Application of Blueberries

The main advantage of blueberry extracts is that they deliver the fruit phytochemicals in correct and simple doses with good flavor. These properties of the blueberries prove that they have potential to prevent heart strokes and related diseases (Norton et al., 2005). In one of the in vitro investigations, it was mentioned that there was reduction in the synthesis of matrix metalloproteinases enzymes, which play a crucial role in malignant tissue metastasis in human prostate cancer cells due to exposure to extracts of blueberry antioxidants. For this reason, the investigators proposed that a blueberry diet might support prevention of tumor metastasis (Matchett et al., 2005).

Apple juice has good quantities of low molecular weight natural phenols, such as flavonols and chlorogenic acid and procyanidins, which are very essential in fighting against diseases related to aging. Because of antioxidant characteristics apple juice can reduce the chance of cancer development and Alzheimer's disease.

3.8 Black Seed: A Potential Nutraceutical for Healthier Brain

Black seed is a well-known nutraceutical for its natural healing characteristics to fight against many diseases and it has been in use for many thousands of years. We get this seed from the plant of family *Ranunculaceae* (*Nigella sativa*), which is an annual flowering plant. Some of the properties of this black seed are bitter in taste, aromatic, contain proteins, fats, carbohydrates, and minerals in very balanced quantities with good therapeutic and nutritional values.

As mentioned earlier, the black seed of *N. sativa* is increasingly gaining and constant popularity worldwide as a medicinal spice in both conventional and nonconventional cooking purpose and in herbal medicine to cure a wide range of diseases. This seed has different names throughout the globe. In India and Pakistan its common name is *Kalonji*, black coriander in some parts of Asia, and black caraway in Turkey.

3.9 Health Benefits of Black Seed

We all know that hypertension (high blood pressure) can produce very serious damages, such as peripheral vascular diseases and several heart diseases. Daily uptake of 100–200 mg of black seed can decrease the systolic and diastolic blood pressure to 110 and 60 mmHg from 140 and 90 mmHg and it can lead to changes in hemogram that is linked to an increase in hemoglobin and packed cell volume. Moreover, it can also lead to a decrease in plasma concentrations of bad cholesterol triglycerides (low-density-lipoprotein) and glucose to basic levels (Huseini et al., 2013; Leong et al., 2013). In many reports it was stated that black seed has the ability to fight against hepatotoxicity age-related endothelial dysfunction of the mesenteric artery and nephrotoxicity (Idris-Khodja and Schini-Kerth, 2012).

Not only has this black seed had medicinal properties to heal constipation, nasal congestion, odontalgia (toothache), and cephalgia (headache), in women it helps to boost the immune system and increase the milk flow in lactating females. It also has properties to fight against different cancers. Some of the examples of its antiangiogenic effects and antitumor of thymoquinone on osteosarcoma in vivo and in vitro studies and in animal models of aggressive prostate cancer (Kaseb et al., 2007; Koka et al., 2010; Peng et al., 2013; Yi et al., 2008).

Black seed oil extracts have many good therapeutic properties, such as antiosteoporotic, antipyretic, antipsoriatic, antiplasmodial antioxidant, antinoniceptive, antineoplastic, antimicrobial, antimalarial, antiinflammatory, antidiabetic, antiatherogenic, antiasthmatic, and antianxiety (Ahmad et al., 2013; Alemi et al., 2013; Al-Wafai, 2013; Das et al., 2012; Perveen et al., 2009; Shuid et al., 2012).

3.10 Role of Black Seed Against Brain Inflammation

Inflammation is a general state that produces innate response to illness, stress, and injury. In the case of inflammation, destructive proinflammatory chemicals are released by particular cells, such as macrophages. These cells are very specific and named differently depending on their residency, function, and morphology. In brain tissues we can locate many types of macrophages.

The most common macrophages found are oligodendrocytes, astrocytes, and microglia, which upon activation produce neurotoxic factors like proinflammatory cytokines, nitric oxide, and reactive oxygen species, though the research on the brain inflammation and black seed is in its beginning stage. The antiinflammatory properties of black seed in several other immune cells have been researched broadly. The aqueous extract of black seed can effectively suppress the production of Th1 cytokines and support Th2 cytokines to balance the immune system. Therefore, it is reported that black seed contains an efficiency to enhance the immune system (Majdalawieh et al., 2010).

3.11 Black Seed in Treatment of Alzheimer's Disease

Progressive degeneration of nerve cells is the main symptom of Alzheimer's disease, which is linked by brain shrinkage and dysfunction, changes in behavior, and loss in memory. It is estimated that 5.4 million Americans are living with Alzheimer's disease. Alzheimer's disease is the one of the leading causes of death. One of the major symptoms is the inability to remember newly learned information due to shrinkage of hippocampus and cortex, which is the main culprit for memory loss and learning. But the correct reason is not known. Another indication of Alzheimer's disease is that the number of synapses and neurons are fewer when compared to that of healthy brains.

Many researchers had proved that Alzheimer's disease is linked to the formation of neurofibrillary tangles and fibrillar extracellular deposits of amyloid β-peptide between the already existing nerve cells that interrupt passages of signals and leads to inflammation. The aggregates of hyperphosphorylated tau proteins are called neurofibrillary tangles, which make the microtubules break up and become unstable. The common physiological function of amyloidal β-peptide, which is derived from the amyloidal precursor protein, is to activate kinase enzymes and to control cholesterol transport, avoid infection, and fight against stress.

At present there is no good treatment existing in the market to cure Alzheimer's disease. Currently, there are several drugs that are approved by the Federal Drug Administration (FDA, 2012), such as GTS-21, Tacrine, Rivastimine, Memantine, Galantamine, and Donepezil, which are in Phase II clinical trials; however, there may be many side effects associated with these drugs. Now the researchers from both clinical and basic research are

concentrating on the herbal medicines. Among the herbal medicines they are focusing mainly on the black seed, as it is very effective. The major and active component of black seed is thymoquinone, which is known to exhibit protection of hippocampus and cortical neurons of rats to fight against amyloid β1–42 toxicity and stimulated neuronal cell proliferation (Gurung et al., 2010).

3.12 Black Seed in Epilepsy Treatment

Epilepsy is a neurological disease, which at present has affected 50 million people throughout the globe. The unexpected rupture of electrical impulses in the brain leads to epileptic seizures due to an incompatible flow of messaging between the neuronal cells. This disease can be cryptogenic, idiopathic, and symptomatic depending on the epileptic action on the part of the brain.

The epileptic activity in the brain leads to fluctuation of inhibitory and excitatory amino acid neurotransmitters, oxidative stress, inflammation, and irregular levels of substances like blood sugars and ions.

Some of the reasons for epileptic activity are decrease in the concentration levels of sodium, aspartate, glutamate, and potassium, and reduced superoxide dismutase, glutathione, ATPase, and catalase activities. Interestingly, black seed has exhibited the ability to restore the balance of antioxidant levels by reversing all the unfavorable changes to improve neurological defects and by preventing the epilepsy seizures in pediatric and adult individuals (Akhondian et al., 2011; Arafa et al., 2013; Ezz et al., 2011). From clinical and preclinical reports, it is shown that recurrent or prolonged seizures and brain damage can lead to neuronal excitability, increases proinflammatory cytokines levels and initiates BBB breakdown (Xu et al., 2013). Recent literature on black seed had reported that treatment of epilepsy with black seed can suppress epilepsy-related illness and it can act as very good anticonvulsant agent.

3.13 Black Seed as Antitumor Agent

In the United States, 100,000 people are diagnosed with brain cancer each year, which generally spreads via metastasis. Glioblastoma are tumors that occur from the glial cells and astrocytes and are the very aggressive and a regular type of malignant brain tumor in humans with a typical survival rate of 15 months from the day of identification. Many studies have demonstrated that antitumour function of bioactive compound of black seed known, as thymoquinone is unique for cancer cells have demonstrated to inhibit autophagy and initiate apoptosis.

Moreover, the thymoquinone of black seed, which as antitumorigenic property suppresses the autophagy in glioblastoma cells through perturbation of the cathepsin translocation and

lysosomal membrane from the lysosomal lumen to the cytosol, at which the thymoquinone shows the antiproliferative effects by initiating DNA damage, arrest cell cycle, which leads to apoptosis in glioblastoma cells. Thymoquinone also supports the changes in telomere length in glioblastoma cells. Healthy cells are having no side effects by treating with Thymoquinone (Gurung et al., 2010; Racoma et al., 2013). It also reported that thymoquinone treatment showed antineoplasic activity, which decreases adhesion and invasion through the downregulation of focal adhesion kinase protein in both CCF-STTG1 and U-87 cells (Kolli-Bouhafs et al., 2012).

For a long time black seed has been nutritionally important. It also has other good pharmaceutical effects and nutritional value and efficacy. In the near future we can expect exciting new applications of black seed in the treatment of several neurological diseases and disorders (Table 2.2).

3.14 Momordica charantia *(Karela) in Treatment of Diabetes*

Diabetes, which is also called diabetes mellitus (DM), is a group of metabolic disorders that have symptoms, such as high levels of sugar in the blood. The increased level of sugar in blood sugar is due to a lack of synthesis of insulin or in short of response of the cells toward insulin. Beta cells in the islets of Langerhans of pancreas produce insulin, which helps for the successful utilization of glucose in the human body. Generally, two types of diabetes mellitus are type 1 and type 2. Type 1 diabetes mellitus is generally referred as juvenile diabetes and insulin-dependent diabetes mellitus. It is generally seen in children.

Type 2 diabetes mellitus generally results from insulin resistance, or the body's incapability to use insulin. Usually it is seen in adults; hence, it is called as noninsulin-dependent diabetes mellitus or adult onset diabetes. During pregnancy a third type of diabetes may occur, due to improper utilization of insulin by the body. This condition is usually stabilized after delivery of the baby, but the mother is at a high danger of developing type 2 DM later in life.

Diabetes is not simply a disease of abnormal blood sugar levels in the body causing cardiac abnormal functions but can indirectly or directly affect the nervous system in human body. Since glucose is the main energy for brain cells, it is crucial for glucose to be successfully and correctly used to prevent abnormalities in the nervous system. Various investigations and data describe the severe effects of diabetes in the brain.

Sometimes diabetes will result in atherosclerosis, in which the blood supply for several parts of the human body is restricted and that can result in heart stroke. Diabetes also leads to neuropathy where the end of the nerve does not work correctly, which results in pain, numbness, and imbalance (Kieran, 2011). Diabetic cerebral malfunctions have manifested in structural, electrophysiological, neurochemical, and neurobehavioral levels (Biessels et al., 1994).

Table 2.2: Nutrient adjuncts for neurodegenerative diseases.

S. No.	Photochemical	Source	Disease/Disorder	Effects	Mechanism
1.	Kaempferol glycosides	Strawberries	Neuroinflammation/ brain injury	Neuroprotective	Inhibits NF-κB and STAT3
2.	Fisetin	Strawberries	LPS-induced microglial activation and neurotoxicity	Antiinflammatory activities	Suppresses production of TNF-α, NO, prostaglandin E2
3.	Secoisolariciresinol	Strawberries	HIV-induced neurocognitive disorders (HAND)	Reduced neuroinflammation and oxidative damage subsequently reducing HAND progression	Under investigation; probably mimics estrogenic receptors
4.	Gallic acid	Strawberries	Transient global ischemia/reperfusion in Wistar rats	Beneficial effect on behavioral impairments after brain injury induced by ischemia/ reperfusion; cerebroprotective properties	Enhances antioxidant defense
5.	Ellagic acid	Strawberries, blackberries, raspberries	Cognitive hippocampal long-term potentiation (LTP) and brain inflammation in rat with traumatic injury	Prevents cognitive and LTP deficits	—
6.	Chlorogenic acid	Strawberries, blueberries	Brain damage and edema in rat model of focal cerebral ischemia	Reduces brain damage	Radical scavenging activity, inhibitory effects on MMP-2 and MMP-9
7.	Pterostilbene	Blueberries	Isoflurane induced neurodegeneration (apoptosis in neurons)	Neuroprotective effects	By regulating JNK and PI3K/Akt pathway in neonatal rats
8.	Quercetin	Raspberries	Hypobaric hypoxia induced hippocampal neurodegeneration; memory dysfunction	Reverses hypoxia-induced hippocampal neurodegeneration and improves memory function in the rat	Antioxidative and antiapoptotic mechanisms
9.	Cyanidins	Raspberries, plum	Ethanol neurotoxicity	Ameliorates ethanol neurotoxicity in developing brain	Inhibits GSK-3β activity in neurons

Table 2.2: Nutrient adjuncts for neurodegenerative diseases. (*cont.*)

S. No.	Photochemical	Source	Disease/Disorder	Effects	Mechanism
10.	Pelargonidins	Raspberries	Parkinson's disease, 6-hydroxydopamine (6-ODHA) toxicity	Neuroprotection in interstratial 6-ODHA lesioned rats; neuroprotection against 6-OHDA toxicity; reduced thioubarbituric acid reactive species (TBARS)	Attenuates oxidative stress, decreases TBARS production and lipid peroxidation
11.	Catechins	Raspberries	Brain senescence with cerebral atrophy and cognitive dysfunction in SAMP10 mice	Suppressive effect on morphological and functional regression of the brain in aged mice with accelerated senescence	Preventive effect on oxidative DNA-damage
12.	Lutein	Plum, prunes, orange	Acute ischemic damage	Protection of inner retina from acute ischemic damage	Reduced oxidative stress
13.	Ursolic acid	Prunes	Experimental subarachnoid hemorrhage	Alleviates early brain injury	By suppressing TLR4-mediated inflammatory pathway
14.	Rutin	Oranges	Brain damage in rats with chronic cerebral hypoperfusion	Neuroprotection against cognitive deficits and brain damage	Multitargeted therapeutic potential
15.	Ellagitannins	Pomegranate	Alziemer's disease (AD)	Neuroprotective against AD	Mediated by urolithins—ellagitannin derivative derived by gut microbes

Diabetes can also lead to elevated levels of dopamine content and alteration of dopamine receptors. This modified monoaminergic neurochemical function will raise the chances of development of major affective disorders. The patients suffering from the diabetes have mitochondrial malfunctions, vascular lesions, and loss of neurons. The loss of neurons will lead to a reduction in brain size (Biessels et al., 1994).

Momordica charantia and its actions on insulin have been broadly researched. Bitter gourd possesses bioactive substances like vicine, charantin, triterpenoids with antidiabetic potential and also a few antioxidants. Several investigations on animal models have stated that the bitter gourd has activity of lowering the blood glucose levels.

M. charantia has the ability to increase the population of beta cells and also to initiate insulin release from the beta cells because of some membrane functions. The seed extracts of the plant have antihyperglycemic protein, which is very much used in the treatment of diabetes. The key factor behind the hypoglycaemic effect is lectin, which shows its effect on the peripheral tissues and suppresses appetite (Choudhary et al., 2012).

Many research studies have proved the antidiabetic effects of karela on insulin function. Hafizur et al. (2011) reported that pancreatic beta cells were changed by ethanolic extract of karela fruit pulp, which improves beta cell function and both serum insulin and fasting blood glucose levels (Hafizur et al., 2011). Abdollahi et al. (2011) proved that supplementation of streptozotocin induced diabetic neonatal rats with fruit extract of *M. charantia* resulted in renewal of pancreatic beta cells, preventing pancreatic damage, reduction of glucose levels in blood, and an increase of insulin levels in serum. The extracts of *M. charantia* are available in the market with different names and forms. Some are available in the form of capsules, a few in the form of tea, and a few in the form of syrup. These commercially available syrups, capsules, and extracts are extremely good for people who live a fast-paced life with nerve-racking professional jobs.

4 Challenges With Natural Nutrient Supplement

Most of the nutraceuticals suffer severely from reduced bioavailability, thus reducing efficacy of the supplement. Bioavailability can be defined as the scale up to which active components of the drug is absorbed and available at the site of action, in contrast to the amount of drug administered. The rapid metabolism and low absorption of such nutraceuticals contribute to its reduced bioavailability at biological interface.

There are numerous factors that affect the bioavailability of the nutraceuticals:

1. Molecular weight: Large macromolecules have reduced absorption rates at intestinal–blood interface, for example, oligomeric apple proanthocyanidins
2. Hydropathy index (HI): Macromolecules with high hydropathy index are less soluble in the blood plasma, thus reducing bioavailability of the compound. The nutrients with high HI include fat soluble vitamins, vitamin D3. Baicalin, a hydrophobic flavonoid isolated from the scutellariabaicalensis, induces apoptosis in colorectal cancer cells under in vitro condition, but under in vivo conditions its biological availability is challenging (Wang et al., 2013).
3. Interaction with gut micro biota: Few nutraceutical compounds are known to be metabolized by gut microbes and then they are absorbed efficiently across the intestinal blood barrier, for example, anthocyanins are catabolized to protocatehuic acid (Moco and Rezzi, 2012), which exhibits antioxidant and antiinflammatory activities (Liu et al., 2002). In significant cases, this metabolism in the gut reduced the therapeutic efficacy of the natural compound, but unfortunately very little investigation has been done their role in malnutrition.

5 Allopathic Drugs and Challenges

Currently, it is a well-established fact that most of the chemical drugs available in the market pose adverse side effects leading to bad consequences on health in the long term. In some senses, increasing awareness regarding the side effects of drugs made people switch to other alternatives like ayurveda and other herbal supplements. The contribution of chemical drugs in increased life expectancy of a person can't be neglected but from the prospective of long-term health benefits there is a story of failure as well. Drugs are usually symptomatic treatment, which is used depending on the symptoms normally to suppress the disease, but nutrients as pointed out previously prevent the disease/disorder.

6 Nutrients as Adjunct for Chemical Drugs

The early clinical research for the role of nutrient intervention in the chemotherapy and other treatments started in 1980s (Arthur et al., 1982). Cameron (1978) reported the beneficial effects of vitamin C in cancer therapeutics. Several other natural products are found to act as adjuncts in cancer therapeutic agents, for example, vitamin C. According to Dr. Rath at Rath Institute of Cellular Medicine, "cancer can be controlled in a most effective way through nutrient synergy" (Niedzwiecki et al., 2008). Rath's group investigated several adjunct nutrients along with standard cancer therapy and reported to reduce the side effects of chemotherapy and fast recovery of patients (Niedzwiecki et al., 2008) (Table 2.3).

7 Nutrient Adjuncts for Cancer Therapy

Vitamin C: Vitamin C has been found to help in the recovery of cancer patients but still there are not many well-established reports, which can make vitamin C acceptable for therapeutic value. Several mechanisms have been proposed for anticancer activity of vitamin C.

1. Antioxidant activities, that is, by scavenging ROS species
2. It disrupts mitochondria, cell membrane, and increases phagolysosome formation

Table 2.3: Strategies of special nutrients.

S. No.	Nutrient	Challenges	Strategies
1.	Curcumin	Poor absorption, biodistribution, bioavailability	Cucumin-loaded biopolymeric nanoparticles like cellulose, PLGA, dipeptides
2.	Daidzein	Poor solubility and intestinal absorption	Borneol/methanol eutectic mixture with microemulsions
3.	Green tea catechins	Poor bioavailability	Steamed rice supplementation
4.	β-Carotenoids	Low bioavailability	Emulsification into digestible, long chain triglycerides (LCT)

3. Positive upregulation of genes like fra-1 glutathione S-transferase pi (GSTpi) and Mut L homolog-1 (MLH-1). Positive upregulation of these genes make cells susceptible to apoptosis when treated with DNA-damaging chemotherapeutic agents (Blumenthl et al., 2000; Catani et al., 2002; Ferguson, 1996; Yam and Shinitzky, 2001).

7.1 Epigallocatechin Gallate

Several green tea extracts have been reported to exhibit anticancer activities, for example, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate (EGCG) is the most potent anticancer agents among all other active compounds in leaf extracts. EGCG has been reported to act through the following mechanisms:

1. Inhibits vascular endothelial growth factor (VEGF) expression. VEGF is required for angiogenesis (Jung et al., 2001).
2. Inhibits activities of gelatinases, metalloproteinases 2 and 9, required for metastasis (Garbisa et al., 2001).

7.2 Selenium

Selenium acts as the cofactor for few enzymatic antioxidants like glutathione peroxidase.

7.3 Arginine

Arginine acts as the precursor for nitric oxide (NO), which in turn induces apoptosis in cancer cells (Lu Yu et al., 2013). Md Waheed Roomi et al. (2005) reported that antitumor effect of arginine, lysine, proline, ascorbic acid, and green tea extract cocktail on pancreatic cancer cell line MIA PaCa-2 (Long Tai Zheng et al., 2008).

8 Advancement in Food Processing for Nutrients

The modern sedentary and stressed working hours is one of the human excuses for their inability to maintain a balanced healthy diet. Researchers are trying hard to boost up the nutrient levels in the natural products. One of the recent reports suggest that nanoparticles (zinc oxide and titanium oxide) increase the mineral and light absorption in tomato plants, which in turn boosts up the antioxidant contents of the tomatoes (Ramesh Raliya et al., 2015). A few food products with increased nutrient levels have attempted to supply in the market, for example, golden rice. Another challenge that persists today is the bioavailability of these nutrients. A proper understanding of interactions between nutrients can help us to frame better dietary plans. Molecular biology has opened new avenues in the area of nutritionally improved agricultural crops. Proper experimental models to study the bioavailability and absorption of nutrients are also lacking. Tamara Zietek et al. (2015) developed intestinal

organoids to study the nutrient uptake across the intestinal barrier, which could open new avenues in our understanding of nutritional studies. Several other derivatives of nutrients are being developed to improve the bioavailability of nutrients without trampling its therapeutic effects, for example, poly(lactic-*co*-glycolic acid) PLGA encapsulated curcumin (Anand et al., 2010). The bioavailability of the nutraceuticals can also be increased by either chemical modification, chemical delivery agents, or by administering them with other food supplements, for example, the administration of carrots and tomatoes with digestible lipids increases bioavailability of carotenoids probably by forming micelles in the intestines (Colle et al., 2013; Moelants et al., 2012).

9 Conclusions

Nutrients and dietary supplements play an important role in the health of human beings. Epidemiological studies established the relation between disease, lifestyle, and diet. The chronic diseases, such as cancer, GI disorders, and cardiovascular diseases, which are causes of death in most industrialized countries and these high-risk conditions are reduced significantly with intake of dietary fruits, vegetables, and whole grains. Herbal nutraceuticals can be developed as a powerful toolbox for maintaining health and to act against nutritionally induced acute and chronic diseases (within their acceptable recommended dietary intakes), thereby promoting optimal health, longevity, and quality of life.

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SECTION II

Nutrition

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CHAPTER 3

Lipids as Nutraceuticals: A Shift in Paradigm

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1 Introduction

Lipids are a chemically diverse group of molecules with a common property of being soluble in organic solvents and insoluble in water. Over the centuries, lipids have traversed a long way from just being an energy source to their beneficial aspect in various physiological phenomenon. Initially, intake of dietary fats was speculated as being directly related to weight gain and associated health problems. However, in 1929, study by George and Mildred Burr challenged this notion by proving that rats fed a fat-free diet developed scaly skin, retarded growth and mortality, which was reversed by addition of linoleic acid to the diet. They identified linoleic acid as an essential nutrient and coined the term "essential fatty acids" (Smith and Mukhopadhyay, 2012). Another important study by Nunn and Smedley-Maclean (1938) demonstrated that rats on a fat-free diet had very low levels of arachidonic acid, which were normalized on feeding methyl linoleate. Further studies confirmed that arachidonic acid was synthesized from linoleic acid, which is the primary unsaturated fatty acid required in the diet (Mead et al., 1953). $n-3$ Polyunsaturated fatty acids were recognized as another class of essential fatty acids with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) with potential health benefits. Recent studies have strengthened the concept of lipids as nutraceuticals or functional foods not only as major nutrients but also for their role in reducing the risk of various diseases.

2 Classification of Lipids

The term "lipid" was coined by a German chemist Bloor in 1943. The most widely accepted classification of lipids is based on their chemical composition (Fig. 3.1).

2.1 Simple Lipids

Lipids with esters of fatty acids and alcohols are considered as simple lipids. They are further classified as the following.

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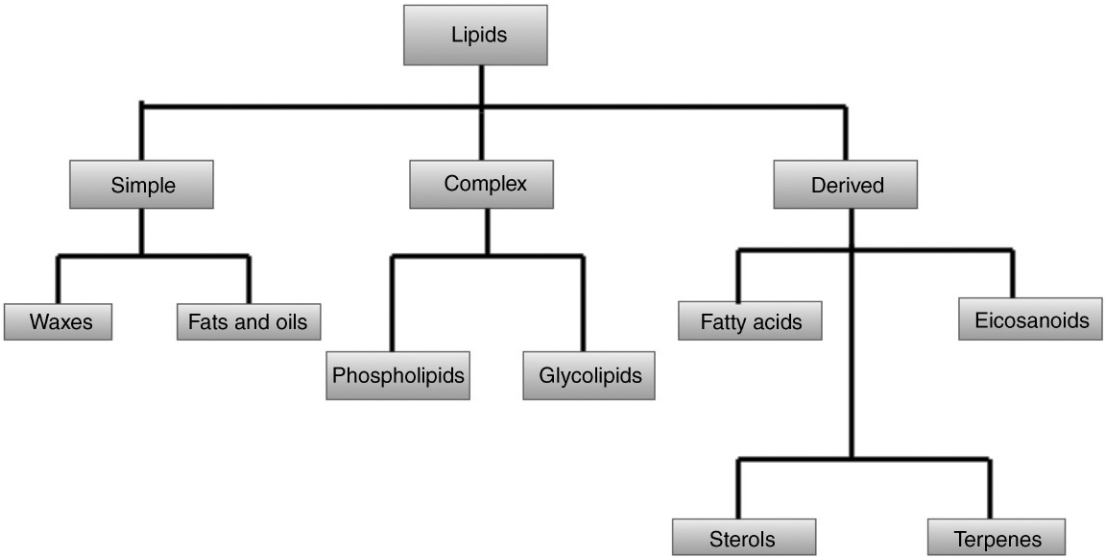


Figure 3.1: Classification of Lipids.

2.1.1 Fats and oils (triacylglycerols)

Fats or triacylglycerols (TAGs) are the storage form of body lipids. They are nonpolar and hydrophobic and are classified as "neutral lipids." Chemically, TAGs consist of glycerol molecule esterified with fatty acyl chains. TAGs are nonpolar because the hydroxyl group of glycerol is linked to carboxyl group of hydrocarbon chain via an ester bond. Fatty acids linked to glycerol may be the same (simple TAGs) or different (complex TAGs).

Triglycerides exist either as fats or oils. These physical properties depend on the length and degree of unsaturation of the fatty acid chains. Triacylglycerols with longer chains of saturated fatty acids exhibit higher melting points and exist as solids at room temperature. Most of the animal fat is rich in saturated fatty acids with the exception of fish fat. Plant fats with high degree of unsaturation exist as oils and are a major source of dietary triglycerides (Fig. 3.2).

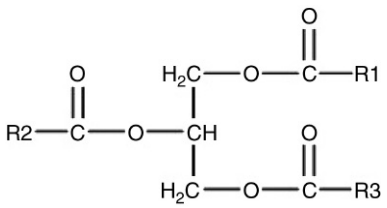


Figure 3.2: Structure of Triacylglycerols.
Simple TAGs-R1 = R2 = R3; Complex TAGs-R1≠R2≠R3.

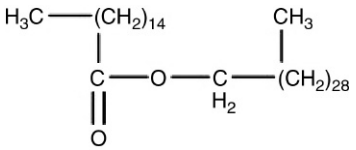


Figure 3.3: Structure of Beeswax.

2.1.2 Waxes

Waxes are esters of long chain fatty acids (C_{14}–C_{36}) and long chain alcohols (C_{16}–C_{30}). Waxes are extremely hydrophobic owing to their long, nonpolar carbon chains. Waxes have comparatively higher melting temperatures (Tm >60°C). These properties make them important lipid components in the protective coating present in insects and animals. Moreover, waxes are major metabolic fuel in planktons, which constitute the bottom of the food chain for marine animals. Waxes can be classified as natural or synthetic. Natural waxes are further classified into vegetable, animal, or mineral waxes. Some important waxes include beeswax, lanolin, carnauba wax and candelila wax. These have a wide range of applications in various industries like cosmetics, food, and polishes (Fig. 3.3).

2.2 Complex Lipids

Complex lipids are esters of fatty acids and alcohols containing additional groups. They are subdivided into following groups.

2.2.1 Phospholipids

Phospholipids are most abundant in biological membranes due to their amphipathic nature. Phospholipids are composed of fatty acids attached to a backbone, a phosphate, and a simple hydroxyl group or a more complex organic base like choline linked to phosphate. Fatty acid chains form the "hydrophobic" tail of the phospholipid and the phosphate group is responsible for the hydrophilic head. Phospholipids can be further divided into phosphoglycerides and sphingolipids depending on the presence of glycerol or sphingosine as the backbone moiety.

2.2.1.1 Phosphoglycerides

Glycerophospholipids or phosphoglycerides are predominantly present in plasma membrane. The carboxyl groups of two fatty acid chains are esterified to C-1 and C-2 of glycerol molecules and a highly polar group is attached to a third carbon via phosphodiester bond. This resulting structure with no further additions is known as phosphatidate or diacylglycerol-3 phosphate, which is the simplest phosphoglyceride. Phosphatidic acid is the parent molecule for synthesis of various phosphoglycerides. However, it is not present in a significant amount in the biological membranes. Further addition of various moieties to the parent structure of phosphatidate is required to form the phosphoglycerides that constitute the major proportion of membranes (Fig. 3.4; Table 3.1).

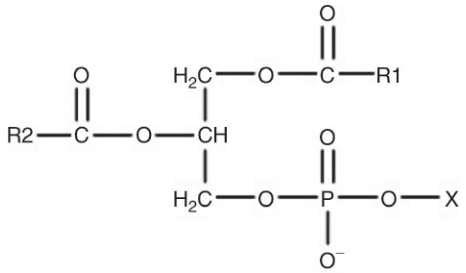
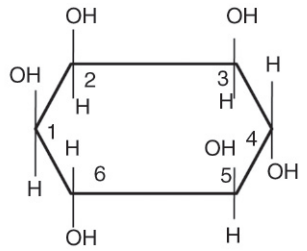
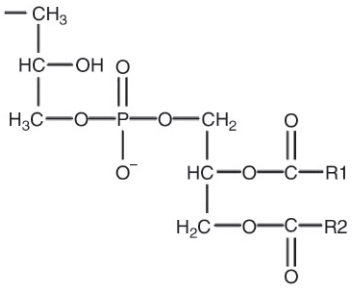


Figure 3.4: General Structure of Phosphoglycerides.

Table 3.1: List of phosphoglycerides.

Name of X-OH	Name of Phospholipids	Formula of −X	Net Charge
Water	Phosphatidic acid	−H	−1
Ethanolamine	Phosphatidylethanolamine; cephalin	$-CH_2CH_3NH_3^+$	0
Choline	Phosphatidylcholine; Lecithins	$-CH_2CH_2N(CH_3)_3^+$	0
Serine	Phosphatidylserine	$-CH_2CH(NH_3^+)COO^-$	−1
Glycerol	Phosphatidylglycerol	$-CH_2CH(OH)CH_2OH$	−1
Myo-inositol	Phosphatidylinositol		−4
Phosphatidylglycerol	Cardiolipin		−2

Phosphatidylcholine, also known as lecithin, are the most abundant phospholipids of the membrane. Phosphatidylcholine is mostly present in the outer leaflet of the plasma membrane whereas phospahatidylserine and phosphatidylethanolamine are present in the inner leaflet, which provides bilayer asymmetry. *Phosphatidylinositol* is a precursor for phosphatidylinositol 4,5-bisphosphate, which is involved in intracellular signaling.

Ether lipids, some animal tissues and unicellular organisms have fatty acid chains linked by ether rather than ester bond to glycerol. These are known as *plasmalogens* and are present in large amounts in heart tissue (Braverman and Moser, 2012). Another important ether lipid with the biological importance is platelet-activating factor (PAF). It acts as a molecular signal with vital roles in inflammation and allergic response (Fig. 3.5).

2.2.1.2 Sphingolipids

Sphingolipids are derivatives of sphingosine, an amino alcohol with long unsaturated hydrocarbon chain. When the fatty acid chain is linked to $-NH_2$ on the second carbon by an amide linkage, the resulting compound is *ceramide*. Ceramide is the parent structure from which other sphingolipids are derived. *Sphingomyelin*, a representative of this group, is structurally similar to phosphatidylcholine. It is found in animal membranes and is majorly present in myelin that insulates the axonal membranes. In *Niemann–Pick disease*, sphingomyelins accumulate in the brain in large quantities due to the absence of enzyme sphingomyelinase (Fig 3.6).

2.2.2 Glycolipids

As the name suggests, glycolipids are lipids containing sugar moieties. They make an essential part of cell membranes and are particularly found in the outer leaflet of the bilayer. Glycolipids are involved in various important functions like cell–cell recognition and tissue immunity. The basic structure of the glycolipids constitute ceramide and one or more sugars.

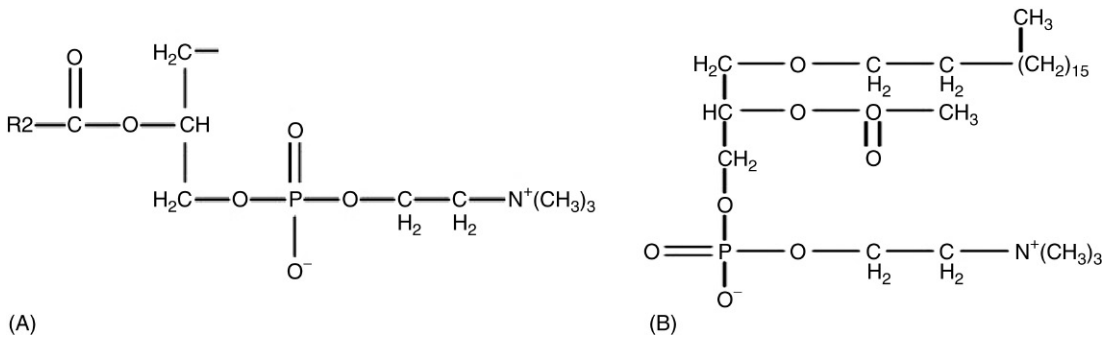


Figure 3.5: Structure of Ether Lipids.
(A) Plasmalogen; (B) platelet activating factor.

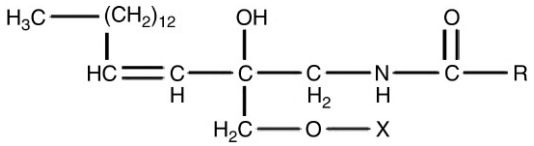


Figure 3.6: Structure of Sphingolipids.
When X-H-ceramide, X-phosphocholine-sphingomyelin.

Table 3.2: List of common glycolipids.

Name of Glycolipid	Group Attached	Charge	Function
Galactosylcerebroside	Galactose	Neutral	Predominant in brain and other nervous tissues
Glucocerebroside	Glucose	Neutral	Mostly present in extraneural tissues, involved in cell–cell recognition, tissue immunity
Gangliosides	Complex oligosaccharides	Negative	Nerve impulse transmission; present in outer surface of membrane for cell recognition; receptors for toxins

Glycolipids differ from phospholipids in that they have sphingosine instead of glycerol backbone and they lack a phosphate group. The simplest glycolipid, *cerebroside*, has glucose or galactose bound to the primary hydroxyl group of sphingosine. Galactosylceramide is the principle glycolipid in the brain and other nervous tissues. The addition of sulfate group to the cerebroside results in formation of *sulfatide* and is the major component of myelin sheath. *Gangliosides* are the more complex glycolipids. They are ubiquitously expressed with abundant expression in the nervous tissues. These are derived from glucosylceramide with one or more molecules of sialic acid. *N*-acetylneuraminic acid is the major sialic acid attached to these glycolipids and renders them the acidic character, unlike other neutral glycolipids (Table 3.2).

2.3 Derived Lipids

Derived lipids are compounds derived from simple and complex lipids upon hydrolysis. Derived lipids include fatty acids, steroids, terpenes, and eicosanoids.

2.3.1 Fatty acids

Fatty acids are the simplest form of the lipids. They are composed of hydrocarbon chains with a methyl group at one end and carboxyl group at the other end of the chain. This structure gives fatty acids the "amphipathic" property, that is, they have a polar, as well as a nonpolar end. The length of hydrocarbon chain in fatty acids ranges from about 4 to 24 carbon atoms.

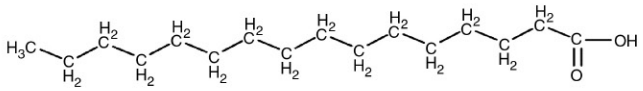
Fatty acids with unbranched hydrocarbon chain are commonly known as *saturated fatty acids*. Among the saturated fatty acids, palmitic acid (16:0) is most widely distributed in plants, animals, and microorganisms. The addition of one or more double bonds leads to the formation of *unsaturated fatty acids*, which are further classified as monounsaturated fatty acids and polyunsaturated fatty acids respectively. Monounsaturated fatty acids (MUFAs) are usually 16–22 in length with one carbon–carbon double bond in *cis* confirmation. The most common monounsaturated fatty acid is oleic acid (18:1, ω-9). *Long chain polyunsaturated fatty acids (LC-PUFAs)* contain more than one double bond and are classified usually on the basis of the position of its last double bond from the methyl end. These are called $n-3$ PUFAs if the double bond is found between the third and the fourth carbon atom and $n-6$ PUFAs if it is between the sixth and seventh carbon from the methyl end. Some PUFAs are produced only in the plants and phytoplanktons and not in mammals, thus must be obtained from diet. *Linoleic acid (LA)* and *α-linolenic acid (ALA)* are essential fatty acids because humans lack enzymes Δ^{12} and Δ^{15} desaturases, which are required to introduce double bonds at these positions. LA and ALA are found in almost all dietary fats and are the major components of vegetable oils. *Arachidonic acid* (AA) is considered an important but conditionally essential $n-6$ PUFA as it can be synthesized in mammals from linoleic acid. *EPA* and *DHA* are vital $n-3$ PUFAs and are majorly obtained from marine lipids, such as fish oil (Fig. 3.7).

2.3.2 Sterols

Sterols are amphipathic molecules with a main frame composed of a planar nucleus and side chain (Nes, 2011). The steroid nucleus has three cyclohexane rings fused with a cyclopentane ring. Sterols, though rarely found in prokaryotes, are ubiquitously synthesized in eukaryotes. The most common sterol present in animal tissues is *cholesterol* ($C_{27}H_{45}OH$). It occurs either in free or esterified form in animal cells. The structure of cholesterol consists of a polar hydroxyl group at C-3 position and a nonpolar steroid nucleus with a hydrocarbon chain at C-17 position. Cholesterol, a constituent of cell membrane, plays a vital role in membrane fluidity and architecture and is a precursor for bile acids and steroid hormones, such as progesterone, testosterone, estradiol, cortisol, and vitamin D (Cruz et al., 2013). Serum cholesterol levels below 200 mg/dL are considered desirable. Both the biosynthesis and dietary fat intake contribute to the body cholesterol. Cholesterol is synthesized from acetyl CoA by a set of complex reactions with β-hydroxy methyl glutaryl CoA reductase (HMGCR) being the major regulatory step. HMGCR catalyzes the synthesis of mevalonate, which further undergoes phosphorylation and then decarboxylation to produce isopentenyldiphosphate. Six molecules of isoprenoid subunits are required to form straight chain squalene, which is finally converted into cholesterol by multiple intermediary reactions.

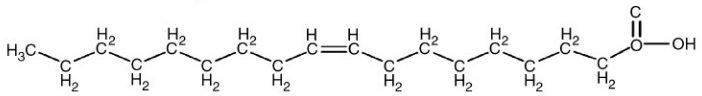
Phytosterols are plant-based sterols and include stigmasterols, sitosterols, and brassicasterols. These are structurally related to cholesterol but differ in the nature of the side chain attached to them. Like cholesterol, these are also synthesized from acetyl CoA and form squalene but

Saturated fatty acids



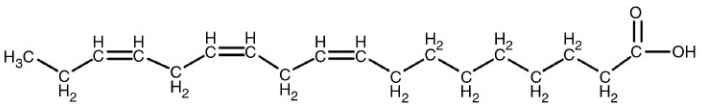
Structure of palmitic acid

Monounsaturated fatty acids

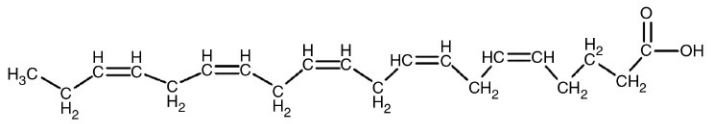


Structure of oleic acid

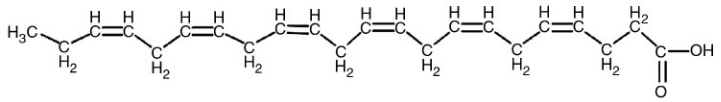
n-3 PUFAs



Structure of linolenic acid

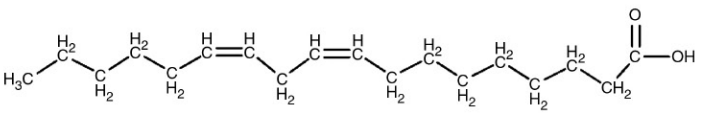


Structure of EPA

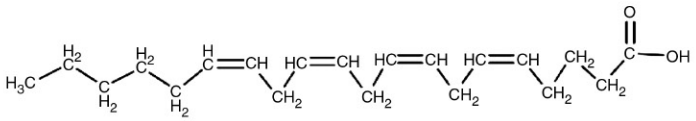


Structure of DHA

n-6 PUFAs



Structure of linoleic acid



Structure of arachidonic acid

Figure 3.7: Structure of Common Fatty Acids.

involve a unique sterol intermediate, cycloarterol. Phytosterols are known to have cholesterol-lowering properties and, therefore, added in the food products as free or esterified forms. Ergosterol, also called provitamin D_2, is a major component of fungal membrane. It is important as precursor of vitamin D. Upon ultraviolet irradiation, ring B is ruptured, which subsequently forms ergocalciferol or vitamin D (Fig. 3.8).

2.3.3 Terpenes

The term *terpen* was used to describe $C_{10}H_{18}$ hydrocarbons that were present in turpentine oil. Otto Wallach was the first to propose "isoprene rule," which states that terpenes are made up of isoprene (2-methyl-1,3-butadiene) subunits. Further, Ingold suggested that these isoprene units were linked in head to tail. A *monoterpene* (C_{10}) consist of two isoprene units,

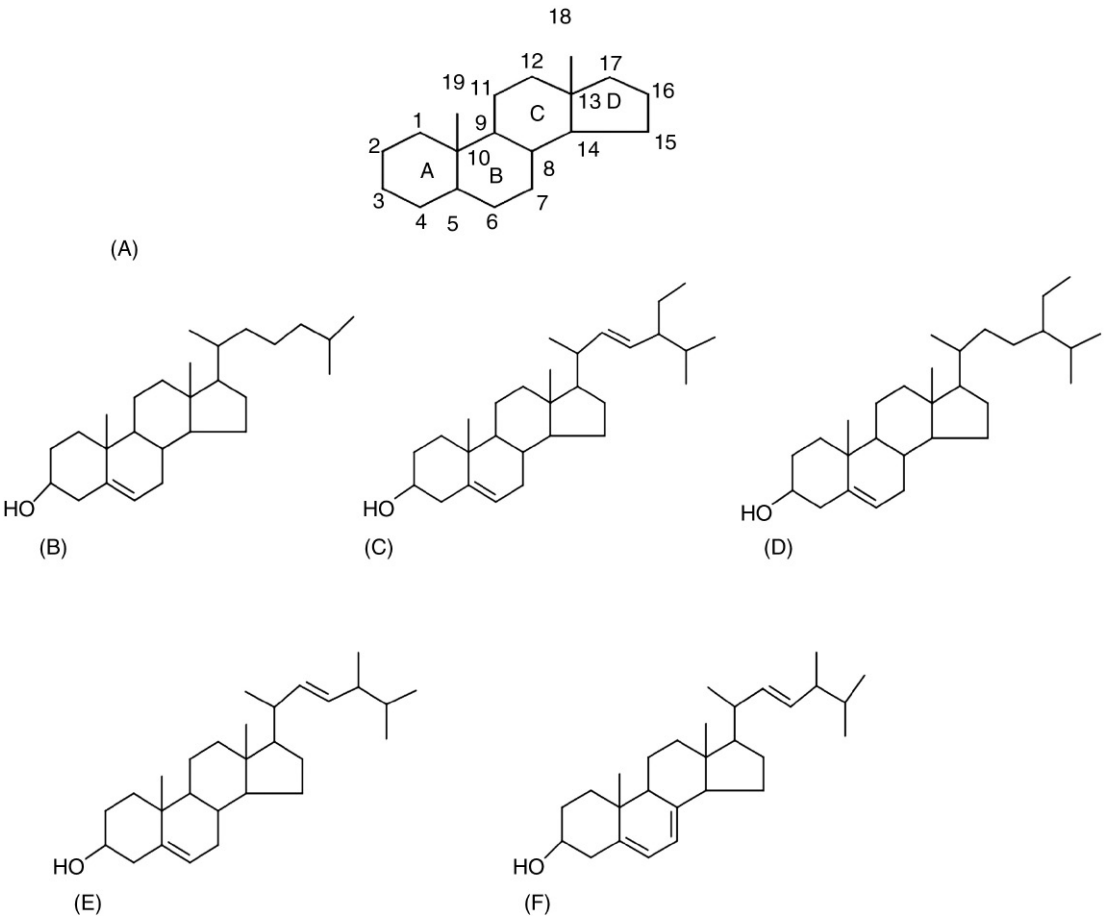


Figure 3.8: Structure of Sterols.
(A) Steroid nucleus, (B) cholesterol, (C) stigmasterol, (D) β-sitosterol, (E) brassicasterol, (F) ergosterol.

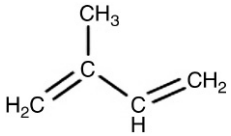


Figure 3.9: Isoprene: Basic Unit of Terpene.

Table 3.3: Classification of terpenes.

Classification	Number of Isoprene Subunits	Carbon Atoms
Monoterpenes	2	10
Sesquiterpenes	3	15
Diterpenes	4	20
Sesterterpenes	5	25
Triterpenes	6	30
Carotenoids	8	40

sesquiterpene (C_{15}) has three, and *diterpene* (C_{20}) has four isoprene units. Monoterpenes and sesquiterpenes are the major components of essential oils. *Triterpenoids* (C_{30}) consist of one of the largest classes of higher terpenoids and include compounds like squalene and lanosterol, which are precursor to steroids in both plants and animals. *Tetraterpenes* (C_{40}) form a separate class called "carotenoids," which includes hydrocarbons "carotenes" and their oxygenated derivatives "xanthophylls" (Fig. 3.9; Table 3.3).

2.3.4 Eicosanoids

Eicosanoids are large family of oxygenated C_{20} fatty acids with *cis*, methylene interrupted bonds, which include *prostaglandins (PGs)*, *thromboxanes (TX)*, and *leukotrienes (LX)*. They act mainly as local hormones and regulate biological processes like inflammation, bronchoconstriction, bronchodilation, and smooth muscle contraction. The most common precursor for the synthesis of eicosanoids is arachidonic acid, an $n-6$ PUFA (C20:4) (Fig. 3.10).

3 Biological Functions of Lipids

Lipids cover a broad range of biological functions, which include their role in energy storage, intracellular signaling, thermogenesis, and structural integrity.

3.1 Energy

TAGs are highly reduced and yield high energy upon oxidation. These are the source of highly concentrated biochemical energy as compared to carbohydrates as they are anhydrous in nature. In vertebrates, TAGs are stored in specialized cells called adipocytes, which are

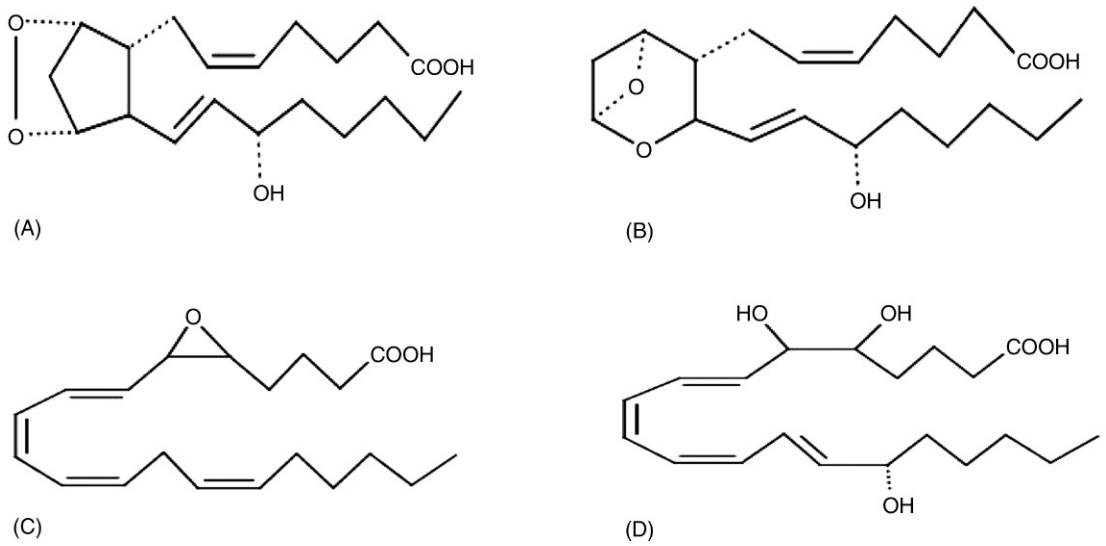


Figure 3.10: Structure of Common Eicosanoids.
(A) PGH_2; (B) TXA_2; (C) Leukotriene A_4; (D) Lipoxin A_4.

primarily found under the skin, in the abdominal cavity, and mammary glands. Triglycerides are stored as fat droplets in the cells and are hydrolyzed by "lipases" to release fatty acids. Further, fatty acids are catabolized into acetyl–CoA by the process of β-oxidation, which then enters Kreb's cycle. The oxidation of one molecule of palmitic acid yields nets 129 ATP molecules whereas one molecule of glucose yields only 32 ATP molecules.

3.2 Role in Membrane

The biological membranes are composed of a lipid bilayer packed with intercalated *trans*-membrane proteins. Phospholipids and cholesterol are the major components of the biological membranes. They provide the membranes a permeability barrier between external and internal milieu of the cells.

Another important component of membranes is lipid rafts. Lipid rafts are small, distinct microdomains that are rich in cholesterol and sphingolipids and have been known to play important roles in signal transduction. Cholesterol is the dynamic glue that helps in the association of various proteins to the raft. The elevated levels of cholesterol and sphingolipids in lipid rafts influence lipid–protein interactions by increasing the thickness of the lipid bilayer. This helps the lipid rafts to compartmentalize several cellular processes by the activation of signaling cascades. The lipid rafts serve as organizing centers for the assembly of multiple signaling molecules involved in cell proliferation and cell death and thus in the pathogenesis of several diseases, such as cancer and neurodegenerative disorders.

3.3 Role of Eicosanoids

As discussed in the previous section, arachidonic acid serves as the main precursor for the synthesis of eicosanoids. Arachidonic acid is esterified to the membrane phospholipids and is released upon activation of membrane bound phospholipase A_2 or C. Upon its release, the fate of arachidonic acid is decided by the metabolic pathway it enters. The first pathway "*cyclooxygenase pathway*" yields prostaglandins and thromboxanes (prostanoids). The primary step in the synthesis of prostanoids is catalyzed by the enzyme *prostaglandin H synthase (PGHS)*, also known as *cyclooxygenase(COX)*, which has both cyclooxygenase and peroxidase activity and synthesizes PGH_2. PGH_2 is then reduced by specific isomerases into biologically active prostaglandins: prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). PGH_2 may also be converted to thromboxane TXA_2 by the enzyme thromboxane synthase. The production of prostanoids is tissue-specific with one of these as the dominant product. For example, PGI_2 is expressed in vascular endothelium, TXA_2 in blood platelets, and collecting tubule cells produce PGE_2. *Aspirin*, a nonsteroidal antiinflammatory drug (NSAID) blocks the synthesis of prostaglandins by inactivating prostaglandin synthase. The second pathway is known as "*lipoxygenase pathway*" via which leukotrienes and lipoxins are formed. Lipoxygenase enzyme incorporates an oxygen molecule at carbon atoms of one of the several double bonds to form hydroperoxyeicosatetraenoic acids *(HPETE)*. The synthesis of leukotrienes in the leukocytes, platelets and macrophages begins with the formation of HPETEs. The major leukotrienes are synthesized by *5-lipoxygenase*, which incorporates oxygen at the fifth position in arachidonic acid. Lipoxins, a family of conjugated tetraenes, are synthesized by the consecutive action of 15-lipoxygenase and 5-lipoxygenase on arachidonic acid. The third is the "*epoxygenase pathway*," which is involved in the synthesis of epoxides. This pathway involves cytochrome p450 epoxygenases, which form *epoxyeicosatrienoic acids (EET)*. These epoxides function as potent lipid mediators in cardiovascular and renal system (Spector and Kim, 2015).

Eicosanoids are known to have a crucial role in maintaining homeostasis and mediating inflammatory roles. Inflammation is the body's immune response to infection and injury. However, prolonged inflammatory response predisposes the body to more tissue damage and is implicated in various diseases, including arthritis, cancer, neurodegenerative, and cardiovascular diseases (Ricciotti and Fitzgerald, 2011). Inflammation is treated by steroidal as well as nonsteroidal antiinflammatory drugs. NSAIDs are usually COX inhibitors while steroidal drugs target phospholipase A_2 and both inhibit the formation of prostaglandins and leukotrienes. The drugs developed specifically against the inducible COX-2 enzyme have grabbed attention due to their clinical efficacy (Wakabayashi, 2000). Accumulating evidences have suggested the key role played by COX-2 in the pathogenesis of cancer. COX-2 was found to be highly expressed in colorectal tumors as compared to normal intestinal mucosa (Dubois et al., 1998). Moreover, over expression of COX-2 is linked to

increased synthesis of PGE_2, which elicits diverse effects on cell proliferation, apoptosis, and tumor invasion (Sobolewski et al., 2010). Eicosanoid production associated with chronic inflammation could be subsided by intake of $n-3$ PUFAs. Therefore, $n-3$ PUFAs are known to be potent antiinflammatory agents. The increased consumption of long chain $n-3$ PUFAs incorporates EPA and DHA in the inflammatory cells and decreases the substrate availability for eicosanoid production (Calder, 2006). Moreover, $n-3$ PUFAs derived resolvins and protectins are highly potent antiinflammatory and proresolving agents as these exert their action primarily on the innate immune system. (Serhan and Petasis, 2011). The role of eicosanoid production in various diseases are discussed in detail later in the chapter.

3.4 Role in Signaling Pathways

Extracellular signals, such as growth factors, hormones, and nutrients stimulate a set of endogenous lipid modifying enzymes, for example, phospholipases, cyclooxygenases, lipooxygenases, prostaglandin synthase, phosphoinositide-3-kinase, sphinogoshone kinase, and sphingomyelinase. These enzymes constitute a complex lipid-signaling network and generate various signaling lipids, such as eicosanoids, phosphoinositides, and sphingolipids. The signaling lipids thus regulated a diverse range of physiological processes, including cell proliferation, apoptosis, metabolism, inflammation, neural development, and atherogenesis. Imbalance in lipid-regulated pathways has been implicated in a number of human diseases, such as cancer, obesity, diabetes, cardiovascular, and brain disorders (Corda and De Matteis, 2013).

3.4.1 Phosphoinositides

The spectacular research in last three decades has established the central role of Phosphoinositides (PIs) in almost all aspects of cell fate (Kimura et al., 2016). PIs regulate vesicle trafficking, lipid distribution and oncogenic processes. Phosphatidylinositol (PtdIns) accounts for 10% of total cell glycerophospholipids in mammalian cells. PIs are further phosphorylated by phosphoinositide kinases (PIKs) at D3, D4, and D5 hydroxyl group of PtdIns generates a diverse range of phosphoinositides, such as PtdIns 4-P, PtdIns 3-P, PtdIns 4,5-P2, PtdIns 3,4-P2, and PtdIns 3,4,5-P3. Three classes of PIKs have been reported with class I being the most extensively studied. Class I PIK is a heterodimer composed of a catalytic subunit (p110) and an adaptor/regulatory subunit (p85). On activation, PI3K converts PtdIns 4,5-P2 into PtdIns 3,4,5-P3, which acts as a second messenger and recruits a set of Pleckstrin homology (PH) domain containing signaling proteins, such as 3-phosphoinositide-dependent protein kinase 1(PDK1) and Akt/protein kinase B(PKB) (Laplante and Sabatini, 2012). The activated Akt/PKB promotes evasion of apoptosis by inactivating apoptotic molecules, such as Bad, procaspase-9, and forkhead family transcription factors that induce Fas/Fas L expression.

In addition to abrogation of apoptosis, Akt also promotes cell survival by activating IkB kinase (IKK), which leads to sustained expression of transcription factor NF-κB. Finally, Akt also positively regulate cell cycle progression by enhancing cyclin D1 expression and inactivating GSK3β (Hers et al., 2011). Mutation in p53 is also linked to alterations in acyl chain composition of PIs. In addition, increased monounsaturation of membrane, which facilitates more efficient conversion of PIP2 to PIP3 due to enhanced expression of stearoyl–CoA desaturase has also been associated with p53 mutation (Abraham and O'neill, 2014).

3.4.2 Ceramides and sphingosine-1-phosphate

Sphingolipids, another major category of lipids, are major players in cell signaling pathways (Pyne et al., 2016). Under normal conditions, ceramide is hydrolyzed to sphingosine by ceramidase. During stress, however, ceramides are produced from sphingomyelin by the action of neutral sphingomyelinase and acid sphingomyelinase. Both ceramide and sphingosine diminish cell growth and are proapotptotic. External environmental stress or agents that cause DNA damage and apoptosis enhance ceramide concentration, which promotes clustering of death receptors and eventual cell death. Ceramides have also been shown to inhibit PI3K/Akt/mTOR pathway by activating protein phosphatases, such as ceramide-activated protein phosphatase (CAPP). However, on conversion to ceramide-1-phosphate (C1P) and sphingosine-1-phosphate by ceramide kinase (cerK) and sphingosine kinase (sphK), respectively, their function is reversed; that is, they promote cell growth and decrease apoptosis (Fyrst and Saba, 2010). Maintenance of equilibrium between ceramide and C1P appears to be crucial for tissue homeostasis and accumulation of one or other results in disease development.

4 Lipid Transport

Lipid transport is a prerequisite in order to meet the tissue's requirement for performing various biological functions. Due to their insolubility in water, lipids cannot be transported as such and has to be associated with specific proteins to form lipoproteins. Lipoproteins consist of a monolayer of phospholipids, unesterified cholesterol, and protein components called "apolipoproteins" surrounding a core of hydrophobic lipids (triglycerides, cholesteryl esters, and a small amount of unesterified cholesterol). Lipoproteins are classified on the basis of their density, which in turn is dependent on its lipid and protein composition. The higher the protein content, the higher is the density of the lipoprotein. Five major types of lipoproteins include chylomicrons, very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Chylomicrons and VLDL are predominantly composed of triacylglycerols whereas LDL and HDL primarily carry cholesterol and phospholipids.

Postdigestion dietary fatty acids and cholesterol are absorbed by the intestine where they are reesterified into triacylglycerols and cholesterol esters and are then transported in the form of *chylomicrons*, which are the major vehicle for the transport of all dietary lipids. Chylomicrons enter circulation through lymphatics and download their cargo of lipids to various extrahepatic tissues by the action of enzyme lipoprotein lipase. The resulting *chylomicron remnant* mainly consists of cholesterol and cholesteryl esters and has low levels of triacylglycerol. This remnant is removed from the bloodstream and delivered to liver. VLDL is assembled in the liver and is responsible for the transport of both dietary and endogenously synthesized lipids. With the removal of TAGs from VLDL by lipoprotein lipase, a transient IDL is formed before its final conversion to a cholesterol rich LDL particle. The chylomicron remnants and LDL are taken up by the liver with the help of *Apo-E* specific receptors and the triglycerides and phospholipids in these particles are hydrolyzed by hepatic lipase. LDL is the major carrier of cholesterol for extrahepatic tissues. LDL bind to tissue-associated LDL receptors, which are then internalized by endocytosis and fused with lysosomes. The cholesteryl esters present in LDL are then hydrolyzed by lysosomal acid lipase and released into the tissues. The unesterified cholesterol is then either used for membrane biogenesis or reesterified for storage purposes.

Another lipoprotein that plays a vital role in the lipid homeostasis is HDL and is known to mediate *reverse cholesterol transport*. HDL synthesized in intestine contains *Apo-A* whereas HDL formed in the liver contains both *Apo-E* and *Apo-C*. Both these lipoproteins are transferred from lipid HDL to intestinal HDL before it enters the plasma. The discoidal nascent HDL collects the unesterified cholesterol from the tissues and other lipoproteins and converts this into the esterified form by the enzyme lecithin: acyltransferase (LCAT). The accumulation of cholesteryl esters converts HDL into spherical particles. Another protein associated with LCAT-HDL is cholesterol ester transfer protein (CETP), which catalyzes the transfer of these cholesteryl esters to LDL and VLDL. The remaining cholesteryl esters in HDL cores are delivered to the liver and are further hydrolyzed by cholesteryl esterase and excreted as bile in the bile salts. This is the major route of excretion of cholesterol from the body.

Lipid profiling involves the measurement of blood levels of total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides and act as a cornerstone for clinical diagnosis and risk assessment for various diseases.

5 Lipids in Pathogenesis of Diseases

5.1 Cancer

Cancer is a complex disease involving transformation of normal cells into abnormal cells that have the potential to proliferate indefinitely and invade or spread to other parts of the body. Though genetic mutations are the prime cause for generation of cancer cells, environment,

and lifestyle changes also have a great impact on cancer development. It has been predicted that dietary and lifestyle modifications can reduce the cancer risk by approximately 35%.

Lipids constitute a significant component of our diet and have an important physiological role in cell growth, proliferation, and signaling. The high growth and proliferation rate of cancer cells demand large amounts of lipids as building blocks for biological membranes. The increased lipid biosynthesis is found to be associated with metabolic reprogramming, which is now established as a hallmark of cancer (Beloribi-Djefaflia et al., 2016). Accumulating evidence suggests that cancer cells show specific alterations in lipid metabolic enzymes and their pathways. Various regulatory factors, enzymes, transporters that are involved in lipid synthesis, transport, and degradation are dysregulated in cancer cells. This depicts the fundamental role played by lipids in maintaining normal function in healthy cells. Recent advances in lipid analytical and imaging technologies, such as electrospray ionization, Raman scattering microscopy, matrix-assisted laser desorption/ionization, and tandem mass spectrometry (MS/MS) provide crucial information on tumor lipid phenotype. In order to determine the complete spectrum of lipids within cancer cells, it is important to study the role of different lipid classes in cancer.

5.1.1 LC-PUFAs

LC-PUFAs are a group of essential fatty acids that are important players in cell membrane structure, fluidity, and cell signaling. The two families of LC-PUFAs, $n-3$ and $n-6$ PUFAs have antagonistic function in cancer physiology. $n-3$ PUFAs have a negative effect on tumor growth while $n-6$ PUFAs have been known to support carcinogenesis. The interaction of $n-3$ and $n-6$ PUFAs and their balanced ratio in dietary intake is important for normal growth of cells. An imbalance in the dietary intake of $n-3$ versus $n-6$ PUFAs has been proposed to be responsible for increased incidence of breast, prostate, and colon cancer. The consumption of higher amounts of $n-6$ PUFAs in Western diets than that of $n-3$ PUFAs is considered a contributory factor in increased burden of number of diseases including breast cancer. It has been suggested that modified dietary strategy incorporating more of $n-3$ PUFAs in diet may reduce the incidence of many diseases (Renuka et al., 2015).

In 2015, a study on women suffering from breast cancer was done to scrutinize the effects of polyunsaturated fats on breast cancer. It was revealed from the studies that the consumption of large amounts of $n-3$ PUFAs led to a reduction of 25% in breast cancer events (Fabian et al., 2015). $n-3$ PUFAs have pleiotropic effects and predominant mechanisms include impairment of cell proliferation or increase in cell death or combination of both.

$n-3$ PUFAs have a significant role in regulation of the cell cycle. In the cell cycle, the transition of one phase into another is regulated by a number of checkpoints. The mechanism of action of these checkpoints includes coordinated activities of protein kinases known as the cyclin-dependent kinases (CDKs) and their regulator proteins known as cyclins (Rhind and Russell, 2012). Mutation in the genes encoding these proteins results in the cell proliferation and development of tumor. $n-3$ PUFAs can arrest the growth of cancer cells by modulating

the expression of cyclins and tumor suppressor gene TP53 (Takahashi et al., 2011). A major reason for cancer-associated mortality is metastasis and drug resistance. Supplementation of $n-3$ PUFAs with 5-FU and other chemotherapeutic drugs, such as oxorubicin, epirubicin, and tamoxifen has been reported to arrest cell cycle at S phase and block G1/S progression (Geelen et al., 2007; Rani et al., 2014). In addition to its chemopreventive actions, $n-3$ PUFAs also have a role in increasing the chemosenstivity of tumor cells to chemotherapeutic regimens (Sala-Vila et al., 2010).

Apoptosis is a sequential process of programmed cell death by an unwanted cell. The oncogenic signals disrupt apoptosis and thus lead to tumor initiation, progression, and metastasis. The two apoptotic signaling pathways are: extrinsic or death receptor pathway and intrinsic or mitochondrial pathway. The death receptor pathway involves Fas and Fas ligand (Fas/FasL), which leads to the formation of death-inducing silencing complex (DISC) and initiates downstream cascade of caspases (Sharma et al., 2016). On the other hand, intrinsic pathway occurs inside the cell and involves alteration in mitochondria structure and function. The mitochondria provide platform for several processes, such as oxidative phosphorylation and formation of free radicals from electron transport chain (Orrenius, 2007). Free radicals further damage the mitochondrial membrane, triggering the release of proapoptotic factors, such as cytochrome *c* from inner mitochondrial space (Sharma et al., 2013). Both the pathways finally merge at caspase-3, which further activates the downstream cascade for initiation of apoptosis. The apoptotic pathway is also regulated by members of the Bcl-2 family, which includes both pro- (Bid, Bak, Bax) and antiapoptotic (Bcl-Xl, Bcl-2) members.

There is ample evidence to support the role of $n-3$ PUFAs in modulating the apoptotic pathways. An increase in desaturation of membrane by incorporation of DHA into mitochondrial membrane makes it susceptible to oxidation by ROS and augments the release of proapoptotic factors (Narayanan et al., 2001). Recently, Abdi et al. (2014) demonstrated that $n-3$ PUFAs induced apoptosis in myeloma cells through mitochondrial disruption and caspase-3 activation. Dietary $n-3$ PUFAs have also been shown to modulate the expression of Bcl-2 family proteins in colon cancer cell lines (Calviello et al., 2005; Danbara et al., 2004; Llor et al., 2003). Sun et al. (2013) reported that $n-3$ PUFAs induce apoptosis in human hepatocellular carcinoma cells by upregulating caspase-3 and Bax expression levels and downregulation of Bcl-2 and Bim expression.

The growth and proliferation of normal cells are regulated by a network of signaling pathways. An important aspect of cancer includes dysregulation of growth factor-dependent signaling pathways. Epidermal growth factor receptor (EGFR) is a tyrosine kinase that plays significant role in regulation of signaling cascades involved in cell proliferation. The important pathways include Ras-induced Raf/MEK/Erk1/2 and the phosphatidylinositol 3-kinase/Akt (PI3K-Akt) and any aberration in these pathways may lead to development of cancer. Ras is a protooncogene that has to be transported to the membrane by posttranslational modification, such as farnesylation. Mutations in Ras gene

are an early event in the colon carcinogenesis (Worthley et al., 2007). *n*−3 PUFAs inhibits localization of Ras gene to the plasma membrane by decreasing the activity of farnesyl protein (Turk et al., 2012). The PI3K/Akt signaling pathway transmits signals from growth factors to secondary messenger by modulating the conversion of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-4,5-biphosphate (PIP3) that phosphorylates Akt (Liu et al., 2011). Activated PI3K/Akt stimulates cell survival and tumor development and is negatively regulated by phosphatase and tensin homolog deleted on chromosome 10 (PTEN). PTEN catalyzes dephosphorylation at the 3′OH group converting PIP3 into PIP2 hence inducing apoptosis (Steelman et al., 2004). *n*−3 PUFAs have been reported to decrease Akt activation in breast and colon cancer by augmenting PTEN expression (Kansal et al., 2014a).

Another mechanism of action of *n*−3 PUFAs in prevention of carcinogenesis is regulation of inflammation by inflammatory mediators, in particular NF-κB. The nuclear factor NF-κB is a transcriptional regulator, which affects the expression of various inflammatory cytokines, COX-2, vascular endothelial growth factor (VEGF), and provides a critical link between inflammation and cancer. NF-κB is activated and translocated to nucleus on phosphorylation and proteosomal degradation of its inhibitory protein IκB. *n*−3 PUFAs decrease the phosphorylation of IκB, which in turn decrease NF-κB activation. The dietary intake of *n*−3 PUFAs modify the substrate pool for the action of COX-2 by decreasing the availability of arachidonic acid for eicosanoid synthesis and hence reduce inflammation. COX-2 enzyme also modulates the expression of VEGF involved in the process of angiogenesis and thus plays a pivotal role in cancer development. Increased expression of VEGF has shown to be related to the poor prognosis, metastasis, and is considered as a major proangiogenic factor associated with the progression of colorectal cancer. *n*−3 PUFAs have been reported to reduce VEGF expression by targeting COX-2 enzyme (Calviello et al., 2004). A reduction in VEGF expression was demonstrated by *n*−3 PUFAs supplementation in experimental colon cancer (Kansal et al., 2014b) (Figs. 3.11 and 3.12).

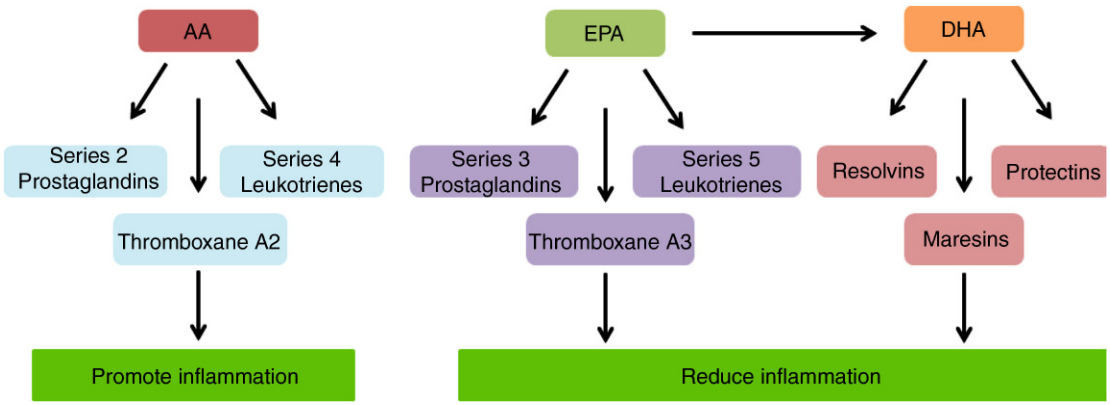


Figure 3.11: *n*−3 Versus *n*−6 PUFAs in Development of Inflammation.

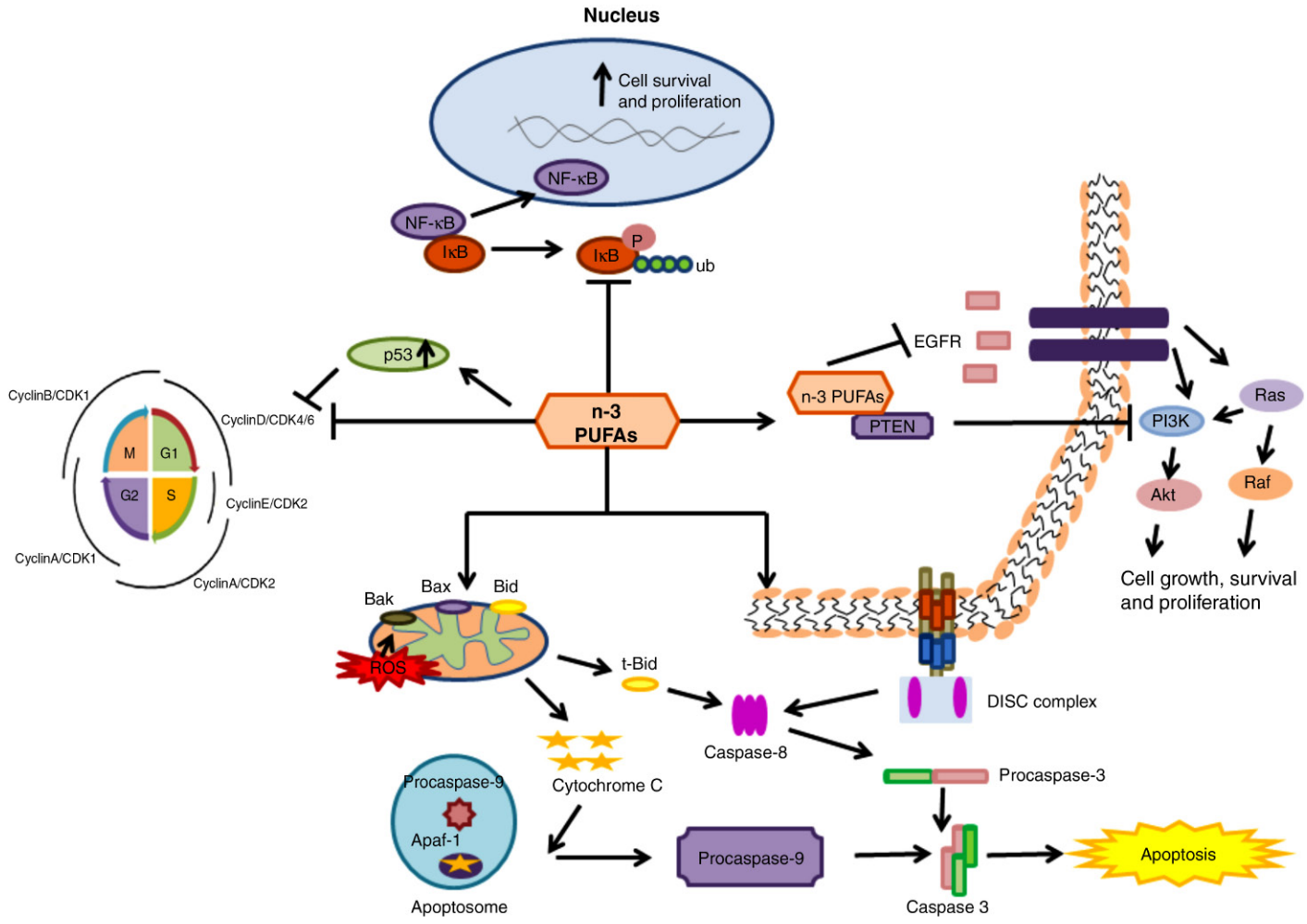


Figure 3.12: Role of $n-3$ PUFAs in Cancer Prevention.

5.1.2 Cholesterol

Cholesterol homeostasis plays a critical role in carcinogenesis. Tumor cells require excess cholesterol to sustain the tumor proliferation, migration, and metastasis (Silvente-Poirot and Poirot, 2012). The increased requirement of cholesterol can be met either by de novo synthesis or influx and efflux (Cruz et al., 2013). The possible mechanism includes an abundant availability of its precursor, acetyl-CoA via glycolysis (Biswas et al., 2012). Acetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is further converted to mevalonate by the enzyme HMG-CoA reductase. The cancer cells exhibit enhanced HMG-CoA reductase activity and are regulated at the transcriptional level by sterol regulatory element binding proteins (SREBPs). Inhibition of mevalonate pathway was suspected to be potential target for the therapeutic strategies in cancer. This led to the use of statins, which are competitive inhibitors of HMG-Co A reductase. In addition, they also have an inflammatory role that interferes with tumor growth and development. However, the recent clinical trials have suggested that statins may promote hematological malignancies in certain segments of the population. This may be attributed to the fact that the use of statins leads to a constant increase in T-cells, which in turn weakens other tumor fighting immune response (Pradelli et al., 2015).

Cholesterol transport involves the use of lipoproteins particularly LDL and HDL. Lipid profiling of cancer patients made it clear that among the two, HDL levels are more affected in the development of tumor. HDL facilitates the removal of excess cholesterol from peripheral tissue with the help of ATP-binding cassette transporter (ABCA1). This indicates that there is negative correlation between HDL levels and risk of cancer. In fact, SR-B1 (HDL) receptor is overexpressed in cancer tissues (Gorin et al., 2012; Lacko et al., 2002; Llaverias et al., 2011) highlighting the role of HDL particles as major suppliers of cholesterol to cancer cells.

Cholesterol is the major component of the specialized membrane domains known as lipid rafts. The lipid rafts act as molecular platforms that organize membrane receptor molecules, such as EFGR and CD95 (Fas) (Simons and Toomre, 2000). This arrangement is of crucial importance in the regulation of inflammation and apoptosis. Depletion of cellular cholesterol disrupts lipid rafts and modulates the activity of these signaling pathways. Therefore, low cholesterol levels in lipid rafts are considered as a therapeutic approach for cancer therapy. Reports suggest that $n-3$ PUFAs are readily incorporated into tumor cell membranes and alter the membrane raft properties by decreasing their cholesterol content (Schley et al., 2007). Turk and Chapkin (2013) reported that the cholesterol content of lipid rafts in colonocytes was reduced by 46% in mice fed on a diet rich in 3-PUFAs.

5.1.3 Sphingolipids

Sphingolipids, particularly ceramides and sphingosine1-phosphate (S1P), have been defined as reciprocal regulators of signaling pathways. Ceramides activate key proteins involved in apoptosis that induce the cell death via Bid, Bak, and Bax. In addition, ceramides also

modulate Protein kinase C (PKC), which triggers cytochrome *c* release and caspase-9 activation (Sumitomo et al., 2002). In contrast, S1P negatively regulates ceramide-mediated apoptosis by changing mitochondrial membrane potential and inhibiting cytochrome *c* release. S1P also downregulates the expression of Bad and Bax by inhibiting their translocation to mitochondria.

5.2 Obesity

Obesity has become a major public health issue and an important contributor to the global burden of chronic disease and disability (WHO, 2000). According to the WHO World Health Statistics Report 2012, globally one in six adults is obese and nearly 2.8 million individuals die each year due to overweight or obesity (WHO, 2012). Any individual whose BMI $\geq$ 30 kg/m^2 is considered clinically obese or overweight. The major factors responsible for obesity epidemic worldwide are westernization of lifestyle and changes in dietary habits. Lipids are considered as main rogue factors in obesity and their role have been studied extensively.

5.2.1 LC-PUFAs

Numerous studies have generally considered that a change in ratio of $n-6/n-3$ PUFAs from 1 to 4:1 to about 10–20:1 in recent times due to westernization of diet and habits is the major cause for obesity (Patterson et al., 2012). An imbalance in dietary LC-PUFAs, which involves low serum EPA/AA ratio, leads to visceral fat accumulation (Inoue et al., 2013).

$n-3$ PUFAs are also known to have a putative role in promoting lipolysis and fatty acid oxidation and thus inhibiting lipogenesis. In obesity, the rate of de novo lipogenesis is elevated due to overexpression of SREBP-1, which in turn increases the mRNA levels of the genes encoding lipogenic enzymes. However, $n-3$ PUFAs are known to reverse the obesity associated upregulation of lipogenic enzymes, such as perilipin, SREBP-1, lipoprotein lipase (LPL), and fatty acid synthase (FAS) (Peng et al., 2016).

Obesity is a consequence of several factors, such as increased energy consumption and reduced physical activities (Rodriguez-Hernandez et al., 2013). Many studies implicate chronic low-grade inflammation in the interplay between obesity and metabolic complications (Orpana et al., 2010). Obesity-linked inflammation plays a major role in the pathogenesis of various diseases, such as type 2 diabetes, cardiovascular disease, hypertension, stroke, gallbladder disease, and some forms of cancers (Kaplan et al., 2003). Patterson et al. (2012) suggested LC-PUFAs as important modulators dealing with local and systemic inflammation in obesity. The $n-3$ and $n-6$ PUFAs have been known to exhibit opposing effects in inflammation. High intake of $n-3$ PUFAs elevate their levels, which in turn replace AA in membrane and decrease their availability for eicosanoid

production. Supplementation with $n-3$ PUFAs also increases acetylcholine levels, which block the release of proinflammatory cytokines, such as, tumor necrosis factor-α (TNF- α), interleukin-6, and interleukin-1. On the other hand, $n-6$ PUFAs increase the production of inflammatory eicosanoids in the membrane rafts. Therefore, dietary modifications incorporating a higher amount of $n-3$ PUFAs can be an important strategy in reducing the risk of obesity (Simopoulos, 2002).

A novel strategy to alleviate obesity is conversion of white adipose tissue to brown adipose tissue. The brown adipose tissue plays a role in thermogenesis by uncoupling the electron transport chain and hence dissipates the excess energy as heat. Various reports have shown that supplementation of diet with $n-3$ PUFAs led to an increase in levels of UCP-1 (uncoupling protein in mitochondrial inner membrane) (Janovska et al., 2013; Oudart et al., 1997). Thus, $n-3$ PUFAs aid in browning of the white adipose tissue, which is a potential strategy in weight reduction (Fig. 3.13).

5.2.2 Cholesterol

Obesity is often found to be associated with hypercholesterolemia, which indicates a positive correlation between obesity and serum cholesterol levels (Tanner, 1951). Overweight individuals tend to have high total cholesterol, triglyceride, and LDL levels and low HDL levels (Franssen et al., 2011). The adversely altered HDL levels in obesity have been

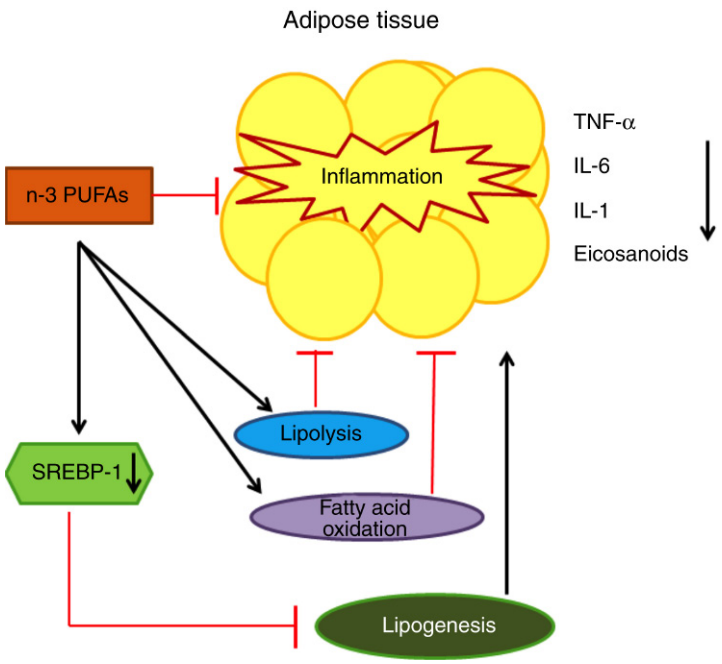


Figure 3.13: Role of $n-3$ PUFAs in Prevention of Obesity.

attributed to increased catabolism of apolipoprotein A-1 (Rashid and Genest, 2007). The metabolic syndromes of obesity and dyslipidemia are corrected by lowering LDL and increasing HDL concentrations. Statins—a class of lipid lowering medications—have assumed the central role in treatment of hypercholesterolemia (Stein, 2003) and are used to reduce LDL and triglyceride levels and increase HDL cholesterol (Singh et al., 2011).

5.3 Role of Lipids in Nervous System

The brain is the second most lipid-rich organ in the body after adipose tissue. Lipids constitute 50%–60% of the brain's dry weight (Müller et al., 2015). In addition to their quantitative abundance, lipids also show enormous structural diversity in brain. Lipids are synthesized in large amounts during early development of the brain due to their crucial role in brain development (Veloso et al., 2011). Synthesis of myelin sheath is crucial for nerve transmission as the speed and quality of signal transmission depends on the health of myelin sheath of neurons. Numerous reports have suggested that a fat-deficient diet during development results in hypomyelination in brain (Salvati et al., 2000). Myelin sheaths are highly specialized plasma membrane characterized by unusual enrichment of lipids (Schmitt et al., 2015). As myelin sheath plays an important role in functioning of nervous system, its lipid composition has been extensively studied. Cholesterol, glycerophospholipids, and glycosphingolipids are the major components of myelin sheath present in a fixed molar ratio (2:2:1). Similarly, lipid to protein ratio in myelin is also fixed for 1:1 in central nervous system (CNS) and 5:1 in peripheral nervous system (PNS). Qualitative and/or quantitative alterations in these ratios result in myelin dysfunction and ultimately a neurological disease (Cermenati et al., 2015). The analysis of lipid content during development from different regions of a rat brain showed unique distribution of sphingomyelins and phosphotidylcholines (Lohmann et al., 2010). Recently, lipidome of human CNS myelin revealed the presence of 700 different types of lipid species. Major classes of lipids include phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, cerebrosides, and sulfatides; however, the study also identified 40% unreported species of lipids in myelin (Gopalakrishnan et al., 2013).

5.3.1 Brain lipid composition

Apart from major forms of lipids, such as phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, cerebrosides, and sulfatides, the brain also contains certain other type of lipids, which are specific in location, as well as function. These low proportion lipids mostly are derivatives of cholesterol and $n-3$ PUFAs.

5.3.1.1 Cholesterol

Cholesterol, a major component of all mammalian cell membrane, is synthesized via 37 complex series of reactions through de novo pathway (Martin et al., 2014). Most of the brain cholesterol is unesterified (99.5%), which is mainly present in myelin sheath as

compared to neurons and glial cells (Vila-Rodriguez et al., 2011). Neurons and glial cells exhibit de novo synthesis of cholesterol and are independent of serum cholesterol due to inability of lipoproteins to cross blood–brain barrier (BBB). Significance of cholesterol in brain development is also highlighted by the fact that its estimated half-life is 5 years in humans (Vance, 2012). Reports from animals and human studies have shown that brain cholesterol is independent of serum cholesterol as free cholesterol or lipoproteins do not cross the BBB therefore the brain relies on de novo synthesis of cholesterol in glial cells and neurons (Zhang and Liu, 2015). Cholesterol homeostasis in the brain is strictly maintained by regulating its synthesis, uptake, and efflux mechanisms. The major efflux mechanism of cholesterol is via its conversion to 24(S)-hydroxycholesterol by cytochrome P_{450} dependent enzyme cholesterol 24-hydroxylase (CYP46A1) present in the brain. The second mechanism involves the efflux of free cholesterol to the blood by membrane transport proteins, such as ABCA1. The cholesterol transport between astrocytes and neurons is also networked through ABCA1 (Chen et al., 2013; Do et al., 2011). In addition, SREBP cleavage-activating protein mediated activation of SREBPs also regulates cholesterol biosynthesis in brain (Shao and Espenshade, 2014).

A further role of cholesterol in the nervous system is that it acts as precursor of signaling molecules, such as neuroactive steroids and oxysterols. Neuroactive steroids modulate neuronal survival in CNS and myelin protein synthesis in PNS, thus contributing to myelin maintenance (Roglio et al., 2008). Oxysterols, such as 24 (S)-hydroxycholesterol and 27-hydroxycholesterol through activation of liver X receptors (LXRs) regulate the expression of ABCA1. Beside activation of LXRs, treatment of human neuroblastoma cells and primary cortical neurons expressing human amyloid precursor protein (APP) with 24 (S)-hydroxycholesterol (<10 μM) has also been reported to inhibit amyloid β (Aβ) production (Urano et al., 2013). Collectively, all these processes are under tight control, suggesting that this molecule is very essential and any disturbance in its homeostasis leads to brain dysfunction.

Several other epidemiological and biochemical studies have provided a strong link between cholesterol levels and Aβ production and deposition in brain (Wang et al., 2012; Yu and Zheng, 2012). Levels of plasma LDL have also been found to be higher in patients with Alzheimer's disease (AD) (Wolozin, 2004). Animal models fed on high cholesterol diet showed increased production and deposition of Aβ. Recently, the role of cholesterol involving lipid rafts in Aβ deposition, localization, and transport of APP has been elucidated (Hicks et al., 2012). This promotes interaction of APP and β-site APP cleaving enzyme (BACE-1) and therefore responsible for generation of Aβ peptide. Production of Aβ "seeds" in the initial stages of AD has been reported to be cholesterol dependent (Puglielli et al., 2003). Therefore, pharmacological removal of cholesterol from lipid rafts may be an attractive strategy to reduce risk associated with AD. Early nonrandomized clinical trials using cholesterol lowering agents, such as statins have indeed shown positive results as indicated by reduction in risk of AD (Shepardson et al., 2011a,b) (Fig. 3.14).

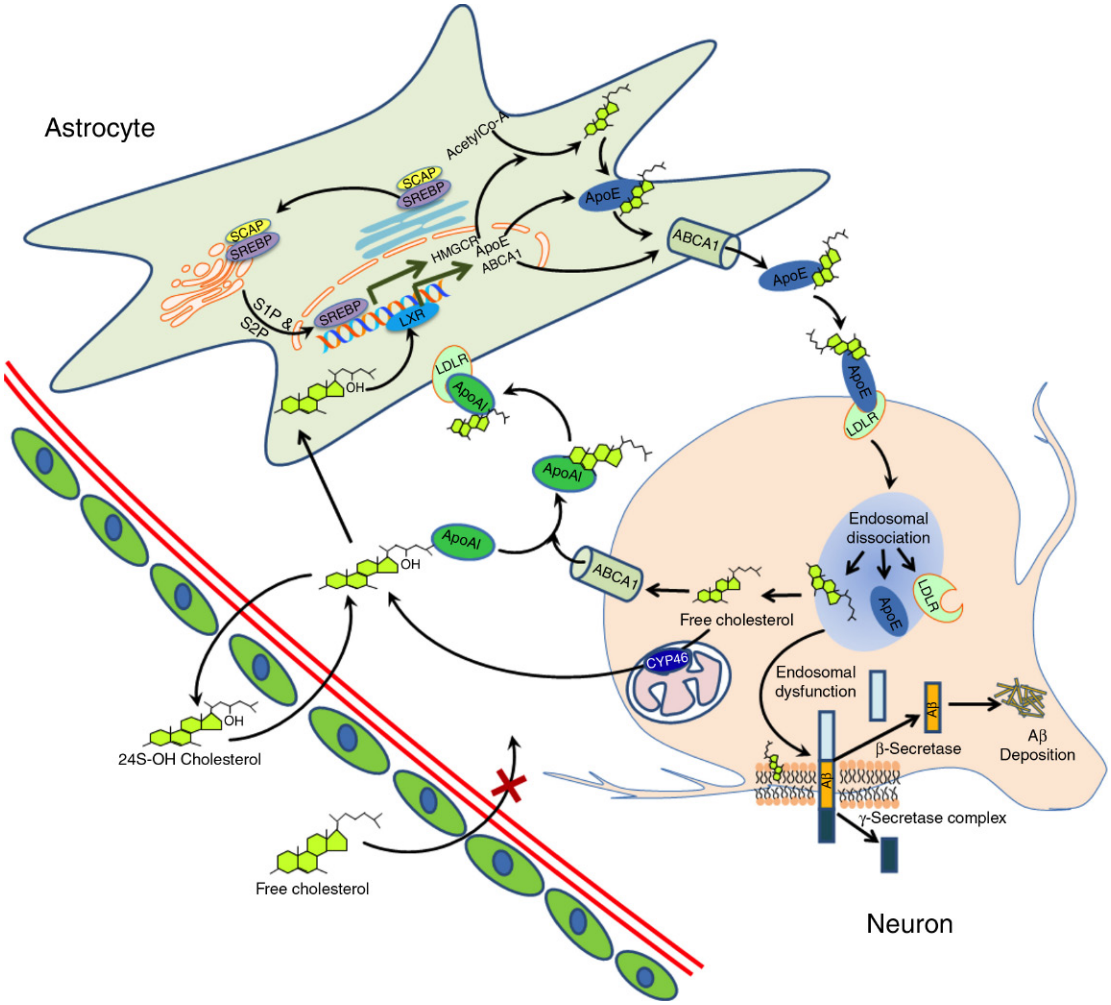


Figure 3.14: Lipid Homeostasis and Transcriptional Regulation in Brain.
Cholesterol scarcity induces the cleavage of SREBPs through S1P and S2P proteins in golgi complex of astrocytes. The cleaved SREBPs induce production of HMGCR which enhances the cholesterol synthesis. Cholesterol thus produced is transferred to neurons via ABCA1 and LDLR. The cholesterol and other bound proteins are released from endosome. The free cholesterol is converted into 24-S hydroxycholesterol, which can pass BBB and returned back to astrocytes. The endosomal dysfunction results in increased production and deposition of amyloid β proteins in neurons.

5.3.1.2 LC-PUFAs

The two major LC-PUFAs in the brain are AA and DHA representing $n-6$ and $n-3$ PUFAs, respectively. LC-PUFAs accounts for 35% of brain lipids suggesting their importance for normal development of brain. AA is a crucial component of brain where it plays important role in cell division and signaling. The rapid conversion of AA to adrenic acid, which is crucial

for neural tissue development, has been reported in infants (Hadley et al., 2016). Dietary AA has been shown to influence plasticity and preserve hippocampal membrane fluidity (Fukaya et al., 2007). In addition, AA mediated activation of peroxisomal proliferator—activated receptor-γ may also provide some protection against oxidative stress (Wang et al., 2006). DHA represents $n-3$ PUFAs in brain that accumulates at a high rate during the third trimester of neonatal development, a period of active neurogenesis, neuroblast migration, differentiation, synaptogenesis, and gray matter expansion (Dubois et al., 2014). Dramatic growth in the brain weight occurs in the first year after birth where breastfeeding is the sole source of DHA suggesting the unquestionable role of DHA in brain development. During early childhood development, the continuous DHA accrual in the frontal cortex implies its role in growth and expansion of frontal cortex gray matter, myelination, and neurocognitive processes, including attention and executive function (Dobbing and Sands, 1973). DHA accounts for approximate 15% of total cortical fatty acid composition in adolescence stage, coinciding with frontal cortex synaptic pruning, white matter expansion, and maturation (Carver et al., 2001). A close relationship between $n-3$ PUFAs/DHA and mental health disorder was implicated when it was observed that AD patients had lower serum DHA levels as compared to healthy individuals (Conquer et al., 2000; Cunnane et al., 2012). Dietary intake of DHA has been shown to improve Aβ clearance (Grimm et al., 2013), cognitive function, neuronal activity, immediate memory, and attention score (Weiser et al., 2016). However, clinical trials of longer duration and larger groups of subjects need to be conducted to get more insight into the therapeutic potential of LC-PUFAs in slowing down cognitive decline in AD patients.

5.3.1.3 Galactosylceramides/sulfatides

These are typical lipids found in myelin membrane that are stabilized by multiple interactions in all the directions and an indirect association of these lipids have been reported with myelin basic proteins (MBP) (Fitzner et al., 2006). The rate of deposition of these lipids increases with myelin synthesis highlighting their role in brain development (Schmitt et al., 2015). Major myelin abnormalities in paranodal junction and minor abnormalities, such as thinning of myelin sheath were observed on genetic manipulation of biosynthetic pathway of these lipids (Dupree et al., 1998; Popko, 2000; Salzer et al., 2008; Thaxton et al., 2011). These studies suggested that not only are these lipids essential for myelin biosynthesis but they also are required for stability and maintenance of myelin architecture during aging.

5.3.1.4 Ganglioside

Gangliosides is a large family of lipids with more than 100 different species of which GM1, GD1a, GD1b, and GT1b accounts for 96% of the gangliosides in the human brain (Schnaar et al., 2014). Besides their accumulation in the brain during development, their complexity also increases indicated by the degree of sialylation. The rate of deposition of ganglioside reaches at its peak during the later stages of fetal development and continues up to 2 years after birth, which coincides with the most active period of myelination in human development

(Ryan et al. 2013). To decipher the function of ganglioside, genes involved in biosynthesis of different gangliosides were deleted conditionally or in combinations that resulted in drastic neurodegenerative phenotype, vacuole formation in brain, and ultimately death in few months (Jennemann et al., 2005; Schnaar et al., 1998; Sheikh et al. 1999). Thus, gangliosides are crucial for axon-glial interactions and other maintenance.

5.3.1.5 Plasmalogens

Another distinguishing feature of brain lipid composition is the presence of high amounts of ethanolamine plasmalogens. Just like gangliosides, rodent models have been developed with deletion in genes involved in plasmalogens biosynthesis to get further insight into the specific function of each plasmalogen in brain function. Glycerophosphate-O-acyltransferase (GNPAT), and alkylglycerophosphate synthase (AGPS) are two specific enzymes, which initiate the plasmalogen biosynthesis in peroxisomes. Mutations that target AGPS resulted in depletion of plasmalogens (De Vet et al., 1998; Ofman et al., 1998). GNPAT knockout rodent model developed completely plasmalogens deficient myelin sheath (Rodemer et al., 2003). Another study showed that deletion in gene (pex 7) that encodes AGPS in nerve causes instability in the absence of plasmalogens (Brites et al., 2003). A double knockout of GNPAT and pex7 showed axonal loss and myelin loss in older age (Bottelbergs et al., 2012) suggesting the important role of plasmalogens in maintaining axonal-myelin unit and brain functioning.

5.3.1.6 Phosphoinositides

Phosphoinositides (PIs) are structurally related and functionally diverse phospholipids with many important roles in the nervous system (Boss and Im, 2012). Phosphorylation of parent phosphotylinositides leads to formation of seven different PIs, consisting of PI4P, PI(4,5)P2, PI(3,4,5)P3, PI(3,4)P2, PI(3,5)P2, PI3P, and PI5P (Pendaries et al., 2003). While considering the role of PIs in neurological disorders, it is important to note that each PIP performs a specific function in neuronal signaling and trafficking. PI(3,4,5)P3 is a small but crucial signaling molecule generated by the phosphorylation of PI(4,5)P in brain. Several neurological diseases including autism, epilepsy, and cancer arise from aberrant activation of PI3K/Akt/mTOR signaling due to deregulated activation of either PI3K or loss of phosphatase activity of PIPs (Chappell et al., 2011; Yap et al., 2008). The recruitment of receptor tyrosine kinases or GPCR at membrane activates PI3K, which relay the signal inside the cell through deployment of PDK1 and Akt. The involvement of this signaling pathway has been established in myelination, which is a key process to maintain neuronal transmission, survival, and brain recovery from trauma (Porta et al., 2014).

Gathering evidence implicate the involvement of PIs in development of Alzheimer's disease. A recent report claimed that PI is the only 1 out of 10 serum lipids that can accurately predict the memory loss with an approximate 90% success (Mapstone et al., 2014). However, it is not clear whether the raised PI serum levels were actually the result of either altered

PI metabolism or it is just due to the increased cell membrane breakage during isolation. The neurofibrillary tangles are enriched in PI(4,5)P2 and PIPs metabolizing enzymes, such as PTEN (Nishikawa et al., 2014; Sonoda et al., 2010). In Down syndrome, early onset of AD has been linked to reduced levels of membrane PI(4,5)P2 and concomitant reduction in Aβ clearance (Voronov et al., 2008). An increase in PI4P production by naturally occurring plant triterpene (ginsenoside/panaxoside) has been shown to fasten the Aβ clearance in murine model of Alzheimer's disease (Guo et al., 2014).

To summarize, recent advancement in developing knockout animal models and technologies in lipidomics have helped a lot in deciphering the specific role of different lipid species in different areas, structures, and cell types in the nervous system. In the future, by integrating lipidomics with other omics approaches, such as transcriptomics and proteomics, will give a more clear and comprehensive understanding of functions of complex lipids in myelin architecture, neuronal function and pathophysiology during different neurological diseases.

5.4 Role of Lipids in Cardiovascular Diseases

Cardiovascular diseases (CVD) term is given to a class of diseases that include myocardial infarction, coronary heart disease, cerebrovascular disease, and peripheral vascular disease (Mendis et al., 2011). CVD is one of the major causes of death worldwide and 23.3 million deaths per annum are estimated by 2030 (Mathers and Loncar, 2006). One of the characteristic features of CVD is atherosclerosis, a process where the arteries are gradually blocked by atheromas or plaques. Atheromas are composed of condensed deposit of cholesterol, cholesterol esters, triglycerides, and phospholipids at the endothelial base of artery (Sherer and Shoenfeld, 2006). CVD results in reduced or negligible supplies of blood and oxygen to the organ due clogged arteries. Arteries usually become rigid and narrower with age; however, this process can be accelerated by the factors, such as sedentary lifestyle, high cholesterol intake, obesity, diabetes, and high blood pressure. Another key event responsible for CVD is the rupturing of unstable plaque. It is speculated that unstable plaques generate an inflammatory response, which further causes weakening and rupturing of atherosclerotic plaque and ultimately blocks the artery (Kromhout et al., 2002).

5.4.1 Cholesterol

Despite the biochemical importance of cholesterol, its abnormal metabolism can lead to serious health problem, such as CVD (Lecerf and De Lorgeril, 2011). The cholesterol is metabolized mainly in the liver and transported to a different part of the body via five lipoproteins (chylomicrons, HDL, VLDL, LDL, and IDL) (Oram and Vaughan, 2006). Deposition of LDL-C has now replaced the total cholesterol as the principal cause of atherogenesis (Tomkin and Owens, 2012). The multifactorial nature of atherogenesis ranging from oxidative stress to inflammation, thrombosis, and lipid lowering renders it a tough target for drug development (Libby et al., 2011). So far, cholesterol lowering is the most

successful target and HMG-CoA reductase inhibitors (statins) are gold standard drugs to treat CVD. Statins have been reported to increase the LDL-Rs with a simultaneous blocking of cholesterol synthesis in liver (Reiner, 2013). HDL and LDL are now considered as indicators of CVD risk and a low HDL/LDL ratio has been linked to promote atherogenesis (Hao and Friedman, 2014). The ability of statins to lower the circulating LDL-C improves the HDL/LDL ratio, which may further contribute to antiatherogenic effect of statins (Millán et al., 2009). The cholesterol treatment trailists (CTT) has found that a reduction in LDL-C with statins resulted in significant decline in risk of major CVD events (Mihaylova et al., 2012). The double-blinded randomized control trial Scandinavian Simvastatin Survival Study (4S) using simvastatin resulted in a reduction in major coronary events by 44% and a decline in total mortality of 30% (Ray et al., 2014). Recently however, rosuvastatin has been found to be more effective and promising for reducing LDL-C levels (Gandhi et al., 2009). The daily recommendation of statins has been subsided now by rosuvastatin (10–20 mg/day) due to its longer half-life. Currently, rosuvastatin is attaining the NCEP ATP III LDL-C goals than other statins. Compared to simvastatin 20–40 mg/day, pitavastatin 2–4 mg/day has a similar efficacy for LDL-C reduction (Ose et al., 2009). Besides reducing the risk of CVD, statins have also been shown protective effects against certain cancers, such as colorectal, oesophageal, gastric, and prostate cancer (Reiner, 2013). Though the cost-effective therapeutic potential and tolerability of statins support their use as first line treatment for hypercholesterolemia, however, the adverse effects of statins as observed in several trails during the treatment of patients suffering from CVD cannot be overlooked. Some of the side effects of statin include early onset of diabetes, myopathy, and rhabdomyolysis (Kwak, 2014; Ose et al., 2009; Reiner, 2013). Alternative strategies other than statins have also been developed, such as inhibitors of dietary cholesterol absorption (stanols, sterols, and ezetimide) and proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) inhibitors (Giunzioni and Tavori, 2015; Gylling et al., 2014). The recent American College of Cardiology/American Heart Association and National Lipid Association guidelines recommend statins as the first choice to be used in addition to healthy life style for treating dyslipidemia (Mahmood et al., 2015) (Fig. 3.15).

5.4.2 LC-PUFAs

Targeting cholesterol lowering with statins would not be the only remedy for CVD, therefore, other strategies that lower the cholesterol levels without side effects will be more promising. The use of diet with low fat and low cholesterol has long been advised to keep the heart healthy. Accumulating evidence has suggested the use of dietary lipids specifically LC-PUFAs can actually be beneficial. Epidemiological studies have shown that certain populations (Inuit population of Greenland) have remarkably lower incidence of CVD (Bjerregaard et al., 2003). A further investigation revealed that higher fish intake in diet is common among these populations. Thereafter, substantial evidence has supported the benefits of including fish especially oily varieties, such as salmon, trout, and sardines, in the diet to

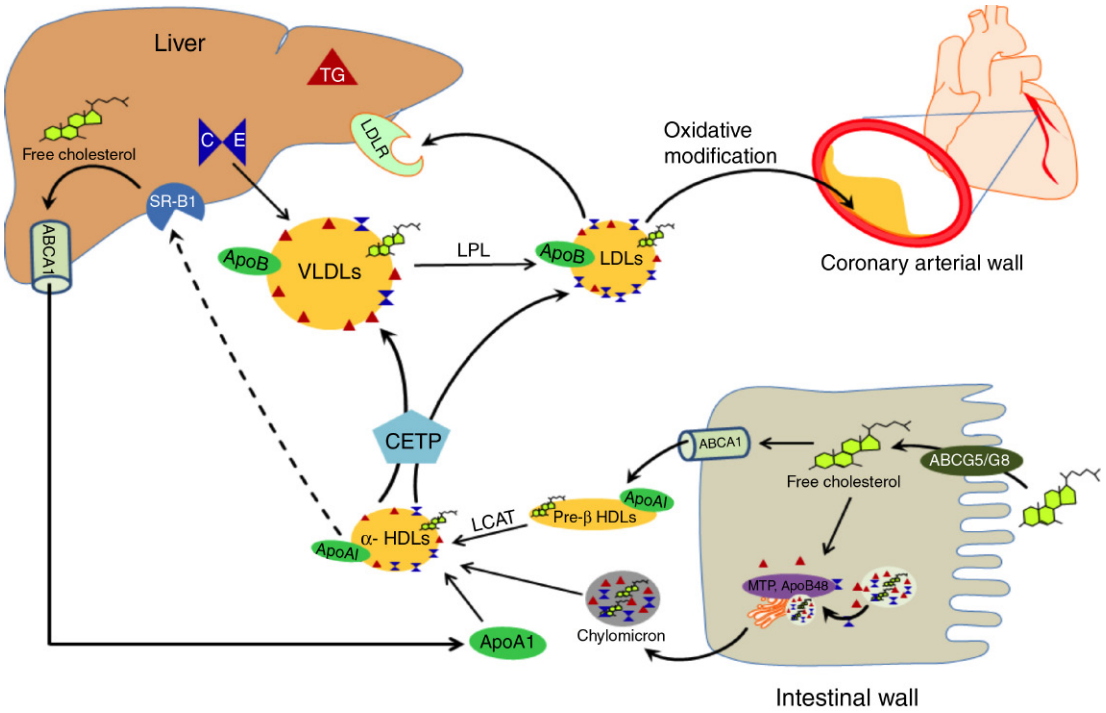


Figure 3.15: Cholesterol Absorption, Transportation and LDL Deposition During Atherogenesis. Cholesterol is absorbed from intestinal lumen through ABCG5/G8 transporters. The association of cholesterol, triglycerides, and cholesterol esters form chylomicrons. ApoAI bound discoidal pre-HDL is converted to spherical α-HDL through LCAT. The α-HDL is converted to VLDL and LDL through CETP. The ApoB helps in transport of VLDL and LDL in blood stream. The LPL converts VLDL to LDL, which gets deposited into the arterial wall and form atheroma. The LDL is absorbed in liver through LDL-R present on liver. The α-HDL can also be absorbed to liver by SR-B1 and free cholesterol in liver is excreted through ABCA1 back to ApoI poor pool.

reduce the risk of CVD. The largest metaanalysis with more than 35,000 participants showed a reciprocal relationship between fish consumption and mortality from CVD (Chowdhury et al., 2012). Consumption of one fish per week was shown to reduce the fatal CVD risk by 16% (95% CI 0.75–0.95), while 2–4 servings per week reduced the risk up to 21% (95% CI 0.67–0.92) (He et al., 2004). A higher fish consumption (>5 servings per week) did not show any statistically significant protective effect (95% CI 0.68–1.01), but this could be due to the fewer number of studies in this regard. Another study involving 1373 participants, long-term fatty fish consumption reduced the risk of sudden cardiac death up to 54% (95% CI 0.27–0.78) independent of age (Oomen et al., 2001).

The beneficial effects of fish consumption are attributed to the presence of high quantities of PUFAs, particularly LC-PUFAs (Sahena et al., 2009; Weichselbaum et al., 2013). A high serum level of $n-3$ PUFAs in native Japanese population was stated to be associated with

low atherosclerotic plaque burden (Sekikawa et al., 2008). $n-3$ PUFAs act as precursors of certain eicosanoids (resolvins, protectins, and maresins), which are antiinflammatory in nature (Tousoulis et al., 2014). In a randomized controlled trial using $n-3$ PUFAs (4 g/day EPA and DHA) supplementation for 6 weeks lowered the VLDL-C, and TAG levels by 20% and 18%, respectively (Woodman et al., 2003). Diet containing an extra 10% $n-6$ PUFAs has been shown to induce adverse effects and promote CVD progression, probably due to their proinflammatory nature (Hammad et al., 2016). Higher levels of IL-6, C-reactive protein, TNF-α were also designated to higher intake of $n-6$ PUFAs. Supplementation of $n-3$ PUFAs to these patients surprisingly elevated the levels of these inflammation mediators, suggesting the importance of $n-3/n-6$ ratio of LC-PUFAs on CVD (Li et al., 2014). It has been shown that a higher ratio of $n-6/n-3$ PUFAs is related to pathogenesis of CVD whereas ratio close to 1 has protective effects (Wijendran and Hayes, 2004). Lower ratios of $n-6/n-3$ PUFAs have been reported to suppress the platelet aggregation, mediators of inflammation and vascular endothelial growth factor expression (Simopoulos, 2008). Conversely, recent clinical trial using $n-3$ PUFAs in cardiac patients failed to show any protective effect and questioned the dietary supplementation of $n-3$ PUFAs (Kimmig and Karalis, 2013). Although it was later pointed out that the dose (1.5 g/day) taken in the trial was not sufficient to elicit protective effects. Despite some negative outcomes, majority of evidence still supports the beneficial effects of LC-PUFAs on cardiovascular related morbidity and mortality risk in low doses. Dietary guidelines for Americans recommend a daily intake of 5%–10% of energy from $n-6$ and 0.6%–1.2% of energy from $n-3$ PUFAs till the time the discrepancy is resolved (Kris-Etherton and Fleming, 2015).

5.4.3 PAF and polar lipids

The accumulating evidence suggests that the lipid fraction of food has a clear role in development of atherosclerosis (Scherr and Ribeiro, 2011). Platelet activating factor (PAF) belongs to a family of lipids composed of fragmented phosphotidylcholines, which mimics cognitive PAF and work as ligand for PAF receptor. These PAF-like lipids regulate the inflammation, thrombosis, and oxidation (Demopoulos et al., 2003). PAF is an important mediator of inflammation and synthesized in various cell types on stimulation, for example, endothelial cells, monocytes, platelets, and foam cells. The lipid component of food that acts as antagonist of PAF would be able to inhibit the process of atherogenesis. Recently, polar lipids from gilthead-sea bream (Sparusaurata) downregulated PAF biosynthesis in a rabbit model of atherosclerosis (Nasopoulou et al., 2011). Other studies have also reported polar lipids as principal component of Mediterranean diet that exhibit antiinflammatory activity (Widmer et al., 2015). The polar lipids inhibit the inflammation without changing the levels of LDL-C in blood, thereby, challenging the generalized statement "rise in circulating cholesterol level are the primary event in atherosclerosis." In a mechanistic study, the effect of fish polar lipids was investigated in hypercholesterolaemic rabbit. The rabbit fed with polar lipids of gilthead sea bream (0.06% w/w) developed less atherosclerotic

lesions (76% $P < 0.05$) in arteries as compared to animals fed on atherogenic diet (1% cholesterol) (Nasopoulou et al., 2011). Furthermore, decreased activity of both PAF-cholinephosphotransferase (CPT) and lyso PAF-acetyltransferase (AT) were noticed in leukocytes and platelets of animals fed on polar lipids (Kolovou et al., 2012). Collectively, suggesting that fish polar lipid supplementation to rabbit inhibit PAF biosynthesis which plays crucial role in developing atherosclerosis in these animals.

6 Lipids as Nutraceuticals

Nutraceuticals are defined as health food products or parts of foods that have been clinically proven to possess health benefits, including prevention and treatment of disease. These are delivered in the form of dietary supplements, functional foods, or functional food ingredients. As discussed earlier, lipids play an important role in pathology and prevention of numerous diseases like cardiovascular, neurological diseases, obesity, and metabolic syndrome, such as obesity, thus lipids have emerged as mainstream nutraceutical products. Lipids as nutraceuticals are safe and have potential therapeutic and health benefits, which may influence the outcome of many debilitating diseases.

6.1 n−3 PUFAs

The potential role of $n-3$ PUFAs in prevention and treatment of a number of diseases has made $n-3$ PUFAs a very attractive nutraceutical option. $n-3$ PUFAs comprise of alpha-linolenic acid obtained mainly from plants; and EPA and DHA from marine sources. Oils from plant origin, such as flaxseed, walnut, perilla, and canola are rich sources of α-linolenic acid. In addition, seaweeds and marine fish, particularly fatty fish, such as herring, salmon, mackerel, tuna, are rich source of EPA and DHA. α-Linolenic acid is converted to EPA and DHA but studies have found that bioconversion of α-linolenic acid to EPA and DHA in adult men is limited to approximately 8% for EPA and extremely low <0.1% for DHA. Therefore, it is mandatory to supplement these fatty acids in diets to meet the required levels of EPA and DHA. Earlier the recommended daily allowance for EPA and DHA was limited approximately to 0.2% of energy or 1–2 g/day (Garcia, 1998). Later in 2012, World Health Organization recommended a total $n-3$ PUFAs intake of 0.5%–2% of energy. For adults 0.250 g/day of EPA plus DHA is recommended. For pregnant and lactating females, the minimum intake for optimal foetal and infant development is 0.3 g/day EPA+ DHA, of which at least 0.2 g/day should be DHA (FAO, 2008). Dietary sources of ALA include flaxseed, walnut, soybean, pumpkin seeds, and rapeseed (canola) oil. The intake of various oils needed to meet current recommendations of ALA for women and men varies from 2.2–3.2 mL/day of flaxseed oil to 158–229 mL/day of olive oil. Another source of $n-3$ PUFAs are the seaweeds, such as kelp, laver, and wakame that range in EPA content from 0.004 to 0.186 g/100 g. DHA-rich microalgae (10%–40% DHA by dry weight) are also a direct source of DHA and

are recommended to be taken at 100–300 mg/day (Davis and Kris-Etherton, 2003). Marine fish oil is another major source of $n-3$ PUFAs and its different fish-derived oils vary widely in amounts of EPA and DHA. The most common fish oil capsules currently available in market provide 180 mg EPA and 120 mg DHA per capsule (Kris-Etherton et al., 2002). In addition to these sources, a variety of food products are now being produced by fortifying them with $n-3$ PUFAs. The US Food and Drug Administration (FDA) allowed a number of fish and algal oils to be generally recognized as safe for food fortification. Various functional foods being fortified with $n-3$ PUFAs include milk-based products, eggs, juices, table spreads, salad dressings, sauces, breakfast cereals, baked goods, sauces, infant formulas, baby foods, and juices (Howe et al., 2002; Lewis et al., 2000; Mantzioris et al., 2000). The $n-3$ fatty acid content of animal feed is increased with the addition of fishmeal, flax, and $n-3$ PUFAs and new $n-3$ PUFAs enriched products are produced including bakery products, eggs, mayonnaise, margarines, pasta, milk, meat, and poultry (Gebauer et al., 2006; Simopoulos, 1999). $n-3$ PUFAs are now also available as approved prescription drugs and are known as RxOM3FAs. MaxEpa, Vascepa, Lovaza, and omtryg are a few main $n-3$ PUFA capsules available commercially (Ito, 2015).

6.2 MUFAs

Oleic acid and palmitoleic acid are the most common MUFAs. MUFA-enriched foods have been highly recommended for reducing risk of cardiovascular diseases, managing body weight, and producing other health benefits. Dietary MUFA intake has been suggested to bring about 20% reductions in the risk of CVD events (Estruch et al., 2013; Gillingham et al., 2011). The American Heart Association has recently recommended consumption of almost 25% of energy from MUFAs. Oleic acid (C18:1$n-9$) is the predominant dietary MUFA, accounting for up to 92% of dietary MUFAs (Poudyal and Brown, 2015). Plant-based oils, including avocado, canola, olive, peanut, safflower, and sunflower oils are rich sources of MUFAs. The Mediterranean diet is also rich in MUFAs and its 16%–29% of energy is provided as MUFAs. Various epidemiological studies and randomized clinical trials extensively reported the cardioprotective role of diet rich in MUFAs. The effects of MUFAs are attributed to its ability to lower the plasma cholesterol and LDL-C level,s as well as elevating the HDL-C: total cholesterol ratio (Venturini et al., 2015). An increasing body of evidence has indicated an increment in fat oxidation rate, diet-induced thermogenesis, and energy expenditure with higher dietary intake of olive oil (Krishnan and Cooper, 2014). High olive oil diet has been reported to increase tumor latency, decrease tumor incidence, and reduced the tumor content and volume. The study of tumor regression for high olive oil diet showed a protective effect and slow progression of tumor (Escrich et al., 2007). Apart from olive oil, there are other readily available sources of MUFAs, such as canola oil, nuts including cashews, peanuts, almonds, and commercially available products, such as peanut oil, peanut butter, virgin olive oil. Peanut oil and peanut butter are convenient

Table 3.4: List of natural sources of MUFAs and PUFAs.

	Food Source	MUFA (% Cal)	PUFA (% Cal)
1.	Sunflower oil (high-oleic; at least 70%)	86	4
2.	Hazelnut oil	82	11
3.	Safflower oil (oleic at least 70%)	78	15
4.	Olive oil	77	9
5.	Avocado oil	74	14
6.	Almond oil	73	18
7.	Canola oil	62	31
8.	Peanut oil	49	34
9.	Sunflower oil (linolenic less than 60%)	47	42
10.	Sesame oil	42	43
11.	Corn oil	25	61
12.	Soybean oil	24	61
13.	Walnut oil	24	66
14.	Flaxseed oil	21	71
15.	Sunflower oil (linolenic at least 60%)	20	69
16.	Grapeseed oil	17	73
17.	Safflower oil (linolenic at least 70%)	15	78

and inexpensive energy sources, which provide fiber, niacin, magnesium, B-complex vitamins, as well as copper and have been found to reduce cholesterol (Kris-Etherton, 1999; Robertson, 2006) (Table 3.4).

6.3 Phytosterols

Plant sterols differ from cholesterol in the structure of their side chain while plant stanols are 5-α derivative of plant sterols (Gupta and Prakash, 2014). The primary sources of phytosterols are vegetable oils, cereals, nuts, fruits, and seeds (Table 3.5) that contribute 50%–80% of daily plant sterol intake (Gylling et al., 2014). The average daily intake of phytosterols and plant stanols is 300 mg and 17–24 mg respectively (Phillips et al., 1999). Sitosterols and compesterols account for 60% and 20% of the total phytosterols intake respectively (Klingberg et al., 2008).

The intestinal absorption efficiency of phytosterols (≤2%) and phytostanols (≤0.2%) is very low compared to cholesterol (≈50%). The transport of phytosterols and phytostanols in blood is similar to cholesterol, that is, through LDL (70%–80%) and HDL (20%–30%) (Björkhem et al., 2001). Even after very low intestinal absorption, phytosterols, and phytostanols are able to inhibit cholesterol metabolism (Cohn et al., 2010). Dietary intake of phytosterols and phytostanols 2 g/day has been reported to lower the LDL concentration by 8%–10% (Gylling et al., 2014) irrespective of age, gender, or cause of hypercholesterolemia. Several mechanisms have been proposed for inhibitory action of phytosterols and phytostanols on cholesterol absorption including displacement of

Table 3.5: Plant sterol and plant stanol contents in different foods.

Food Item	Phytosterols	Phytostanols
Vegetable oils		
Corn oil	686–952	23–33
Rapeseed oil (canola oil)	250–767	2–12
Soybean oil	221–328	7
Sunflower oil	263–376	4
Olive oil	144–193	0.3–4
Palm oil	60–78	Trace
Cereals		
Corn	66–178	—
Rye	71–113	12–22
Wheat	45–83	17
Barley	80	2
Millet	77	—
Rice	72	3
Oats	35–61	1
Nuts		
Peanuts	320	—
Almond	143	—
Vegetables		
Broccoli	39	2
Cauliflower	18–40	Trace
Carrot	12–16	Trace
Lettuce	9–17	0.5
Potato	7	0.6
Tomato	7	1
Fruits and berries		
Avocado	75	0.5
Passion fruit	44	Not detected
Raspberry	27	0.2
Orange	24	Not detected
Apple	12–18	0.8
Banana	12–18	Not detected

Data are given as mg/100 g (dry weight, either range or mean value).

cholesterol from mixed micelles (the micellar theory), modification of expression of genes encoding sterol transporter proteins Niemann–Pick C1-Like 1 (NPC1L1) or ATP binding cassette (ABCG5 and ABCG8) transporters, promotion of cholesterol efflux from enterocytes back into the intestinal lumen, inhibition of cholesterol reesterification rate in the enterocyte and increased cholesterol removal from the body via the transintestinal cholesterol efflux (TICE) pathway (Gylling and Simonen, 2015). Out of these, only the micellar theory has been accepted (Nissinen et al., 2002) while the intake of phytosterols and phytostanols was found unable to activate the genes involved in sterol metabolism either in animal or human studies (De Smet et al., 2015) and effect on TICE pathway

needs to be experimentally proven in human subjects. The phytosterols and phytostanols are expected to increase the removal of ApoB-100 containing lipoproteins; however, only reduced transport rate of LDL-ApoB-100 has been documented in few kinetics studies (Ooi et al., 2007). The dietary intake of phytosterols and phytostanols has also been shown to reduce serum triglyceride levels without effecting HDL-C, lipoprotein (a) and PCSK9 concentration (Verges et al., 2011).

Bioactive foods and neutraceuticals, which are enriched in phytosterols and phytostanols (Table 3.6) have emerged as potential supplements to protect against cancer, obesity, cardiovascular, and neurological diseases (Das et al., 2012). These sterols can be further hydrogenated/esterified to produce stanols/ester products that have comparable physical and chemical properties of edible fat and oil, enabling them to be added in various processed foods. The Unilever phytosterol enriched "spread" was first launched in 1999 in the USA. Phytostanol esters are marketed under the Benecol brand. The first phytostanol ester enriched product, "a spread," was launched in Finland in late 1995. Since then, phytostanol ester enriched margarines with fat contents from 60% to 65% have been launched in countries in the European Union and in the USA. The first cereal-based Benecol food was dry pasta launched in Finland in the beginning of 2003 and later instant oatmeal was marketed (Weber and Mukherjee, 2006).

Nestlé's JianXin Double Care, a specially designed milk to enhance heart and bone health for middle-aged and senior adults, was launched in China in December 2013 (Nestlé, 2014). The product contains phytosterols, which lower LDL-C, as well as calcium and vitamin D. November 2013, the *New England Journal of Medicine* released a landmark Harvard study with more than 118,000 healthy men and women volunteers reported that nut eaters who regularly consumed a 1-ounce daily serving of walnuts, almonds, cashews, or other nuts had a 20% lower risk of dying of any cause during the 3-decade-long study, compared to those who did not consume nuts (Bao et al., 2013).

Table 3.6: List of commercial food products enriched with phytosterols, phytostanols, and their fatty acid esters.

Sr. No.	Product	Manufacturer	Constituents	Use
1.	Benecol	Raissio/McNeil	Phytostanyl esters	Margarine (United States), spreads, milk, yogurt, mayonnaise, snack bars (Europe)
2.	Take Control	Lipton/Unilever	Phytosteryl esters	Margarine, salad dressings (United States)
3.	Flora Proactive, Becel Proactiv	Unilever	Phytosteryl esters	Margarine, salad dressings (Europe, Canada)
4.	Logicol	Meadow Lea	Phytosterols	Spreads, milk (Australia)
5.	CookSmart	Procter & Gamble	Phytosteryl esters	Cooking oil (United States)
6.	Danacol	Danone	Phytosteryl esters	Yogurts

6.4 Isoprenoids

Isoprenoids are a class of organic compounds playing a wide variety of roles in physiological processes of plants and animals. They are available from both plants and animal sources. The common plant species containing isoprenoids are menthol, citral, camphor, and limonene while the animal sources of isoprenoids include fish liver oils, wool wax, yellow pigments in egg yolk and butterfat. Carotenoids are another important isoprenoids that function as antioxidants and as precursors of vitamin A, which is essential for vision and neural development in animals. Isoprenoids are composed of terpene subunits and have varying degree of structural complexity such as mono-, di-, tri-, and sesquiterpenes. They constitute the largest group of natural products and have been reported to decrease the risk of cardiovascular diseases and carcinogenesis by blocking the mevalonate pathway (Elson et al., 1989). Assorted isoprenoids, such as monoterpenes and sesquiterpenes posttranscriptionally downregulate the expression of HMG-CR enzyme that catalyzes the synthesis of mevalonate and thus help to treat hypercholesterolemia and other associated diseases. In addition to blocking activities, isoprenoids also have efficacy in suppressing the growth of a broad range of tumors. Some dietary isoprenoids having anticancer effects include limonene, geraniol, perillyl alcohol, and farnesol. Burke et al. (1997) reported significant (60%–90%) inhibition of the growth of human MIA PaCa2 pancreatic tumor cells with 25 μM farnesol, 25 μM geranylgeraniol, 100 μM perillyl amine, 100 μM geraniol, or 300 μM perillyl alcohol. Further testing in hamster pancreatic adenocarcinoma revealed that farnesol and geraniol at 20 g/kg diet completely inhibited the tumor growth and were more potent than perillyl alcohol, which inhibited the tumor growth by 50% at 40 g/kg diet. The beneficial roles of isoprenoids in several metabolic processes suggest their use as nutraceuticals. However, the sources of isoprenoids are not yet commercially available.

6.5 Conjugated Linolenic Acid

Conjugated linoleic acids (CLAs) are the isomers of linolenic acid and are found mostly in meat and dairy products. The other common dietary sources of CLA include beef, mutton, eggs, mushrooms, and vegetable oils including hydrogenated soybean oil. The scientific interest in CLAs began in late 1980s due to its anticancer benefits. Studies revealed that CLAs act as a cancer preventive agent by competing with LA in the biosynthesis of AA and eicosanoids that have important role in several events of tumorigenesis, including inflammation and cell proliferation (Ha et al., 1990). Recent findings showed that CLA enhance the levels of inflammation lowering protein and inhibit angiogenesis and metastasis in tumor cells (Mohammadzadeh et al., 2013). In addition to their anticancer role, CLA have also been demonstrated to reduce body fat accumulation (Hargrave et al., 2002). Investigations showed that in human subjects, supplements containing isomers of CLA reduced body fat in men and women (Watkins and Li, 2006).

Table 3.7: Health benefits of conjugated linolenic acid from human clinical trials (Watkins and Li, 2006).

Effect	CLA dose[2] (g/day)	Duration	Subjects
Reduced body fat but not body weight	1.7–4.2	4 weeks–6 months	Healthy or overweight/ obese subjects
Lowered leptin, no effect on fat mass	3	94 days (64 days CLA treatment)	Healthy women subjects
Reduced blood triacylglycerols and VLDL/HDL cholesterol subjects	0.7–3.0	8 weeks	Healthy or normolipidaemic
The *t*10, *c*12 enhanced response to hepatitis B vaccination	1.7 or 1.6	12 weeks	Healthy Caucasian males

Table 3.8: Food content of conjugated linolenic acid in commercial and natural food products (Watkins and Li, 2006).

Products	CLA Content (mg/g fat)[2]
Natural cheeses	
Blue	0.55–7.96
Cheddar	1.36–5.86
Cottage	4.5–5.9
Cougar Gold	3.20–5.17
Monterey Jack	4.80
Mozzarella	4.31–4.96
Swiss	5.45–14.2
Processed cheeses	
Processed	1.81–6.2
Cheese whiz	4.9–8.81
Kraft American singles	3.19
Butter and milk	
Cow milk	0.7–10.1
Butter	4.7–8.11
Fermented dairy products	
Buttermilk	4.66–5.4
Sour cream	4.14–7.49
Yogurt	1.7–9.01
Ice cream	
Ice cream	3.6–4.95
Beef	
Beef products, uncooked	1.2–8.5
Cooked beef	3.3–9.9

Various beneficial effects of CLA on health conditions of humans are summarized in Table 3.7.

Due to its supposed health benefits, the marketing of commercial CLA products has been largely directed toward several health problems. A summary of CLA sources in commonly consumed food products is presented in Table 3.8.

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Further Reading

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CHAPTER 4

Plant Nutrition and Agronomic Management to Obtain Crops With Better Nutritional and Nutraceutical Quality

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1 Introduction

Changes in lifestyles, the magnitude of the human population and life expectancy are imposing challenges that have not been seen before in the production systems and food processing. These challenges, which require the generation of food innovations to obtain a healthier and more diverse nutrition, should be accompanied by the design of resilient production systems with less environmental impact per unit of matter transformed (Godfray et al., 2010). In that sense, obtaining a high-quality nutraceutical food is not only a commitment to the food processing industry but also an opportunity to improve the processes of the primary agricultural industry. Proper agronomic management is necessary to promote, in the field and greenhouse, food production with high nutrient density and nutraceutical quality, with the maintenance of the necessary economic, social, and environmental profitability.

Because phytochemicals occur in significant numbers and diversity in plant foods, and given the enormous complexity of responses and interactions at the transcriptomic, proteomic, metabolomic, and physiological levels, it is difficult in the short term to create a situation in which all nutrient and nutraceuticals needs are covered by synthetic products or preparations. Instead, the answer may be found in the production of plant foods with a higher density and a better balance of mineral nutrients and phytochemicals. For that reason, it is necessary to study the impact of agricultural technology, such as plant nutrition, irrigation, the use of growth regulators, the management of electromagnetic radiation, genetic improvement, and the use of GMOs, as well as the effects of different types of soils or climates on the composition and concentration of phytochemicals in plant foods. This knowledge can be applied to make more efficient and effective use of resources with the goal of obtaining high-quality food.

Therapeutic Foods
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Agronomists must be committed to delivering nutritional quality in appropriate quantity, either directly to the consumer who eats fresh vegetables and fruits or indirectly to the food industry that transforms and improves the products. This approach includes a gradual decline in the use of toxic compounds and the increasing application of ecological knowledge and -omics sciences to optimize the balance between food production and the impact on nonrenewable resources, such as biodiversity, soil, water, and the atmosphere (Tscharntke et al., 2012). Ecological knowledge is applied to understand how the flows of information, energy, and matter determine the behavior of populations of organisms that are present in an agricultural production system. These populations of plants, arthropods and other invertebrates, fungi and bacteria that we usually consider as an agricultural "ecosystem" are always adapting to both the biotic and abiotic environments (Philip Robertson et al., 2014). Interactions at the ecological level could result in adjustments in gene expression that result in biochemical and physiological differences; these same differences can be capitalized upon to make improvements in the nutraceutical quality of plants under proper growth conditions and best agricultural practices, here is where the -omics sciences are involved: primarily but not only with transcriptomics, metabolomics, and ionomics providing the tools to understand and modify the behavior of plants, both in the field and in greenhouses, laboratories and plant factories.

We must make a great effort within many disciplines to contribute to the conception, design, research, and transfer of new agricultural technologies and processes to achieve the aforementioned compromises (Hefferon, 2015; Newell-McGloughlin, 2008). There is also a need for an increased communication between the different sectors, namely the primary agricultural production area, the secondary industrial transformation field, the academic sector for knowledge and innovation generation, environmental management, government, and health, so that the various efforts do not occur in an unconnected way (Godfray et al., 2010), and so that the value chain does not present significant bias toward either end. This chapter includes information which was collected and organized about how the manipulation and control of environmental variables, plant nutrition and the use of signaling compounds allow the production of plant foods of higher nutraceutical quality.

2 The Nutritional and Nutraceutical Quality of Crops

The term "nutritional quality" refers to the components that are traditionally studied in plant foods, including carbohydrates, fats, proteins, vitamins, and minerals, such as Ca, Mg, Fe, and Zn. These compounds are synthesized and metabolized in what is known as the "primary metabolism" from which energy is obtained to generate structures and reserves for plant growth. From an agricultural practice standpoint, this primary metabolism is promoted to obtain high yields. However, in recent decades, this nutritional quality was improved for complementation by a series of mineral compounds and phytochemicals that are involved in what is called the "secondary metabolism," which allows the adaptation of plants to multiple environmental challenges through ultratrace and trace elements, phenolic compounds, terpenoids, glycosides, alkaloids, and various antioxidants (Jain and Ramawat, 2013). This set of phytochemicals

does not contribute directly to biomass or structures; thus it is not included in the yield, but it determines a high-value characteristic, namely the nutraceutical quality of harvested crops.

A nutraceutical is defined as any substance considered as food or a portion thereof that provides medical or health benefits and is useful for the prevention and treatment of diseases. A nutraceutical can be a food extract (such as extracts of garlic or *Ginkgo biloba*) with multifactorial benefits, or food itself when considering the content of individual active compounds (such as tomatoes for their lycopene content) (Ferrari, 2004). This category also includes vitamins, such as folic acid and minerals, such as selenium and iodine; thus, biofortified crops that contain these elements can be characterized as nutraceutical foods (White and Broadley, 2009).

Nutraceuticals are important in human nutrition because the same phytochemicals, vitamins, antioxidants, and trace minerals that plants use to adapt to the environment and to protect against stress can fulfill the essential functions of defense and stabilization of cellular components in humans (Fig. 4.1). The intake of foods with high levels of antioxidants and phytochemical compounds is related to higher levels of these compounds in the human body. Similarly, there are many reports about the different beneficial effects of various plant nutraceuticals on the antioxidant capacity of organs and tissues in the human body, disease

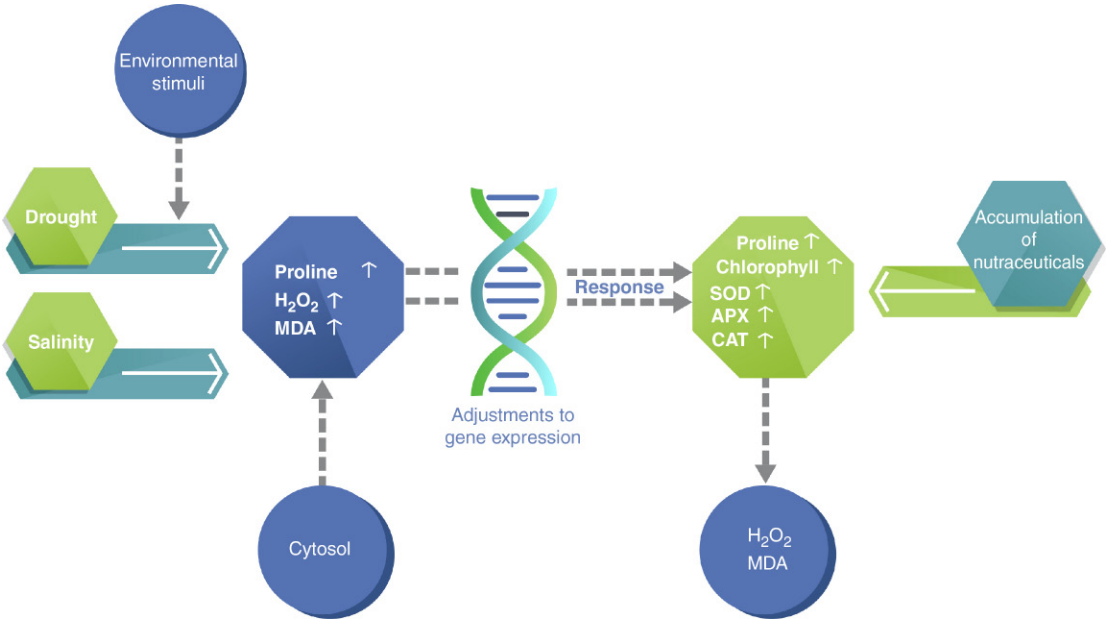


Figure 4.1: Nutraceutical Foods as a Result of Plant Adaptation to Environmental Stress. The changes in gene expression promote the secondary metabolism responsible for the accumulation of protective molecules that acts as antioxidants and stabilizers of membranes, proteins, DNA, and RNA. Nutraceuticals are important in human nutrition because the same phytochemicals, vitamins, antioxidants and trace minerals that plants use to adapt to the environment and to protect against stress can fulfill the essential functions of defense and stabilization of cellular components in humans.

resistance, and prevention of vascular accidents, as well as some degenerative diseases (Ferrari, 2004; Jain and Ramawat, 2013).

The intake of 400 to 600 g/day^{-1} of fruits and vegetables (Heber and Bowerman, 2001), or the equivalent of 9 servings per day of varied fruits and vegetables (Liu, 2013), is associated with a reduction in the incidence of many common forms of cancer and degenerative diseases. The compounds that are responsible for this beneficial effect are phytochemicals, which modulate the gene expression and stability of the membranes, proteins, and DNA–RNA in plants; they have these effects in humans when ingested as food phytochemicals. These compounds in nutraceutical foods inhibit carcinogenesis and degenerative diseases that are associated with the instability and oxidation of biomolecules (Heber and Bowerman, 2001; Liu, 2004).

Some mechanisms of action of antioxidants and phytochemicals that impart nutraceutical quality in plant foods are described (Ferrari, 2004) as:

1. Stabilizers of mitochondrial membranes and mitochondrial function promoters, that is, agents that decrease cell death by apoptosis (programmed cell death) or necrosis.
2. Chelating agents that decrease the oxidative damage caused by free metals in ionic form, which are promoters of Fenton reactions.
3. Antioxidants that reduce cellular damage, stimulate intrinsic antioxidant activity, protect DNA from oxidation, and inhibit cell death.
4. Inducers of apoptosis in preneoplastic and neoplastic cells.

Additionally to the four previous points it is necessary to consider, as established by the "dietary xenomiR hypothesis" (Witwer, 2012), the possible impact of the intake of molecules, such as small microRNAs and other vehicles of epigenomic information found in all plant foods (Khraiwesh et al., 2012; Rock, 2013; Sunkar et al., 2012). It has been shown that these molecules, even when they are from other organisms, are capable of modifying the gene expression profile by changing the human epigenome (Celluzzi and Masotti, 2016). It was also determined that microRNAs are robust during storage, preparation, and passage through the digestive tract, which makes them potentially bioavailable (Philip et al., 2015). Although the potential benefits of microRNAs have not been shown, these molecules may also have nutraceutical effectiveness for preventing and managing cancer and other disorders.

A practical way to recognize the presence of phytochemicals is their color, although this approach has limitations in the case of important phytochemicals, such as polyphenols and vitamin C (Barnes et al., 2013). In general, red fruits and vegetables contain lycopene (the pigment precursor of carotenoid that is found in large quantities in tomatoes), which is associated with prostate health. Yellow and green-yellow produce, such as corn and various leafy vegetables, contain lutein, and zeaxanthin. These pigments are located in the retina and reduce the risk of macular degeneration. Red and purple fruits contain anthocyanins, which are powerful antioxidants found in red apples, grapes, strawberries, raspberries, cherries, and wine grapes. Plant foods with an orange color, including mangos, carrots, squash, and

peaches, contain β-carotene. The yellow-orange color found in oranges, tangerines, and lemons are associated with flavonoids. White foods, such as onions and garlic, contain sulfur compounds with high germicidal and antioxidant activities (Heber and Bowerman, 2001).

Vegetables from the cruciferous family, such as broccoli, Brussels sprouts, and kale contain glucosinolates; these compounds are converted into isothiocyanates by plant myrosinase and the gastrointestinal microflora. Glucosinolates and isothiocyanates block chemically induced carcinogenesis in animals. Isothiocyanates are also inducers of phase 2 proteins, a group of antioxidant proteins associated with reduced susceptibility to carcinogenesis in mammal models for studying cancer (Abdull Razis and Noor, 2013).

There is a great diversity of phytochemicals, and most of them are little studied. It is estimated that plants produce approximately 200,000 different compounds, with some 25,000 different compounds from cultivated plants (Newell-McGloughlin, 2008). Phytochemicals are classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. The carotenoid and phenolic compounds are the most studied of the phytochemicals. Carotenoids are pigments with antioxidant and provitamin roles. Carotenoids have a structure consisting of 40-carbon isoprene units. The carotenoids include compounds, such as lycopene, lutein, and β-carotene. Phenolics are compounds that possess one or more aromatic rings with one or more hydroxyl groups. The phenolics include compounds, such as phenolic acids (hydroxy-benzoic acids, such as salicylic acid and benzoic acid, and hydroxy-cinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoids), stilbenes, coumarins, and tannins (Liu, 2013).

It is a great challenge to develop crops that have a precise balance of phytochemicals and to produce foods that are tailored to each group of people who have a particular range of ages, activities, or metabolic characteristics. It is a difficult goal to reach because of the extraordinary number of interactions that occur in cells, organs, individuals, and ecosystems that give rise to the expression of a certain profile of phytochemicals and certain molar balances between them. However, this process is theoretically possible in greenhouses and food factories with the right mix of efforts from different -omics sciences, computer science, and systems biology.

In the following paragraphs, some phytochemical groups (Fig. 4.2) and their benefits are annotated (Ferrari, 2004):

1. Anthocyanins, catechins, cyanidins and flavonols: these compounds are found in grape peels and juice, berries, eggplants, and red wine. They have antioxidant activity, suppress lipid oxidation, and act as superoxide scavengers.
2. Cacao polyphenols in cacao, cacao butter, and chocolate: these compounds inhibit DNA oxidation and the binding of carcinogens to DNA.
3. Carotenoids, vitamins C and E, selenium, and zinc found in many plants and biofortified foods: these compounds promote immunological responses against cancer.

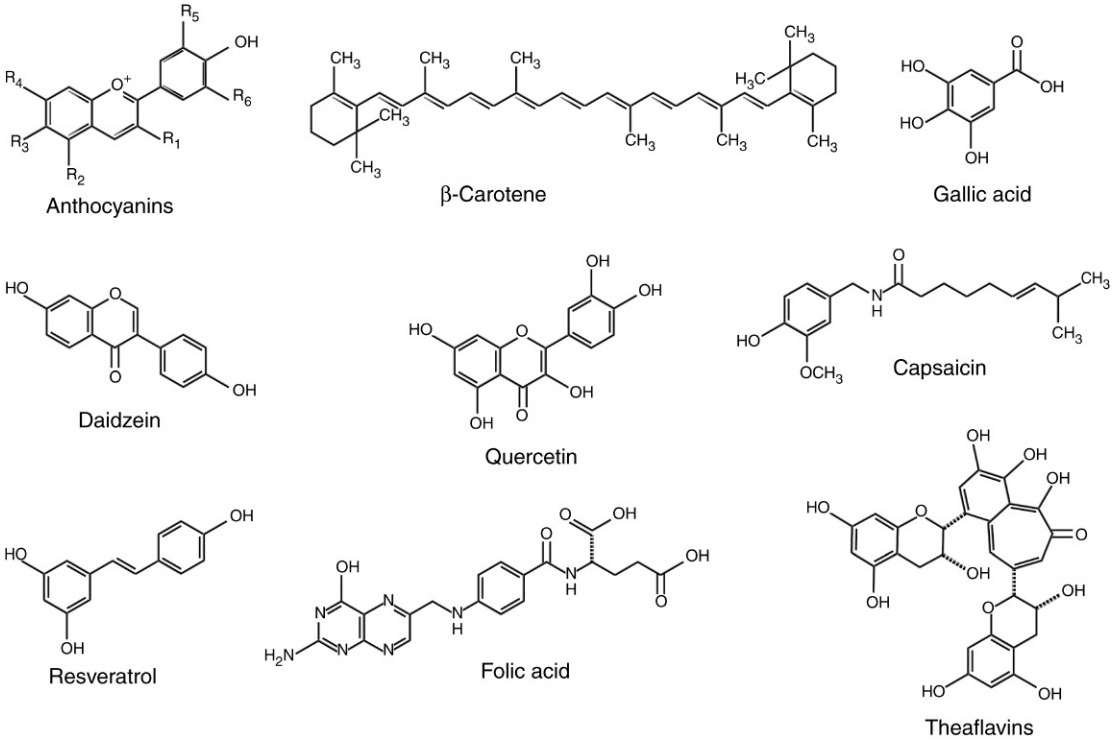


Figure 4.2: The Chemical Structure of Some Phytochemicals.

4. The capsaicin in peppers acts as a scavenger of superoxide anions.
5. Curcuminoids of *Curcuma longa*: these compounds increase DNA repair activity inhibit DNA oxidation and the binding of carcinogens to DNA. They increase the activity of antioxidant enzymes (GSH, GPx, and SOD) and decrease lipid oxidation.
6. Daidzein and genistein (isoflavones) of soybeans: these structures inhibit lipid peroxidation and stabilize DNA-protein links.
7. Fibers found in many plants, namely insoluble fiber, soluble fiber, β-glucan, fructans, and inulins: these fibers restrict caloric intake and absorption; decreasing the time it takes for food to transit the intestinal bulk.
8. The folic acid found in many plants increases DNA repair and DNA stability.
9. Gallic acid, sulfides and the thiols of garlic, onions, and broccoli: these compounds inhibit DNA oxidation and the binding of carcinogens to DNA. They also block carcinogenic cellular division.
10. Kaempferol, isorhamnetin, and quercetin of *G. biloba*: these structures are reactive oxygen species scavengers that remove nitric oxide. They promote the growth and stability of cells.

11. The isoprenoids, terpenoids and tocotrienols found in many plants: these compounds induce apoptosis and contribute to cancer control.
12. Lycopene and other carotenoids from vegetables (tomato, greens, citrus, and maize): these components quench singlet oxygen and other radicals, and they inhibit lipid oxidation.
13. Myricetin, quercetin, and rutin found in teas, grapes, and wines: these compounds inhibit DNA oxidation. They have antioxidant activity, suppress lipid oxidation, and act as superoxide scavengers.
14. Polyphenols found in many plants: phenolics, such as resveratrol, caffeic acid, and ferulic acid have antioxidant-like activities. They induce apoptosis and contribute to cancer control and may reduce the risk of degenerative diseases.
15. Sesaminol of rice and sesame seeds and oil: this compound inhibits lipid peroxidation.
16. Tannic acid found in many plants: this acid has metal-chelating activities and inhibits DNA mutagenesis.
17. Theaflavins of green and black teas: these compounds inhibit lipid peroxidation.
18. Tocopherol, ubiquinol, and tocotrienols found in rice, soy, and olive oils: these components inhibit the peroxidation of lipids.

3 Nutraceutical Value of Plant Crops and Human Health

Consuming a sufficient variety of fruits and vegetables has shown to be essential for a healthy diet. Epidemiological and clinical studies have associated diets that are high in fruits and vegetables with a lower risk of cardiovascular disease. They also offer some protection against metabolic and degenerative diseases, as well as certain forms of cancer, diabetes, and obesity (Campos-Vega and Loarca-Piña, 2010; Liu, 2013, 2004).

This protection is believed to be related to the contents of fiber, vitamins, minerals, and phytochemicals, such as polyphenols, flavonoids, sterols, carotenoids, chlorophyll, anthocyanins, which are responsible in part for the high antioxidant activity of fruits and vegetables (Fig. 4.3) (Andersen and Markham, 2006; Siriamornpun et al., 2012). In fact, it is known that these bioactive compounds can chelate metal ions (such as iron and copper), donating hydrogen atoms and eliminating the harmful free radicals associated with the induction of chronic diseases and thereby protecting human tissues and cells from oxidative damage (Balasundram et al., 2006; Edge et al., 1997; Heim et al., 2002; Johnson, 2002).

It is important to note that in an isolated manner, as in pills and vitamin supplements, phytochemicals do not show the same effectiveness that they do when they act synergistically with the complex of different phytochemicals found in plant foods (Liu, 2004). Even this synergism has been described in simple in vitro models for anthocyanins and polyphenols in beverages (Chung et al., 2016). The nutraceutical quality is

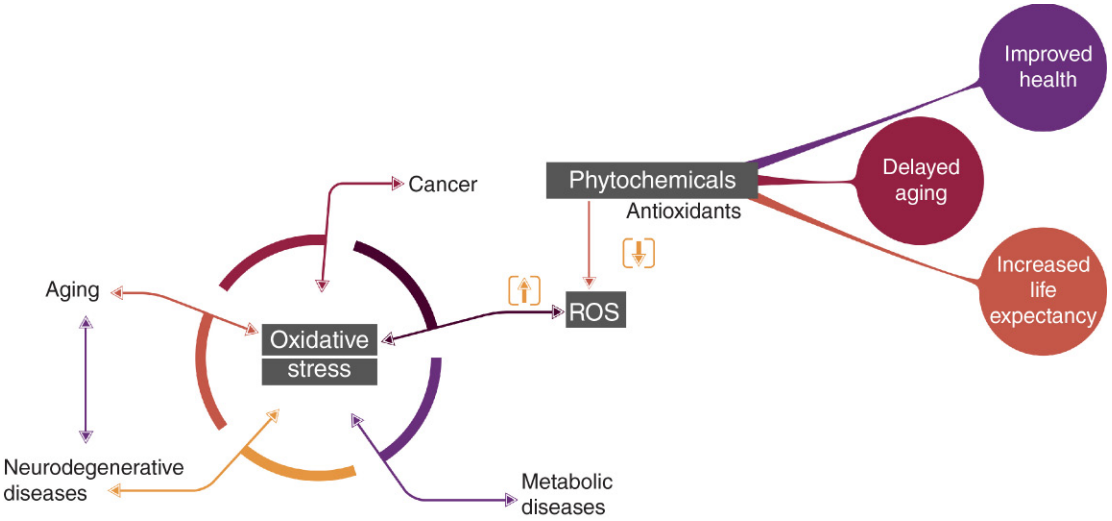


Figure 4.3: Protective effects of phytochemicals, such as polyphenols, flavonoids, sterols, carotenoids, chlorophyll, and anthocyanins, which are responsible in part for the high antioxidant activity of fruits and vegetables. It is known that these bioactive compounds can chelate metal ions (such as iron and copper), donating hydrogen atoms and eliminating the harmful free radicals (ROS) associated with the induction of chronic diseases by oxidative stress.

not defined with regard to one or a few compounds, but it is the result of all the interactions between the phytochemicals and recipient cells or target cells, in this case, the cells of the food consumer. Each food group has a distinctive profile of phytochemicals with respect to the molecular weight, solubility, polarity, bioavailability, and ease of excretion. This group of characteristics has a particular effect on the various organs, tissues, cell types, and organelles in the receiving subject. Thus far, it has not been possible to imitate this process by using dietary supplements. In some cases, it has even been found that adding these compounds in pure and isolated forms in supplements can lead to detrimental responses (Liu, 2013).

An excellent example of the earlier finding is described in a study by Milenkovic et al. (2011), who performed a human nutrigenomics study by examining the effects of consuming orange juice and hesperidin, a pure citric phytochemical that is likely linked to the beneficial effects of orange juice, on the expression of genes in leukocytes in healthy volunteers. Orange juice intake modulated changes in the expression of 3422 genes, hesperidin intake induced the expression of 1819 genes, and 1582 genes were induced in both groups. Many of the genes that were affected by the intake of orange juice and hesperidin are implicated in lipid transport, adhesion, chemotaxis, and cell infiltration, suggesting the lower recruitment and infiltration of circulating cells in the vascular wall and intima, a lower lipid accumulation, and the less frequent formation of atherosclerotic plaque.

3.1 Diseases Related to Oxidative Stress

The relatively recent growth in our knowledge of the role of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that is creating a new era of health and disease management (Aruoma, 2003). Most of the potentially harmful effects of oxygen are caused by the formation and activity of ROS. Among the most important free radicals containing oxygen are the hydroxyl radical ($^{\bullet}OH$), superoxide anion (O_2^-), singlet oxygen (O_2^1), hypochlorite (ClO_2^{-1}), nitric oxide radical ($NO^{\bullet}$), and the peroxynitrite radical ($ONOO^{\bullet}$), in addition to compounds, such as hydrogen peroxide (H_2O_2), which are not free radicals but do show a high oxidizing ability. These molecules are highly reactive, and they are capable of causing damage to biologically relevant molecules, such as DNA, proteins, carbohydrates, and lipids in the nucleus and the membranes of cells (Young and Woodside, 2001). However, ROS also plays a substantial role in signaling cell stress and the redox state, and thus their presence in cells at levels controlled by the antioxidant metabolism is considered necessary. The cellular signaling of the energy state is achieved in part through interactions between ROS and specific associated antioxidants. The differences in the composition and complementation of ROS-antioxidants are considered to be among the factors associated with the specificity of signaling between different organelles, cells, and organs (Noctor and Foyer, 2016). It is likely that these differences also partly explain the existence of a very broad range of antioxidants, as well as the need to consume foods with complex mixtures of phytochemical antioxidants instead of supplements with one or a few antioxidants in pure form.

Free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide, and their biological metabolites also play a significant role in carcinogenesis. ROS induce DNA damage, as in the reaction of free radicals with the DNA-causing modification of the nucleotide bases and protein crosslinks with DNA. There is ample evidence that the participation of free radicals is related to carcinogenesis, mutation, and transformation (Lobo et al., 2010).

Free radicals and other ROS are derived from either normal essential metabolic processes or external sources, such as exposure to radiation, O_3, smoke or snuff, burning wood or industrial fumes, and chemicals (Bagchi and Puri, 1998). Free radical formation occurs continuously in the cells as a result of enzymatic and nonenzymatic reactions. The rise of the cellular ROS concentration has been implicated in the induction and complication of diabetes mellitus, macular diseases, and neurodegenerative disorders, such as Parkinson's disease (Rao et al., 2006).

Virtually all phytochemicals, especially phenolics and carotenoids, have an antioxidant capacity that allows for the mitigation or elimination of the adverse effects of oxidative stress from the organism. However, because the production of free radicals is a continuous process that is a part of metabolism, the intake of fruits and vegetables should occur at the same rate.

Hence, there is a recommendation to eat at least 9 servings daily (four of fruits and five of vegetables) (Liu, 2013).

3.2 Cardiovascular Diseases

The consumption of flavonoids and adherence to the Mediterranean diet was significantly inversely correlated with mortality from cardiovascular events in humans. The total intake of flavonoids in the forms of quercetin, myricetin, kaempferol, luteolin, and ficetin was inversely associated with the concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL) (Liu, 2013). The Mediterranean diet comprises abundant plant foods, minimally processed and locally grown foods, desserts based on fresh fruits, nuts and honey, olive oil, cheese and yogurt consumed in low to moderate amounts, four or fewer eggs per week, red meat consumed in low frequency and amounts, and wine consumed in low to moderate amounts (Serra-Majem et al., 2004).

In the long-term feeding trials, adequate levels of dietary fiber lower the serum cholesterol and LDL in humans; it seems likely that they may provide protection against cardiovascular disease (Marzolo et al., 1993). Dietary fiber can be defined as a heterogeneous group of nondigestible compounds that include fiber (soluble and insoluble), resistant starch and oligosaccharides, such as raffinose, stachyose, and mullein, among others (Cruz-Bravo et al., 2011). For example, a *Phaseolus mungo*-isolated fiber showed a neutral detergent residue (NDR) that presented reductive activity that could increase the cholesterol and bile acid excretion in feces (Thomas et al., 1983).

3.3 Carcinogenesis

Carcinogenesis is a complex, multiple-step process with initiation, promotion, and progression stages. Chronic exposure to agents that promote inflammation and oxidative damage plays an important role in the formation of cancer. At the initiation stage, ROS induce DNA oxidative damage. If the molecules are left unrepaired, the damaged DNA can lead to base mutation, single- and double-strand breaks, and DNA crosslinking. Dietary antioxidants and food phytochemicals prevented or retarded the potentially cancer-inducing DNA oxidative damage in the initiation stage. During the promotion and progression stages of carcinogenesis, food phytochemicals also regulate different signal transduction pathways that are involved in the cell division and propagation of carcinogenic cells.

Between the potential mechanisms by which phytochemicals contribute to cancer prevention are the enhancement of immune function, antiinflammation, the inhibition of cell adhesion and invasion, antioxidant activity that inhibits DNA damage, the regulation and promotion of DNA repair, the inhibition of cell proliferation and the induction of factors that inhibit

the cell cycle, increased factors that inhibit the gene expression of oncogenes and promote apoptosis, the induction of antioxidant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase, and the inhibition of prooxidant enzymes, such as cyclooxygenase, nitric oxide synthase, and xanthine oxide (Liu, 2013).

Epidemiological evidence suggests a protective effect by dietary fiber against colorectal cancer. In rats with induced colon cancer that were given pinto beans, black and white bean-treated animals reportedly developed 4 times fewer tumors than did rats that were not fed beans (Reynoso Camacho et al., 2007).

Bean lectins are proteins or glycoproteins that possess at least one noncatalytic domain that reversibly binds mono- or oligosaccharides. These proteins have been shown to have antitumor activity, immunomodulatory, antifungal, antibacterial, and anti-HIV properties (Sharma et al., 2009). Lectins that bind between molecules and the cell surface or are internalized by the cell involve various necessary signals for the cell, such as binding or cellular aggregation regulation, the induction of apoptosis or cell cycle interruption, the downregulation of telomerase activity and the inhibition of angiogenesis (Ferriz-Martínez et al., 2010). Lectins from *Phaseolus vulgaris* and *Phaseolus acutifolius* exhibit a differential cytotoxic effect against neoplastic cells for certain cancers by inducing apoptosis (Chan et al., 2012; García-Gasca et al., 2012; Valadez-Vega et al., 2011).

3.4 Neurodegenerative Diseases

Parkinson's disease (PD) and Alzheimer's disease (AD) are the most common neurodegenerative disorders. They are characterized by the accumulation of abnormal protein aggregation that leads to oxidative stress and inflammation in the central nervous system. These disorders are caused by environmental and genetic influences. Various types of mechanisms promote these neurodegenerative disorders, namely oxidative stress, protein aggregates in neurons, insufficient synthesis or depletion of neurotransmitters, abnormal ubiquitination, mitochondrial dysfunction, degradation of neurotransmitters by high cholinesterase, and the excitotoxicity of neurons (Rasool et al., 2014).

Several phytochemicals have shown antioxidant and anticholinesterase activity; the phenols, polyphenols, linoleic acid, oleic acids, luteolin, terpenes, flavonoids, quercetin, tannins, and alkaloids are represented in many plant species. Other more specific and potent phytochemicals appear only in certain species, such as *G. biloba* (amentoflavone), *Panax ginseng* (ginsenoside), and *Rauvolfia serpentina* (reserpine) (Rasool et al., 2014).

Moreover, a diet that is high in omega-3 fatty acids is gaining approval to enhance cognitive activity in humans (McCann and Ames, 2005). Additionally, an omega-3-rich diet has shown the ability to promote the upregulation of genes that are vital to sustaining synaptic activity and agility in rodents (Wu et al., 2007).

Brain activity is definitely based on proper nutrition. High risk is reported for mental disorders, such as attention-deficit disorder, dyslexia, dementia, depression, bipolar disorder, and schizophrenia, when humans do not ingest sufficient amounts of omega-3 fatty acids (Freeman et al., 2006). Because the human body is unable to synthesize the omega-3 fatty acid docosahexaenoic acid (DHA), which is an important part of the membranes of neurons, we must rely on DHA from dietary sources, such as those found in plant oils, wild edible plants (Simopoulos, 2004), or the Mediterranean diet (Scoditti et al., 2014).

4 Regulation of the Synthesis and Accumulation of Compounds That Enhance the Nutraceutical Quality of Plant Foods

During evolution, plants developed mechanisms for the induction of responses to various environmental stimuli. One part of these responses, such as the morphology of stomata and other epidermal cells, the forms of the leaves and stems, the type of metabolism (C3, C4, or CAM), among others, are constitutive; however, others appear only under particular inductive conditions. The last responses are observed as reactions over the short term, during plant adaptation to the environment, not necessarily in magnitudes, such as those that cause stress. These responses are triggered by biotic factors, such as pathogens, pests, and symbionts, or by abiotic factors, such as high or low temperatures, radiation, and salinity, among others (Fig. 4.4).

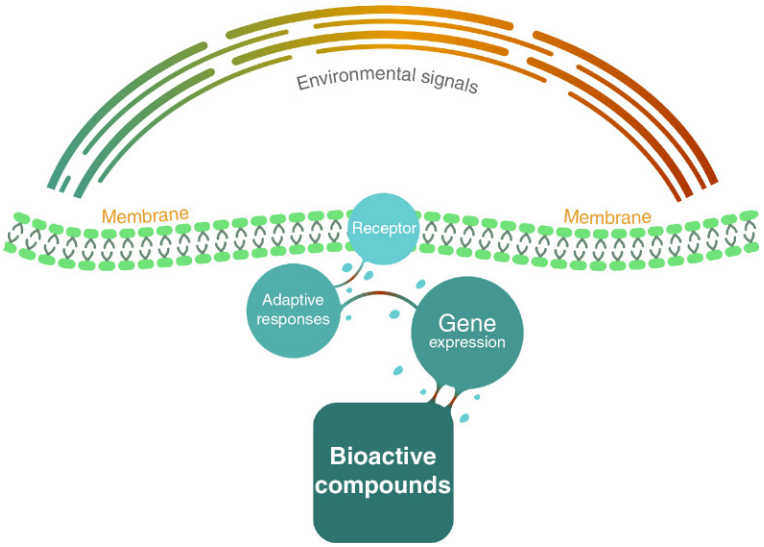


Figure 4.4: Induced Synthesis of Bioactive Compounds by Physical, Chemical, or Biological Signals From the Environment.

Abiotic induction or biotic elicitation is a suitable strategy for inducing physiological changes and stimulates defense or eustress-induced responses in plants. The induction or elicitor treatments trigger the synthesis of nutraceutical compounds in cereals, vegetables, fruits, and herbs.

The growth environment is a constant source of information and resources for plants, which are in a dynamic state of physiological and metabolic change that in turn result in recurrent changes in gene expression. Phenotypes, rather than being immutable, are dynamic and evolving on time scales ranging from minutes to days or months.

Adaptive responses that occur to cope with any environmental factor depend on the action of inducers or elicitors that interact with specific receptors, which are usually located in the membranes surrounding the cytoplasm, the tonoplast, and other organelles. The factor that triggers the response is called the inductor. These inductors can be physical, such as electromagnetic radiation and temperature. If the inductor is a chemical or biochemical compound, whether natural or synthetic, it is called an *elicitor*. An elicitor can be defined as a chemical or biochemical compound that is introduced in small concentrations to a living system to promote the biosynthesis of the target bioactive compound. Examples of elicitors are selenium or silicon, and salicylic acid or cell wall oligomers. A classification of elicitors is presented in Table 4.1.

Table 4.1: Elicitor and inductor classification.

Biotic Elicitors	Abiotic Elicitors	Inductors
Lipopolysaccharides	Acetic acid	Altered gas composition, high pressure, CO_2, and O_3
Polysaccharides: pectin and cellulose in cell walls; chitosan, chitin, and glucans of microorganisms, alginate, arabic gum, guar gum, and yeast extract	Metal ions: Co^{2+}, Fe^{2+}, Al^{3+}, Ag^{2+}, Ag^{+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, and Cd^{2+}	Extreme temperature shock, chilling, high temperature
Oligosaccharides: galacturonides, guluronate, mannan, and mannuronate	Silicon	Drought
Proteins: cellulase, cryptogein, glycoproteins, oligandrin, pectolyase, fish protein hydrolysates, and lactoferrin	Calcium prohexadione	High or low osmolarity
Fungal spores, mycelia cell wall, microbial cell wall, and coronatine (pathogen toxin)	Ethanol and ethene	UV irradiation
Jasmonic acid, methyl jasmonate, methyl salicylate, salicylic acid, ethylene, cytokinin, and gibberellin GA3	Benzothiadazole	Wounding and herbivorism
Extracts of plants (oregano, *Larrea tridentata*)	Inorganic salts: mercuric chloride ($HgCl_2$), copper sulfate ($CuSO_4$), calcium chloride ($CaCl_2$), and vanadyl sulfate (VSO_4)	Saline stress

Modified from Baenas, N., García-Viguera, C., Moreno, D.A., 2014a. Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19, 13541–13563.

The membrane receptor that is activated by the inductor or elicitor modifies the gene expression and induces the synthesis, accumulation, and metabolism of certain secondary metabolites, namely phytoalexins, proteins, or other compounds related to stress responses. In addition, elicitors may induce short-term modulatory changes in the properties of the cell membrane, or they may determine the posttranslational modifications in certain proteins, leading to another signal transduction cascade and more adjustments in plant behavior (Aliferis et al., 2014; Hooper et al., 2010).

The improvement of a crop's phytochemical composition is achieved through the application of environmental stimuli with some degree of control (light, temperature, or elicitors). The elicitors are perceived by specific receptors in plant cells, and they trigger cascades of adaptive responses to these environmental signals that result in a response called eustress (low stress), during which the cells are better able to tolerate and acclimatize to changing environments (Kranner et al., 2010) (Fig. 4.5).

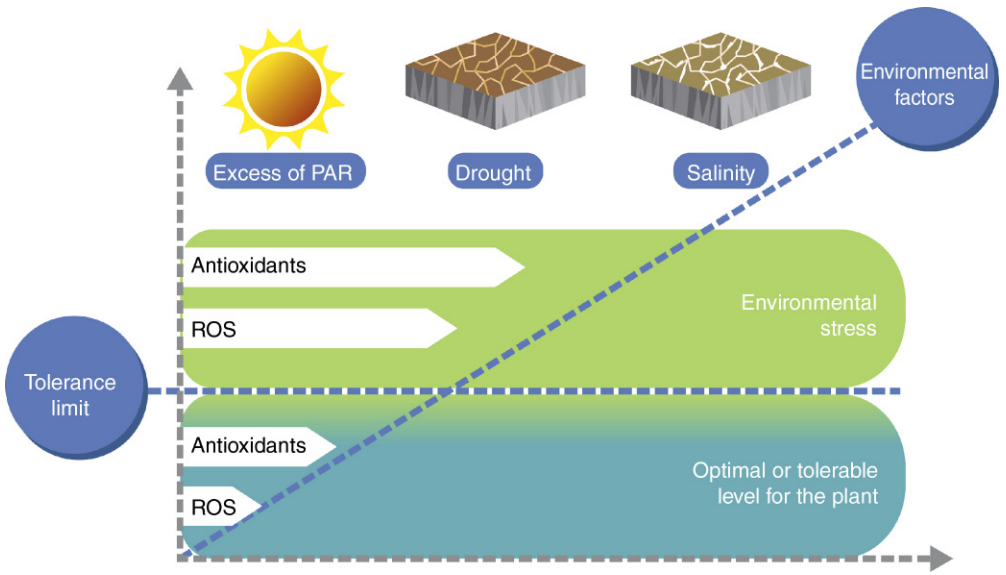


Figure 4.5: The Improvement of a Crop's Phytochemical Composition Through the Application of Environmental Stimuli With Some Degree of Control (Light, Temperature, or Elicitors). The environmental signals below of the tolerance limit are perceived by specific receptors in plant cells, and they trigger cascades of adaptive responses to these environmental signals that result in a response called eustress (or low stress), during which the cells accumulate antioxidants and other phytochemicals, and are better able to tolerate and acclimatize to changing environments. Instead, when the environmental stimuli, for example, high irradiance, drought, or salinity, is applied at high intensity or for a long time, then the system response is negative or in distress (high-stress). In other words, resources (energy and molecules) that must be invested will reach their limit, and the exhaustion of metabolic reserves no longer allows for the biosynthesis of novel defense compounds.

This acclimation is based on a series of metabolic adjustments that lead to the synthesis and accumulation of phytochemicals with nutraceutical value that increases stress tolerance, including antioxidants, such as glutathione and phenolic compounds, osmolytes, such as proline, secondary messengers, such as salicylates, various metabolites that increase the stability or activity of proteins, many volatile compounds, peptides, carbohydrates, and a wide variety of defense compounds against insect pests and pathogens, and so on (Ben Rejeb et al., 2014; Goławska et al., 2014; Kalemba et al., 2015; Scala et al., 2013; Zagorchev et al., 2013). These compounds also have essential functions as free radical scavengers, stabilizers, and protectors of DNA and proteins against oxidative stress (Inzé et al., 1995) in all living aerobic organisms, and in humans, they exert positive health effects (Baenas et al., 2014a; Martínez-Ballesta et al., 2013; Tan et al., 2012; Zeng et al., 2013). Many phytochemicals do not show constitutive expression but depend on induced responses that take place in several well-defined phases, namely alarm, acclimatization, and resistance (Kosová et al., 2015), as shown in Figs. 4.6 and 4.7.

The three phases of the adaptive response to stress or adjustment occur dynamically, and thus the transcriptomes, proteomes, metabolomes, and ionomes are subject to constant modification and change (Kosová et al., 2015). The cellular response is amplified because, from the few cellular receptors that perceive the environmental stimuli, a process of transduction and signaling occurs through a higher amount of secondary messengers and transcription factors. These messengers and factors regulate the expression of several hundreds or thousands of genes, creating a new transcriptome that is built specifically for the particular situation. The mRNA of the new transcriptome can generate several types of proteins from processes, such as alternative RNA splicing and RNA editing (possibly increasing the number of resulting proteins by a factor of 10 or more). Apart from these processes, the obtained proteins, many of which have a particular expression for certain environmental challenges, are subject to subsequent posttranscriptional modifications, such as phosphorylation, acetylation, methylation, ubiquitination, myristoylation. This amplification process makes it difficult to predict the nutraceutical value and physiological behavior based solely on genomic or transcriptomic information.

Many of the obtained proteins are directly associated with resistance to stress (such as antioxidant proteins and chaperonins), but many of them are part of metabolic pathways that are modified or activated to produce a myriad of phytochemicals that come together to form the metabolome, which groups all the low molecular weight compounds (including phytochemicals). The metabolome is the level of complexity of the organism that is directly related to the nutraceutical properties of plants, response to plant stress, and physiological behavior (Fig. 4.7) (Aliferis et al., 2014).

Metabolic stress responses have been found to occur through the coordinated action of groups of transcription factors, which in turn regulates gene clusters (the core metabolic

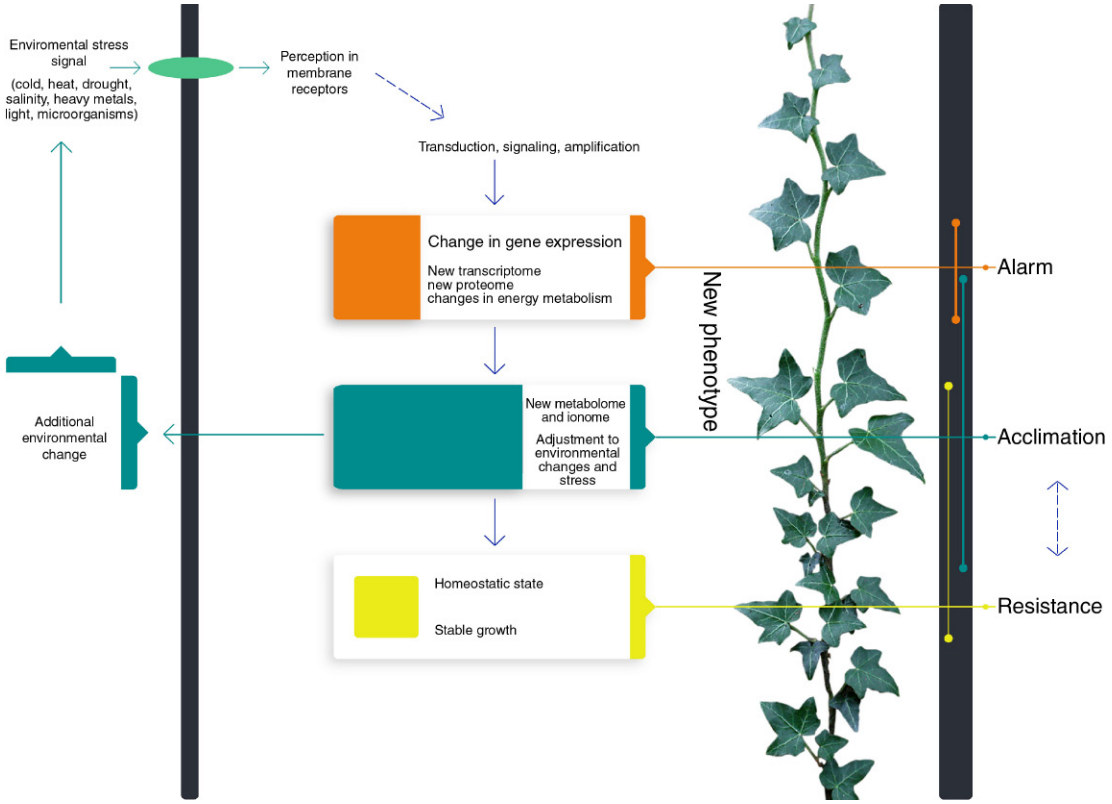


Figure 4.6: Diagram of the Sequence of Actions Performed in a Cell That was Responsive to an Environmental Stimulus.

On the left side, the environmental signals are perceived by specific receptors that are known as the alarm during the first phase, which leads to the transduction, signaling, and amplification of the information via secondary messengers and transcription factors that migrate to the nucleus and modify the expression of genes, resulting in a transcriptome that can respond and adjust to the specific prevailing environmental situation. Later, during the acclimatization phase, changes in the transcriptome generate a new proteome that modifies the energetic metabolism as a preamble to shifts in the metabolome and ionome, which are the phenotypic manifestations that are directly linked to cell adjustments, environmental changes, and stress tolerance. If subsequent changes do not occur in the environment, then a new homeostatic state is reached and accompanied by stable growth and metabolism. If additional changes take place in the environment, then the response restarts, as marked with the arrow on the left side on the bottom. Although the successive phases elapsed over different time periods, substantial overlaps occur between them, as indicated by the vertical boxes on the left.

response). This gene coexpression triggers the expression, at the metabolomic level, of sets of phytochemicals with profiles and concentrations that are different from one botanical family to another (Rabara et al., 2015). This finding likely explains the characteristic differences between crop species with regard to their phytochemical composition, and their relative

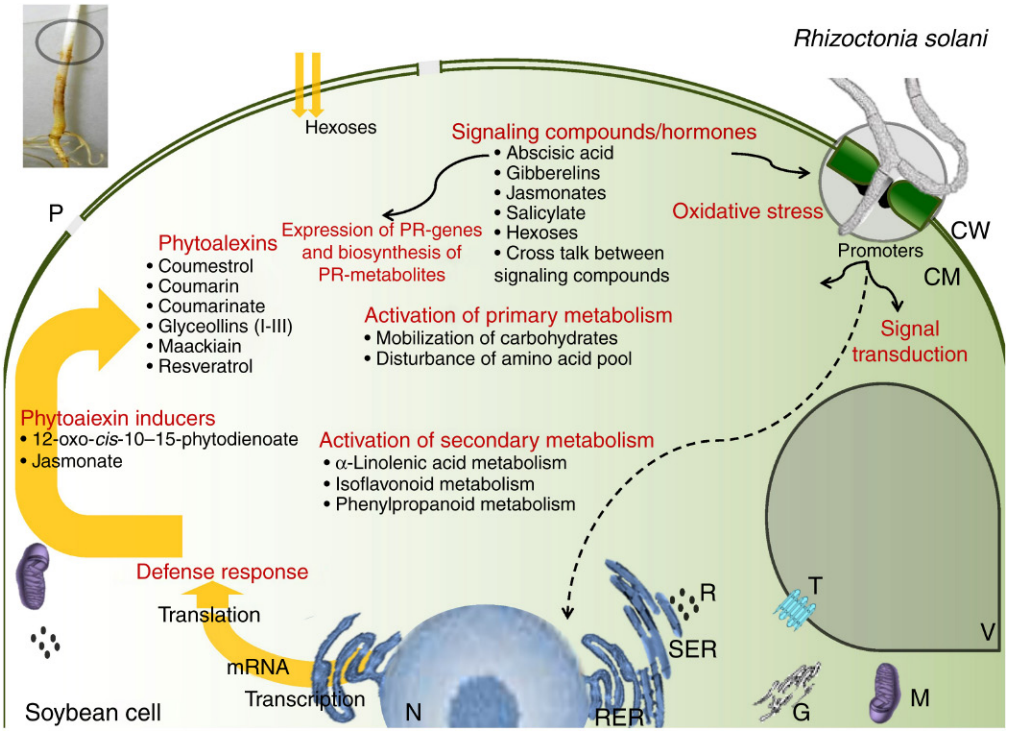


Figure 4.7: Proposed Graphical Model for the Role of *Rhizoctonia solani* in the Activation of Soybean Defense Mechanisms.

The pathogen elicitors trigger a cascade of responses at the transcriptomic, proteomic, and metabolomic levels, changing the energy metabolism and physiological responses of the plant. *CM*, cell membrane; *CW*, cell wall; *G*, Golgi body; *M*, mitochondrion; *N*, nucleus; *P*, plasmalemma; *R*, ribosomes; *RER*, rough endoplasmic reticulum; *SER*, soft endoplasmic reticulum; *T*, transporters; *V*, vacuole. *From Aliferis et al. (2014).*

nutraceutical value. In addition to indicating that the same plant species that are subject to various environmental stimuli or unequal intensities of the same environmental factor will exhibit different phytochemical compositions and, thus, different nutraceutical values. This extensive variation of intra- and interspecies illustrates the need for consuming a varied vegetable diet, including seeds, tubers, leaves and flowers, fruits, and stems. This variation is needed to ensure the necessary consumption, in both quantity and quality, of the essential phytochemicals needed for proper cell function and physiological activities (Lutaladio et al., 2010).

To implement different agricultural practices, with the usage of environmental stimuli (inductors and elicitors), and to determine both how they are applied and the expected response in the form of a higher concentration of phytochemicals, it is necessary to consider that the expression of genes regulated by stimuli that induce stress typically follows the

Table 4.2: Genes involved in a systemic or nonsystemic stress response.

	Total (Root and Shoot)	Nonsystemic	Systemic Type I	Systemic Type II
Genotoxic	257 (220 and 99)	158	37	62
Osmotic	2687 (1185 and 2066)	621	1502	564
Oxidative	28 (11 and 18)	10	17	1
Salt	1934 (1164 and 456)	1478	270	186
UV-B	1166 (146 and 1100)	1020	66	80
Wounding	453 (19 and 440)	434	13	6

Modified from Hahn, A., Kilian, J., Mohrholz, A., Ladwig, F., Peschke, F., Dautel, R., Harter, K., Berendzen, K., Wanke, D., 2013. Plant core environmental stress response genes are systemically coordinated during abiotic stresses. Int. J. Mol. Sci. 14, 7617–7641.

following three trajectories: nonsystemic (regulated in the stressed tissue only), systemic type I (regulated in the nonstressed tissue only) or systemic type II (regulated in both stressed and nonstressed tissues) (Hahn et al., 2013). Each stressor factor induces a particular pattern of response as exemplified in Table 4.2. The important point is that this information clearly indicates that it is indeed possible, based on a better understanding of plant responses within the ambit of different areas as the transcriptome, metabolome or ionome, to design cropping systems that guide the growth and development of plants toward obtaining a specific composition, in this case, for certain concentrations and profiles of phytochemicals.

5 Agricultural Practices for Improving the Nutraceutical Quality of Crops

In the previous section, there was a discussion of how environmental stimuli modifies the synthesis and accumulation of nutraceuticals. The modern research tools from the -omics sciences have allowed a breakthrough in understanding the biosynthesis and metabolism of a large number of compounds whose effects on health and human welfare are just now beginning to be glimpsed. One point to note is that virtually all environmental factors and their interactions are capable of modifying gene expression, as well as the resulting biochemical composition in the form of phytochemicals that define nutraceutical quality (Fig. 4.8). Different combinations of environmental factors are present in large numbers, and they tell us, on the one hand, the wide range of opportunities for using agricultural technology to modify the nutraceutical quality of plants, but on the other hand, they also point to the complexity involved in characterizing all the possible agricultural practices in a systematic and orderly fashion according to this objective.

The response capacity, or the higher or lower synthesis of phytochemicals that occur after applying a particular environmental stimulus, is highly dependent on the differences between species, subspecies, varieties, or hybrids (Guevara-Figueroa et al., 2010; Khanam et al., 2012; Kim et al., 2013). Some characteristics function as markers, such as different colors and shapes in seeds, fruits, leaves and stems, and roots, showing differences in the accumulation of certain phytochemicals (Sun et al., 2007). Another marker of the differential capacity to

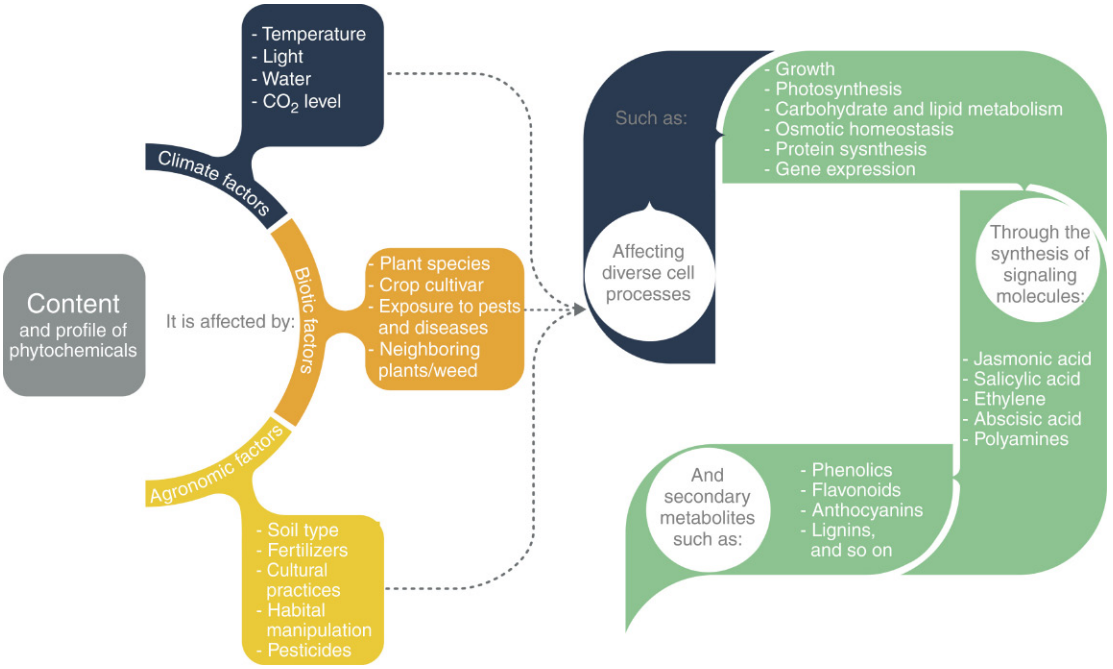


Figure 4.8: Factors That Affect the Content and Diversity of Phytochemicals.

produce nutraceuticals lies in the relative differences in the response of hybrids and varieties to some stresses, and in the growth stability of different environments (Carrera et al., 2014). These basal metabolic and biochemical differences determine the direction of the response to the stimuli that are applied; for example, irradiance or temperature control, and they therefore modify the nutraceutical quality of the resulting product. The same case applies to some techniques, such as grafting, in which the specific rootstock-scion combination determines the synthesis and accumulation ability of phytochemicals (Chávez-Mendoza et al., 2015) and therefore imposes certain limits on responses to environmental stimuli.

It is noteworthy that the potentially positive effect obtained by applying a given environmental stimulus depends on its intensity or concentration, and the time during which the stimulus is maintained (Bloem et al., 2014; Wang and Frei, 2011). When the inducer of an adaptive response factor, for example, a water deficit (Król et al., 2014), is applied at high intensity or for a long time, then the system response is negative or in distress (high-stress) (Kranner et al., 2010). In other words, resources (energy and molecules) that must be invested will reach their limit, and the exhaustion of metabolic reserves no longer allows for the biosynthesis of novel defense compounds.

The use of agronomic practices for environmental handling, such as plant nutrition and the application of regulators that induces metabolic responses, permits the regulation of the intensity of the applied stimulus. As a result, it is possible to reach the state of eustress

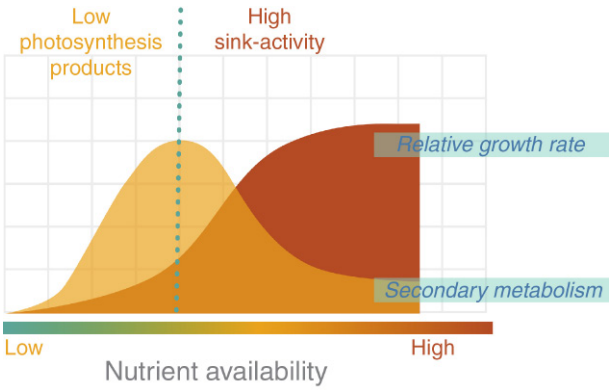


Figure 4.9: Responses of the relative growth rate, net assimilation rate, and constitutive secondary metabolism across a gradient of nutrient availability as predicted by the growth-differentiation balance hypothesis. In source-limited plants, a positive correlation is predicted between growth and secondary metabolism, and in sink-limited plants, the correlation is predicted to be negative.

in which plants accumulate higher concentrations of nutraceuticals in comparison with a system in which no such stimuli are applied, or when the stimuli are in excess (Larbat et al., 2012; Mody et al., 2009; Wang and Frei, 2011) (Fig. 4.5). In the case of fertilizer inputs, CO_2, water and other resources that promote the net assimilation rate (NAR) and relative growth rate (RGR) the most accepted hypothesis, which is known as the "growth-differentiation balance hypothesis" (Herms and Mattson, 1992), states that beyond a certain level of nutrient availability, greater plant growth will be accompanied by a decrease in the concentration of compounds with nutraceutical value (Glynn et al., 2007; Schutte et al., 2013) (Fig. 4.9). For this reason, the environment and particularly plant nutrition must be manipulated to improve the nutraceutical quality, which implies a reduced quantity and improved balance of applied fertilizers. A better balance in fertilizers in turn raises the tolerance of these plants to pests and pathogens (Treutter, 2010). This change results in the more efficient use of natural, technical, and financial resources. The highest nutraceutical quality does not necessarily have to be detrimental to productivity or yield (García-Mier et al., 2013).

The same environmental management systems and the application of different physical, chemical, and biological stimuli used in plants in the field or greenhouses are also applicable at the postharvest stage, in seeds, leaves, fruits, and flowers. These organs remain active after harvest, both metabolically and with the ability to perceive and signal environmental factors that trigger adjustments in biochemical and physiological processes (Aghdam et al., 2012; Wu et al., 2012). Changes may then occur in both the nutritional and nutraceutical quality with respect to the flavor, biochemical composition, concentration and profile of phytochemicals, and physiological activities, such as respiration in accordance with the conditions of storage

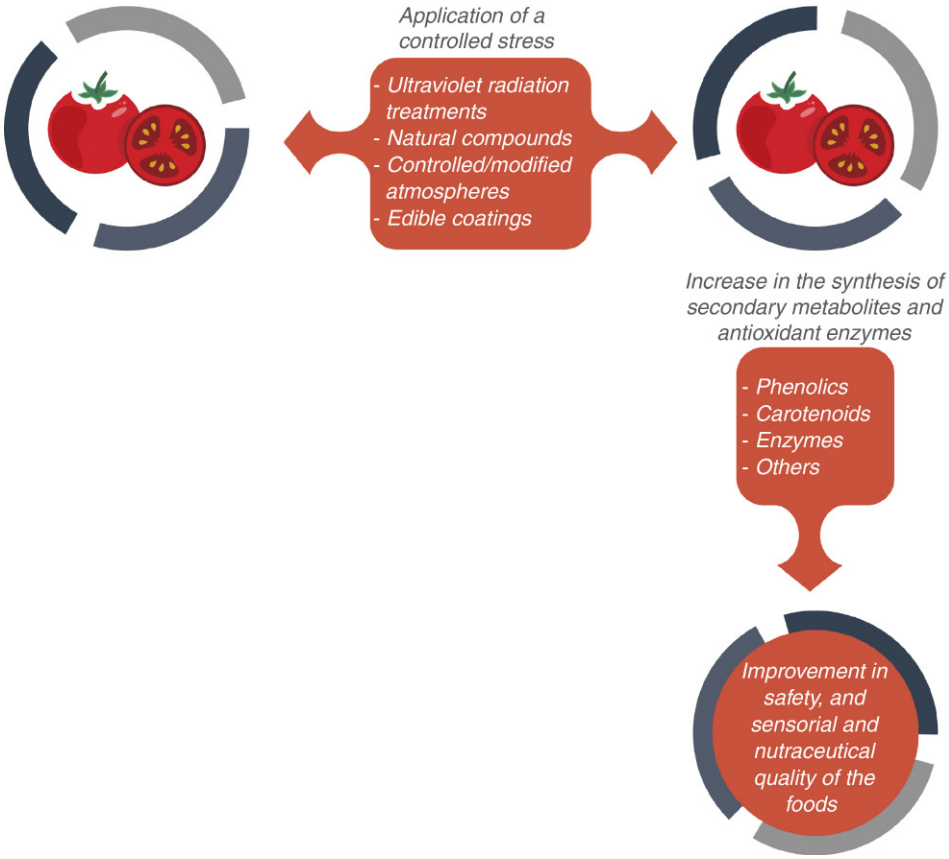


Figure 4.10: Application of Different Physical, Chemical, and Biological Stimuli at the Postharvest Stage—in Seeds, Leaves, Fruits, and Flowers.
These organs remain active after harvest, both metabolically and with the ability to perceive and signal environmental factors. Changes may then occur in both the nutritional and nutraceutical quality in accordance with the circumstances of storage or processing. These changes can improve or be unfavorable to the concentration of phytochemicals and consequently the nutraceutical quality.

or processing (Fig. 4.10). These changes can enhance or be detrimental to the concentration of phytochemicals and consequently the nutraceutical quality (Asghari and Aghdam, 2010; Castagna et al., 2013; Hodges and Toivonen, 2008).

Other techniques for obtaining plant foods with substantial improvements in the nutraceutical quality are based on the use of GMOs (Khurana and Chaudhary, 2010; Park et al., 2013; Yang et al., 2006). In tissue culture, the concept of the "plant-cell factory" (Ramirez-Estrada et al., 2016) is used, and the application of metabolic engineering (Keasling, 2010) and synthetic biology (Comas et al., 2016) are techniques that are employed in combination with environmental manipulation and have promise for achieving substantial progress toward better crops, once there is greater understanding about the regulation and interconnectivity between

different metabolic pathways in plants (Hahn et al., 2013; Rodríguez-Concepción, 2010; Shetty and McCue, 2003; Vallabhaneni and Wurtzel, 2009; Zhu et al., 2013). However, for reasons of space, these issues of transgenic organisms, metabolic engineering, synthetic biology, and the plant cell factory are not considered in this chapter.

6 Results of Applying Agricultural Practices on Nutraceutical Quality

6.1 Preharvest Environmental Factors

The environment in which crop growth is developed, that is, the geographic region that determines the altitude above sea level, relative humidity, temperature, photoperiod, irradiance, precipitation, soil, and water, and so on in combination with seasonal changes play a definitive role in determining the concentration and profile of nutraceuticals in plants. Different studies have shown that the same varieties of cultivated plants at various sites, or in the same location during summer or autumn, can change the phytochemical composition, texture, and flavor as a result of the difference in environmental stimuli (Tuttolomondo et al., 2014). On average, the effects of seasonality in temperate zones lead to a higher concentration of phytochemicals in spring–summer crops in comparison with autumn–winter crops. Similarly, long photoperiods have a positive impact on the amounts of phytochemicals that are synthesized and accumulated by plants (Björkman et al., 2011).

The point discussed earlier is valid, even for greenhouse crops, which, depending on their seasonality, latitude, altitude above sea level, and composition of irrigation water or small differences in fertilizer application, will result in different combinations and concentrations of phytochemicals, which then modifies the nutraceutical value of the crops (Toor et al., 2006b). In a study by Akhatou et al. (2016) three varieties of strawberries were evaluated under soilless cultivation using nutrient solutions with different electrical conductivities, cover types, and substrates. Significant alterations were found in the primary metabolites of the three cultivars, including sugars, such as fructose and glucose, organic acids, such as malic and citric acids, and some amino acids, among others. The changes described, in combination with different environmental signals perceived by plants, will in turn have a profound impact on the secondary metabolism and nutraceutical quality. This multifactorial environmental impact is associated with the geographical effect on the nutraceutical quality of table wines (Vitalini et al., 2011), which has also been shown to depend on specific plant microbiomes (Bokulich et al., 2016).

There are also significant differences in the concentrations and types of phytochemicals present in the same species when considering the various organs of plants, such as seeds, flowers, leaves, stems and roots, or different stages of growth. As an example, the concentration and diversity of glucosinolates in 29 different plant species are much

higher in the roots than in the stems and leaves. In addition, there is a considerable variation between different root sections, with the largest concentration of glucosinolates being found in the inner periderm, and lower levels in the outer periderm and pericycle. Moreover, broccoli and cabbage sprouts can contain 10–100 times more phytochemicals than mature plants. During growth and development, some phytochemicals decrease in concentration while others increase, as with the carotenoids lutein and β-carotene in kale. Lutein is more abundant in kale leaves during the two first 2 weeks of growth of the plant, and β-carotene increases in the foliage after the first 2 weeks of growth. This variation over time depends specifically on the organ of the plant that is interacting with environmental factors, such as temperature, irradiance, and plant nutrition. Therefore, the results obtained during a specific stage of growth cannot be extrapolated to other stages or other plant organs (Björkman et al., 2011).

It is well known that electromagnetic radiation is one of the determinants of plant growth because it directly impacts the photosynthetic process. As explained previously, there is not a direct relation between increased photosynthesis accompanied by greater RGR and the accumulation of phytochemicals (Schutte et al., 2013). Thus, an agronomic approach focused solely on promoting an increased photosynthetic rate will not necessarily result in a higher nutraceutical quality. In the same vein, Pérez-López et al. (2015) evaluated two cultivars of lettuce with different pigmentation (green and red) under various irradiance and CO_2 concentration conditions. In both cultivars, the results showed that high irradiance increased the concentration of soluble proteins and sugars, also generating controlled oxidative stress, which led to a higher concentration of carotenoids, glutathione, total phenols, and anthocyanin levels. By combining high irradiance with elevated levels of CO_2, which is a very common strategy for promoting the growth rate, the results showed decreased levels of Chl-a, carotenoids, total phenols, and anthocyanins.

A different result can be obtained by manipulating the photoperiod, showing that the periods of illumination greatest to 12 h, leads to a positive influence on the level of phytochemicals derived from the roots, stems, and leaves of plants. This effect is especially significant in seed sprouts in which the photoperiods of 18, 22, or 24 h determine a significant change in the concentration of phytochemicals (Björkman et al., 2011). In addition, the spectral balance of the radiation, that is, the molar balance of photons located in different sectors of the spectrum, not only directly influence the growth of plants but also affects the synthesis of nutraceuticals. The work with LED light sources, which permits investigators to obtain different spectral balances, and the use of ultraviolet (UV) radiation, supported the effectiveness of this approach (Samuolienė et al., 2012, 2011). To study UV radiation, (Topcu et al., 2015) different doses (2.2, 8.8, and 16.4 kJ/m^{-2} day^{-1}) of UV radiation (280–315 nm) were applied throughout the development of the broccoli plants. The

investigators found that UV significantly decreased the carotenoids, chlorophyll a, and chlorophyll b contents in the plants. In a counterpart, the contents of ascorbic acid, total phenols and flavonoids increased, as did the sinigrin and glucotropaeolin, especially with high UV doses.

The stress induced by temperature extremes is a factor that induces phytochemical accumulation. Rai et al. (2015) reported a greater accumulation of H_2O_2 and proline in *Lablab purpureus* under high temperatures, as well as an increase in catalase activity as compared with control plants. However, in other enzymes, such as ascorbate peroxidase, glutathione peroxidase, and guaiacol reductase, no differences were observed between the control and treated plants under stress. During cold storage (4°C, 75 days) *Citrus sinensis* fruit accumulated anthocyanins (eightfold compared with the control) in their juice vesicles. In apple fruits, cold storage promotes an accumulation of phenolic compounds, coupled with the increased activity of phenylalanine ammonialyse (PAL), a key enzyme in the phenylpropanoid pathway (Baenas et al., 2014a).

Another environmental factor that commonly influences the phytochemical content is the water deficit. In a study by Odjegba and Alokolaro (2013), the authors found that in *Acalypha wilkesiana*, some phytochemicals, such as alkaloids, flavonoids, and tannins were decreased by a water deficit, and others, such as saponins, increased. In another study using *Withania somnifera*, tolerance to water deficits was associated with the production of secondary compounds (Sanchita et al., 2015).

For the effect of salinity on crops, it is known that salinity can generate two types of stress. The first stress is an osmotic type and directly influences the ability of a plant to take up water, and the latter is a toxic effect caused by the accumulation of elements, such as sodium itself. In the first case, the induction of nutraceuticals in plants is similar to what occurs when a stress is generated by a water deficit. In the second case, sodium intoxication causes a distress that decreases the concentration of phytochemicals. For example, the application of different levels of salinity (0–32 dS/m) in *Portulaca oleracea* induces more phenols (8%–35%), flavonoids (35%), and a significant increase in antioxidant capacity (Amirul Alam et al., 2015). In lettuce, the application of NaCl (50–150 mmol/L) improved some of the structural characteristics of the leaves, increasing at the same time as the concentration of phenols (Garrido et al., 2014).

However, not only abiotic factors can generate greater nutraceutical quality. Baum et al. (2015) wrote a review in which they mentioned that the use of mycorrhizae improves the quality of crops, such as lettuce, tomatoes, peppers, and strawberries, primarily because of the accumulation of secondary metabolites, minerals, and vitamins. Mycorrhization is a type of controlled infection, and the action of the inductor on the plant permits the synthesis and accumulation of different phytochemicals. These authors concluded that the potential of mycorrhizae to promote growth and quality in the host plants is controlled both by the

genotype of the host and the fungus and their interactions. In *Trigonella foenum-graecum*, an endophytic *Bacillus* sp., promoted a higher concentration of diosgenin (Jasim et al., 2015), showing that biotic agents as bacteria also have a significant and direct influence on the nutraceutical quality of crops.

6.2 Postharvest Environmental Factors

Traditionally, foods are preserved using heat treatments, which have the advantage of reducing potential human disease by inactivating microorganisms and enzymes, contributing to increased shelf life. However, these heat treatment methods also have some limitations, such as the deterioration of the product's organoleptic quality (Braddock, 1999), loss of color, and undesirable physical and chemical changes (Arreola et al., 1991), in addition to the loss of nutrients and phytochemicals (Chen et al., 1993).

Fruits and vegetables are highly perishable products (because of their high water content, which is approximately 80%) that deteriorates within a short period if not handled properly (Orsat et al., 2006). In recent years, various methods of food preservation have been developed with nonthermal technologies, such as ultrasound (US), high hydrostatic pressure (HHP), high-pressure homogenization (HPH), UV radiation, and electric field pulses (PEF). The nonthermal technologies can improve the quality and shelf life of fruits, and they have become potential alternatives to conventional methods (Rupasinghe and Yu, 2012). They also have the advantage of being friendly to the environment and nontoxic, unlike other techniques that are used in the industry to improve the shelf life of fruits and vegetables (Kentish and Ashokkumar, 2011). In strawberry fruits, US can not only improve the physical and chemical properties of fruit and increase its shelf life, but it also increases the antioxidant activity (Gani et al., 2016).

Several technologies, such as modified atmosphere packaging, irradiation, and coatings in combination with cold storage are proposed to prolong the life of the fruits in the postharvest stage (Castagna et al., 2013; Xanthopoulos et al., 2012). Edible coatings of chitosan on cherry fruits cause changes in the total contents of polyphenols, anthocyanins, and flavonoids, as well as by modifying the antioxidant capacity (Petriccione et al., 2015). The same result was obtained in tomato fruit with the application of a cover containing chitosan (Ortega-Ortíz et al., 2007).

Drying has been used to extend the shelf life of fruits and vegetables, improve storage stability, minimize packaging requirements, and reduce the transportation weight. Numerous processing techniques have been used for drying fruits and vegetables (Ahmed, 2011). New drying technologies, such as freeze-drying, osmotic dehydration, microwave drying, hybrid drying technologies, drying with infrared radiation, and drying with supercritical carbon dioxide are techniques that represent minimum food processing approaches and can allow foods to retain nutrients and phytochemicals with minimal degradation (Maskan, 2001; Mujumdar, 2007).

All foods, from fresh fruit to processed foods, frequently serve as a source of components that have beneficial effects on human health. In most cases, processing adversely affects the

concentrations of bioactive components in functional foods and nutraceuticals. Therefore, minimally processed products can better serve the health of consumers (Shahidi, 2009). In this regard, greater importance should be conferred to the preparation and cooking of plant foods as a complementary factor to obtaining food with high concentrations of phytochemicals (Fabbri and Crosby, 2016).

6.3 Summary of Environmental Factors, Technology, and Agricultural Practices That Modify the Nutraceutical Quality of Crops

Below we recount some results; we present how the application of certain technical and agronomic management tools allow for the development of crop plants with improved nutritional and nutraceutical qualities. The described techniques are grouped in Table 4.3, which addresses the following different categories:

1. Technology for environmental manipulation or environmental control. Includes abiotic inductors, such as temperature, water availability, irradiance control, LED lighting use, plastic mulches, plastic covers, and shade netting.
2. Induction of nutraceuticals using plant nutrition: use of fertilizers, organic production, and hydroponics.
3. Elicitation with heavy metals and metalloids.
4. Abiotic elicitation with natural or synthetic elicitors, biotic elicitation with microorganisms or their metabolites and plant extracts, use of biopolymers, and application of plant growth regulators.

Table 4.3: Summary of different environmental factors and crop management technologies that modify the nutraceutical quality.

(a) Technology for environmental manipulation or environmental control		
Low temperatures (9–15°C) and cold acclimatization can increase the soluble sugar concentration and improve the taste. Temperatures higher than 15°C, with an average of 21°C, promote more antioxidant activity and phytochemical accumulation.		
Factor under control	Species	References
Temperature	*Brassica rapa*	Justen and Fritz (2013)
Temperature	*Brassica napus*	Johansen et al. (2016)
Temperature	*Brassica olereacea*	Steindal et al. (2015)
Temperature	*Ribes nigrum*	Krüger et al. (2012)
Water regimes (water deficit, irrigation deficit, and well-watered) modify plant responses. The plant biomass increases under well-watered conditions, with a decrease in phytochemicals. A severe water stress diminished both the biomass and phytochemical concentration. A mild irrigation deficit induced higher concentrations of phytochemicals, without affecting the plant biomass significantly.		
Water availability	Brassicales	Zhang et al. (2008)
Water availability	Lettuce	Eichholz et al. (2014)
Water availability	*Prunus persica*	Falagán et al. (2016)

Table 4.3: Summary of different environmental factors and crop management technologies that modify the nutraceutical quality. (*cont.*)

Nonsaturating PAR irradiance levels (400–800 µmol/m^2 s) promote the synthesis and accumulation of phytochemicals. The PAR irradiance range is specific for each category of phytochemicals and plant species. In species that produce essential oils in leaf surfaces, a better response occurs with high irradiance.		
Manipulation of irradiance	*B. oleracea*	Neugart et al. (2016)
Manipulation of irradiance	*B. oleracea*	Neugart et al. (2013)
Manipulation of irradiance	*Lens culinaris*	Świeca et al. (2012)
Manipulation of irradiance	*Origanum vulgare*	Shafiee-Hajiabad et al. (2015)
Use of LED lighting, at nonsaturating irradiances as growing light or supplemental light promotes the accumulation of phytochemicals. Generally, better results were obtained in terms of the growth and phytochemical concentration with higher ratios of red: blue light and with green LED as supplemental light. The system can be applied to the greenhouse and for sprouted seeds.		
Use of LED lighting	Broccoli	Steindal et al. (2016)
Use of LED lighting	Broccoli	Jin et al. (2015)
Use of LED lighting	Lettuce	Son and Oh (2013)
Use of LED lighting	Microgreens of *Brassica* spp.	Samuolienė et al. (2013)
Use of LED lighting	Sprouted seeds of lentil, wheat, and radish seeds	Samuolienė et al. (2011)
Use of LED lighting	*Gynura bicolor*	Ren et al. (2014)
Use of LED lighting	*Valerianella locusta*	Wojciechowska et al. (2015)
Plastic mulch and colored plastic mulches led to a poorer response in comparison with row covers, light-selective plastic greenhouse films, and photoselective shade netting. The best responses in terms of plant growth and yield and the concentration of phytochemicals occur with row covers and pearl and red shade netting (25%–40% shading).		
Plastic mulch	Strawberry	Khanizadeh et al. (2014)
Plastic mulch	Strawberry	Li et al. (2011)
Colored plastic mulches	*B. rapa*	Justen et al. (2011)
Greenhouse plastic films	Strawberry	Miao et al. (2016)
Photoselective shade-netting	*B. rapa*	Justen et al. (2012)
Shade-netting	Tomato	Krumbein and Schwarz (2013)
Photoselective shade-netting	Tomato	Tinyane et al. (2013)
Photoselective shade-netting	Tomato	Ilic´ et al. (2015)
Grafting is a technique with positive results on the concentration of phytochemicals. However, there is a significant interaction of the rootstock, scion, and environment.		
Grafting	Tomato	Riga et al. (2016)
Grafting	Sweet cherry	Milinovic´ et al. (2016)
(b) Fertilizers, hydroponics, organic fertilizers, and metalloids		
The nutrition level of the plants and the application of specific nutrients can change the antioxidant capacity, the concentration of phytochemicals, and the dominant individual phytochemicals. N and Ca fertilization combined with warm temperatures has a positive effect on antioxidant concentrations. The application of metals, such as Zn, Fe, and Mn (2–10 µM) to plants and sprouted seeds raises the antioxidants and certain phytochemicals. The application of heavy metals such as Cd and Ag in cell cultures (1–2 mM) induces oxidative stress and antioxidants.		
Factor under control	Species	Reference
N fertilization	Broccoli	Fabek et al. (2012)
N fertilization and NO_3: NH_4 ratio	*Ocimum basilicum*	Saadatian et al. (2014)
Calcium	Broccoli sprouts	Yang et al. (2016)
Calcium and $ZnSO_4$	Broccoli sprouts	Yang et al. (2015)

(*Continued*)

Table 4.3: Summary of different environmental factors and crop management technologies that modify the nutraceutical quality. (*cont.*)

Manganese Zn Heavy metals	Sweet pepper *Nicotiana tabaccum* *Gymnema sylvestre*	Yasuor et al. (2015) Daş et al. (2016) Ch et al. (2012)
Using hydroponics allows for good phytochemicals production, as long as the production system does not promote the excessive and rapid growth of plants. In some cases, soil cultivation with adequate fertilization gave better results than hydroponics.		
Hydroponics versus soil Hydroponics versus soil Hydroponics Hydroponics	*Salvia plebeia* *Ocimum basilicum* *O. basilicum* *Echinacea angustifolia*	Chen and Kang (2014) Sgherri et al. (2010) Kiferle et al. (2011) Maggini et al. (2012)
Conventional production systems using inorganic fertilizers in soil and hydroponics promotes growth and yield; however, in most cases, the use of organic fertilizers in the soil and the application of compost and vermicompost tea in hydroponics allows for the production of more antioxidants and other phytochemicals. In soil cultivation, a significant interaction commonly occurs between the different treatments and plant species with climate and year factors.		
Low input agriculture Organic fertilizers Organic fertilizers Organic fertilizers Organic system Organic system Organic system Organic system Organic system Organic system Organic system	Durum wheat Tomato Cucumber Wheat Broccoli Cauliflower, endive and zucchini *Lupinus angustifolius* *P. vulgaris* Raspberries Tomato Tomato	Dinelli et al. (2013) Toor et al. (2006a) Santiago-Lopez et al. (2016) Gasztonyi et al. (2011) Øvsthus et al. (2015) Maggio et al. (2013) Jansen et al. (2015) Jakopic et al. (2013) Skupień et al. (2012) Migliori et al. (2012) Lahoz et al. (2016)
Biofortification with the metalloids selenium and iodine increased the total antioxidant activity, antioxidant vitamins, phenolics and other phytochemicals. The application of these metalloids should be performed at suitable concentrations (10^{-6}–10^{-4} M in nutrient solution or 10^{-4}–10^{-3} M applied with foliar spray) because a high amount induces oxidative stress and defense responses and decreases the concentration of some phytochemicals. An effective way to use both metalloids is by applying them to sprouted seeds.		
Selenium Selenium Selenium Selenium Selenium Selenium Selenium Selenium Selenium Iodine	Broccoli Broccoli Broccoli Cruciferous vegetables Lettuce *Opuntia ficus indica* Tomato Tomato Tomato *Opuntia ficus indica*	Robbins et al. (2005) Keck and Finley (2006) Bachiega et al. (2016) Brigelius-Flohé and Banning (2006) López-Gutiérrez et al. (2015) Bañuelos et al. (2012) Companioni et al. (2012) Becvort-Azcurra et al. (2012) Castillo-Godina et al. (2016) García-Osuna et al. (2014)

Table 4.3: Summary of different environmental factors and crop management technologies that modify the nutraceutical quality. (*cont.*)

(c) Abiotic elicitation, biotic elicitation, plant growth regulators, and biopolymers		
Phenotypic plasticity is the capacity to change plant developmental programs in response to environmental cues. The environmental signals may be abiotic or biotic. An environmental stress that is applied to the plants at low or moderate intensity may result in the increase of the phytochemical concentration; this process is named elicitation, and it is used to induce changes in gene expression and to modify developmental programs. However, if the impact of an environmental stress is very high, it decreases the concentration of phytochemicals. Environmental signals can be applied in a controlled manner to seeds and seedlings, changing the growth patterns and the profiles and thereby the concentration of phytochemicals in later stages of plant development. Similarly, the environmental stimuli applied to fruits and flowers cause variations in the postharvest physiology and nutraceutical quality.		
Factor under control	Species	References
Abiotic stress	*B. rapa*	Steinbrenner et al. (2012)
Weather/climate	*Origanum vulgare* ssp. *hirtum*	Tuttolomondo et al. (2014)
UV-B	*Brassica oleracea* var. sabellica	Neugart et al. (2014)
UV-B	*Kalanchoe pinnata*	Nascimento et al. (2015)
UV-B postharvest	Strawberries, blueberries, grapes, cherry tomatoes, white sweet corn, potatoes	Du et al. (2014)
UV-B	Melon	Sosa-Flores et al. (2014)
UV-C	Soybean, wheat, and sunflower	Foroughbakhch-Pournavab et al. (2015)
Laser irradiation of seeds	Sugar beet	Sacała et al. (2012)
Laser irradiation of seeds	Wheat	Jamil et al. (2013)
Salinity	Broccoli	López-Berenguer et al. (2009)
Salinity	*Cynara scolymus*	Borgognone et al. (2014)
Salinity	*Swertia chirata*	Abrol et al. (2011)
Salinity	Radish sprouts	Yuan et al. (2010)
Salinity	*Arabidopsis thaliana*	Martínez-Ballesta et al. (2015)
Salinity	Broccoli sprouts	Guo et al. (2013)
Salinity	*Ocimum basilicum*	Tarchoune et al. (2013)
Heat and hypoxia	Broccoli sprouts	Guo et al. (2016)
Ozone	*Melissa officinalis*	Tonelli et al. (2014)
Microbial elicitors (yeast polysaccharides)	*Vigna radiata*	McCue and Shetty (2002)
Microbial elicitors (cyanobacterial metabolites)	Rice, wheat, maize, cotton	Singh (2014)
Microbial elicitors (yeast extracts)	Lettuce	Złotek and Świeca (2016)
Microbial elicitors of *Rhizopus oryzae*	Soybean	Aisyah et al. (2013)
Mycorrhizae	Tomato	Hart et al. (2015)
Mycorrhizae	*Curcuma longa*	Dutta and Neog (2016)
Mycorrhizae and PGPB	Strawberry	Lingua et al. (2013)
Biotic elicitors (fish protein hydrolysates, lactoferrin, and oregano extract)	*Vicia faba*	Randhir and Shetty (2003)

(*Continued*)

Table 4.3: Summary of different environmental factors and crop management technologies that modify the nutraceutical quality. (*cont.*)

Biotic elicitors (chitooligosaccharides, chitosan, actinomycetes spores, and yeast extract)	Soybean	Al-Tawaha et al. (2005)
Biotic elicitors (yeast, *Escherichia coli, Bacillus subtilis, Agrobacterium rhizogenes*, and *Agrobacterium tumefaciens*)	*Andrographis paniculata* cell suspension cultures	Gandi et al. (2012)
Harpin (bacterial elicitor protein)	Tobacco	Dimlioğlu et al. (2015)
Plant extract (*Moringa oleifera*)	*Vicia faba*	Moussa and Hassan (2016)
Seaweed extract	*Phaseolus vulgaris*	Kocira et al. (2016)
Plant growth regulators are the internal signals that translate the environmental cues that the plant perceives. Plant growth regulators modify the status of different transcription factors that change the gene expression and the developmental programs, changing the profiles and concentration of phytochemicals.		
2, 4-Epibrassinolide	Broccoli sprouts	Guo et al. (2014)
2, 4-Epibrassinolide	Cucumber seedlings	Yuan et al. (2015)
H_2O_2	*Capsicum chinense*	Vargas-Hernández et al. (2016)
Jasmonic acid	*Artemisia annua*	Mehrjerdi et al. (2013)
Jasmonic acid	Ginseng in cell and tissue culture	Rahimi et al. (2015)
Jasmonic acid and benzothiadazole	*Vitis vinifera*	Ruiz-García et al. (2013)
Melatonin	*Malus prunifolia*	Yin et al. (2013)
Melatonin	Cherry	Zhao et al. (2013)
Melatonin	*Arabidopsis thaliana*	Bajwa et al. (2014)
Methyl jasmonate	Broccoli	Ku et al. (2013)
Prohexadione calcium	Tomato	Ramírez et al. (2010)
Salicylic acid, jasmonic acid, and methyl jasmonate	Seed sprouts of *B. oleracea*, *B. napus*, *B. rapa*, and *Raphanus sativus*	Baenas et al. (2014b)
Salicylic acid	Strawberry	Shafiee et al. (2010)
Salicylic acid	Strawberry	Babalar et al. (2007)
Salicylic acid	Cherry	Dokhanieh et al. (2013)
Salicylic acid	*Mentha piperita*	Figueroa Pérez et al. (2014)
Salicylic acid	*M. piperita*	Figueroa-Pérez et al. (2015)
Salicylic acid	*Lycium* spp.	Guan et al. (2014)
The application of chitosan and other biopolymers induces modifications in metabolism and gene expression that raises the concentration of antioxidants and other phytochemicals, in addition to the defense and antioxidant enzymes.		
Chitosan	Citrus fruits	Coqueiro et al. (2015)
Chitosan	Fruits and vegetables in postharvest	Romanazzi et al. (2017)
Chitosan	Spinach	Singh (2016)
Chitosan	Sweet cherry fruits	Petriccione et al. (2015)
Chitosan	*V. vinifera*	Tessarin et al. (2016)
Chitosan	*V. vinifera*	Portu et al. (2016)
Chitosan	*V. vinifera*	Iriti et al. (2011)
Chitosan and benzothiadiazole	*V. vinifera*	Vitalini et al. (2011)
Chitosan and salicylic acid	Tomato	Ortega-Ortíz et al. (2007)
Chitosan, salicylic acid and H_2O_2	*Capsicum annuum*	Mejía-Teniente et al. (2013)
Chitosan, selenium, and methyl jasmonate	Broccoli sprouts	Carvacho et al. (2014)

7 Conclusions

Obtaining more plant foods with a higher nutraceutical quality depends on a set of phytochemicals whose synthesis and accumulation is subject to regulation at different biological levels, such as the transcriptomic, proteomic, ionomic, metabolomic, and physiological levels. The environmental factors that control these activities are many and interact among themselves and with living organisms. Thus, the modeling and prediction of the nutraceutical quality over a range of environmental conditions are still pending tasks. However, the use of relatively simple and accessible agricultural practices makes it possible to increase and vary the concentrations and profiles of phytochemicals in the edible organs of plants.

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CHAPTER 5

Mushrooms as Sources of Therapeutic Foods

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1 Introduction to Mushrooms as Therapeutic Foods

The medicinal use of mushrooms has a very long tradition in the Asian countries, whereas their use in the Western hemisphere has been slightly increasing only in the last few decades. In essence, raw fruiting bodies, fungal extracts of different origin, or the extracted molecules from them are suitable for medicinal purposes. There are many ways to obtain fungal material, whether by collection in the wild, or cultivation of mushrooms in farms and harvesting of the fruiting bodies or by cultivation of mycelium in fermenters with liquid or solid substrates.

The mushrooms have components that can contribute to human wellness and mitigate threats and assaults that render the human body vulnerable to several life-threatening diseases including cardiovascular ailments, cancer, metabolitic (diabetes and obesity), and neurodegenerative disorders (Vikineswary and Chang, 2013).

It is generally accepted that mushroom extracts contain multiple components, each of which may have its own biological or medicinal effects. Mushroom-derived β-glucans have immunomodulatory effects; they affect both innate and acquired immunity. In addition, mushroom triterpenoids and some of the proteins have been referenced as imune-system modulators. It was previously shown that phenolic compounds act as potent antioxidants, but these compounds are also able to produce free radicals in the presence of Cu(II) and Fe(II), which may be a major mechanism for their apoptosis-inducing ability. Proinflammatory effects of medicinal mushrooms as caused by the generation of reactive oxygen species could, therefore be due to the presence of polyphenols rather than to β-glucans alone (Wei et al., 2008). Mushrooms packed with a wide array of bioactive components are excellent antioxidants and antiinflammatory agents. Secondary metabolites of mushroom that may have therapeutic potentials are alkaloids, antibiotics, lectins, lactones, and terpenoids (Wasser, 2010). Primary metabolites, such as enzymes-glucose oxidase, superoxide

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dismutase, peroxidases, and laccases may prevent oxidative stress (Chang and Wasser, 2012; Wasser, 2010).

In recent years, a growing interest has developed in the mechanisms of action of medicinal mushrooms. For over a thousand years, mushrooms have been used in folk medicine in Asia to prevent and cure a multitude of diseases of quite different nature.

The fungal species that showed the best biological potential with great success against different kind of diseases belong to the following genera: *Agaricus*, *Albatrellus*, *Antrodia*, *Calvatia*, *Clitocybe*, *Cordyceps*, *Flammulina*, *Fomes, Funlia*, *Ganoderma*, *Inonotus*, *Inocybe*, *Lactarius*, *Phellinus*, *Pleurotus, Russula, Schizophyllum*, *Suillus*, *Trametes*, *Xerocomus,* and so forth (Patel and Goyal, 2012).

2 Cultivation Technologies for Edible and Medicinal Mushrooms

There are over 2000 varieties of mushrooms reported to be edible, including about 700 with some pharmaceutical activities (Wasser, 2010). Edible mushrooms were only traditionally harvested wild, and collection from wild woodlands is still important in the world (Zhang et al., 2014a). Field collection of large quantities of wild mushrooms is season-dependent and not ecologically sustainable (Yang et al., 2012).

China's mushroom industry has a long history but reliable techniques for cultivation of fruiting bodies are known only for about 65 mushrooms. In recent years, production of mushroom has increased to a new level, especially in the countries with growing economy, such as China, Poland, Hungary, and India (Vikineswary and Chang, 2013) (Table 5.1). The progress of the mushroom industry over these years has made a great contribution to

Table 5.1: World mushroom production (1960–2011).

Year	Total Production (×1000 Tons)
1960	170
1965	301
1970	484
1975	922
1978	1,060
1981	1,257
1983	1,453
1986	2,182
1990	3,763
1994	4,909
1997	6,158
2002	12,250
2009	24,000
2011	Established: 29,000

developing the world rural economy. The total population directly engaged in the mushroom industry is now over 30,000,000. World production of mushrooms varies depending on the culture and continents; Asian countries produce more than 74.64% of world mushroom markets followed by Europe (19.63%), respectively, in 2014 (Rosmiza et al., 2016). In some countries, such as Brazil, where a tradition of mushroom cultivation is not very well established, cultivation is rapidly expanding (Souza-Dias, 2010).

Edible and medicinal mushrooms are possible to be cultivated in specific conditions. Saprobic species are specific in relation to nutrition and environmental conditions. Some grow on fresh wood residues (*Lentinus* spp., *Pleurotus* spp., *Flammulina* spp., *Auricularia* spp., *Agrocybe* spp., *Ganoderma* spp.), others only in slightly composed lignocellulose material (*Coprinus* spp., *Volvariella* spp.). In addition, some of the species grow on the well-composted material and animal manure (*Agaricus* spp.) whereas other grows in the soil and the humus (*Lepista* spp., *Morchella* spp.). It is not uncommon that certain types prefer particular substrates (straw or wood-specific plant species), as well as specific climatic conditions (cold and wet or warm and dry). In addition to these, commercially cultivated species of some mushroom are produced in small quantities in only some regions of the world or are only available through semicultivated techniques because they are mycorrhizal (species of the genera *Tuber*, *Cantharellus*, and *Morchella*).

Mushroom growth dynamics involve some technological elements. After the vegetative phase, the following induction of fruiting is regulated by the environmental factors: switching on the light, providing fresh air, and lowering temperatures. Although the principles of cultivation are commonly similar for all mushrooms, the practical technologies can be quite different for different species cultivated. The technologies have to be modified to the local climatic conditions, materials available for substrates, and varieties of the mushroom used.

The major practical steps/segments of mushroom cultivation are: (1) selection of an acceptable mushroom species; (2) secreting a good quality fruiting culture; (3) development of robust spawn; (4) preparation of selective substrate/compost; (5) care of mycelia (spawn) running; (6) management of fruiting/mushroom development and; (7) harvesting mushrooms carefully (Singh, 2011).

The most extensively cultivated mushroom worldwide is *Agaricus bisporus* followed by *Lentinula edodes* and *Pleurotus ostreatus*. Many other mushroom species are cultivated and commercialized as well due to their edible or medicinal properties: *Pleurotus eryngii, Pleurotus ferulae, Pleurotus nebrodensis, Pleurotus abalonus, Pleurotus pulmonarius, Pleurotus corucopiae, Pleurotus tuber-regium, Agaricus blazei, Agaricus bitorquis, Agrocybe aegerita, Auricularia auricular, Armillaria mellea, Coprinus comatus, Cordyceps militaris, Dictophora duplicata, Flammulina velutipes, Ganoderma lucidum, Grfola frondosa, Hericium erinaceus, Hypsizygus marmoreus, Lignosus* spp. (tiger's milk mushrooms), *Lepista*

nuda, Laetiporus sulphureus, Phellinus linteus, Phellinus baumii, *Tricholoma giganteum, Tremella fuciformis*, *Pholiota namenko*, *Volvariella volvacea* mainly for medicinal uses.

Submerged cultivation of different species of fungi has received increasing attention around the world and is viewed as a promising alternative for efficient production of biomass and valuable metabolites for some mushroom species. Petre et al. (2012) were focused on testing the most efficient food biotechnology to be applied in leading and controlling the edible mushroom submerged fermentation of *L. edodes*, *G. lucidum* and *G. frondosa*. The main advantages of cultivation is the faster production of both mycelial biomass and high value-added metabolites, in a shorter period of time within reduced space and less chance of contamination (De Silva et al., 2013).

As the world population is expected to continue to increase in the 21st century, food availability and adequate medical care to each individual may decrease. Mushrooms that make it possible to convert agro-residues to value-added food, fertilizer, and fuel thus generating a new cheap source of functional food from waste materials.

3 Mushrooms Uses in Traditional Medicine Versus Scientific Confirmation for the Uses

Some countries like China have long-term use of mushroom in their traditional medicines, and there is a huge proportion of natural remedies based on dried mushroom extracts that occupy an important segment, all around the world, on the pharmaceutical market dealing with natural medicines. In traditional medicine, mushrooms are used in the treatment of various conditions, including fatigue associated with aging, aches, pains, heart and lung diseases, and intestinal worms. There is an available literature demonstrating the use of mushrooms in cancer and immunological disorders. Nevertheless, there is a lack of scientific evidence that can fully support traditional uses of mushrooms and their extracts in the therapy of human diseases (Money, 2016). Many studies dealing with mushrooms as therapeutic agents are focused on in vitro data, suggesting that many clinical trials should be conducted to obtain the precise therapeutic value of mushrooms and their respective extracts. So far some of the isolated compounds were investigated in clinical trials giving supporting data just for the several species.

Clinical studies investigating the influence of mushroom preparations on pathological disorders are very rare. One of a very few of them conducted by Japanese researchers has discovered that the mushroom compound lentinan in combination with other drugs elongated life span of the patients suffering from the stomach cancer (Ina et al., 2013). Besides the lack in clinical trials, there are a lot of in vitro scientific evidences (Ferreira et al., 2015) that support mushrooms as sources of medicinal agents, such as antioxidants, antimicrobials, and anticancer agents.

This chapter will mainly focus on in vitro studies demonstrating potential benefits of mushrooms consumption, giving special emphasis on necessity for further clinical investigations to support these data.

4 Agaricus *spp.*

Agaricus genus represents the most important cultivated edible mushroom. *A. bisporus* (J.E. Lange) Emil J. Imbach, a "common button mushroom," has the leading position among edible cultivated mushrooms, whereas *A. brasiliensis* Waser is cultivated all around the world for its medicinal properties (Llarena-Hernández et al., 2013). These species have been demonstrated to express diverse and valuable medicinal properties including antitumor, antiaromatase, antimicrobial, immunomodulatory, anti-QS antiinflammatory, as well as antioxidant, activities (Ferreira et al., 2010; Jeong et al., 2010; Kozarski et al., 2011; Moro et al., 2012; Sokovic et al., 2014). *A. brasiliensis* is an edible fungus belonging to the Brazilian biota, and has traditionally been used to treat cancer and other diseases (Wang et al., 2013). This species is also known as *A. blazei* Murill (ABM) and is a relative newcomer that was first found in 1960 in Piedade (Brazil) (Fig. 5.1).

The medicinal effects of ABM have been attributed to β-glucans (acting as immunomodulators), phenols, and terpenes (redox regulators), both enhance or attenuate the immune system of higher animals by inducing or inhibiting the production of pro- and antiinflammatory cytokines (Hetland et al., 2013). In vitro ABM induces and balances immunity once proinflammatory processes are taking place, whereas in higher animals ABM quickly stimulates innate immunity—that is, it causes rapid proinflammatory effects when under threat by infectious agents or cancer (Smiderle et al., 2013). The antimicrobial effects of ABM as such, have been attributed to the immune system. Fantuzzi et al. (2011) presented the role of ABM in prevention of innate immunity was presented by who showed



Figure 5.1: ***Agaricus blazei*.**

that ABM extract did not promote immunostimulation and protection during experimental *Salmonella enterica* infection in mice. In the last few decades, numerous studies have reported the cytotoxic and antitumor properties of *A. brasiliensis* polysaccharides, which mainly act through immunomodulatory mechanisms but also by direct cytotoxic effects on tumor cells. The effects of agaritine derived from *A. brasiliensis* were studied on human leukemic monocyte lymphoma cells. It was shown that agaritine induced moderate apoptosis in the tested cells, which was demonstrated by using common cellular markers of aptotosis (Akiyama et al., 2011). Stojković et al. (2014) showed that *A. bisporus* and *A. brasilensis* are good sources of bioactive compounds, such as tocopherols, fatty acids, organic, and phenolic acids. Both of the species possessed antioxidant effect tested by different methods, as well as antimicrobial effect tested on different strains of bacteria and fungi. Finally, it was shown that extracts possessed antimicrobial preserving effects when incorporated in yoghurt.

Agaricus bohusii Bon is an edible and prized mushroom especially common in southern Europe. *A. bohusii* extract is a rich source of carbohydrates and proteins, containing the only isoform γ-tocopherol of tocopherols investigated. Polyunsaturated fatty acids also predominated over mono and unsaturated fatty acids. Of the phenolic and phenolic related compounds *p*-hydroxybenzoic, *p*-coumaric acids, and γ-L-glutaminyl-4-hydroxybenzene and cinnamic acid were detected in the sample, respectively. Malic, oxalic, and fumaric acids were the organic acids identified and quantified in *A. bohusii*. High concentration of total phenolics was in correlation with strong antioxidant capacity. Methanol extract successfully inhibited development of *P. verucosum* in cream cheese. Sensory evaluation showed cream cheese in combination with *A. bohusii* extract more acceptable to panelists than cream cheese alone (Reis et al., 2012).

Glamočlija et al. (2015a) investigated nutritional value and biological activity of *A. bisporus, A. bitorquis*, *A. campestris*, and *A. macrosporus* (Fig. 5.2). The studied species are low in fat



Figure 5.2: *Agaricus macrosporus*.

and rich in proteins and carbohydrates, presenting *A. campestris* with the lowest caloric value and total sugar content. Phenolic and organic acids, as well as tocopherols (γ-tocopherol) were found in the highest concentrations in *A. campestris*. Methanolic extracts showed higher antioxidant, but lower antibacterial and antifungal potential than ethanolic ones. The biofilm-forming capability of *P. aeruginosa* was also reduced in a concentration-dependent manner. Furthermore, ethanolic extracts demonstrated statistically significant reduction of virulence factors, such as AQ inhibition zones, twitching, and swimming motility of the tested bacteria. The extracts of the tested *Agaricus* species are a promising source of antioxidant, antimicrobial, and antiquorum sensing compounds and is proved to be healthy foods. Regarding the antibacterial and antifungal potential, the ethanolic extracts were more effective than the methanolic ones. Ozturk et al. (2011) recently published the antimicrobial activity of three *Agaricus* species. They described effects of methanolic extracts against six species of Gram-positive bacteria, seven species of Gram-negative bacteria, and two species of yeasts. Giri et al. (2012) described a methanolic extract of *A. campestris* from India that showed antimicrobial activity against seven bacterial species.

5 Antrodia *spp.*

Antrodia camphorata Gilbertson and Ryvarden is described as a fungal parasite on the inner cavity of *Cinnamomum kanehirae,* the endemic species of Taiwan. *A. camphorata* only grows in Taiwan, and it is being used in Taiwanese traditional medicine for different health-related conditions: cold, influenza, headache, and fever, for twisted tendons and muscle damage, terrified mental state, and many internal diseases (Tzeng and Geethangili, 2011). Previous studies have shown that 78 compounds were isolated and identified in *A. camphorata*. Most of them are represented by triterpenoid compounds with similar structures, having lanostane or ergostan skeleton (Popović et al., 2013). Several other constituents have been described in this species, such as polysaccharides, lignans, benzoids, benzoquinones, sterols, and fatty acids.

Mycelium and fruiting bodies of this species were shown to have antiproliferative effects on different human carcinoma cell lines, tested by in vitro and in vivo methodologies. Studies on extracts showed that they can promote immune responses by exhibiting antileukemic activity in leukemia BALB/c mice and can activate the immunomodulation of macrophages in a human hepatoma cell model (Popović et al., 2013). It has been shown that antroquinonol, isolated from this extract, exhibits anticancer activity against hepatocellular carcinoma through activation of 5'AMP-activated protein kinase and mTOR pathway inhibition (Popović et al., 2013).

Extracted mycelium of *A. camphorata* has shown to have in vivo antihepatitis B virus activity in a dose-dependent fashion without showing cytotoxicity to normal cells, thus suggesting this fungus as a possible therapeutic agent. *A. camphorata* is a strong antioxidant agent and



Figure 5.3: *Boletus aereus*.

also has hepatoprotective features. Methanolic extract of *A. camphorata* at dose of 10 mg/kg showed significant antihypertensive effect in rats induced with hypertension (Tzeng and Geethangili, 2011).

All these findings suggest that *A. camphorata* might be developed in functional therapeutic agents, after careful conduction of preclinical and clinical investigations.

6 Boletus *spp.*

Boletus is a genus of mushrooms, comprised of over 100 species. The genus *Boletus* was originally broadly defined and described by Fries in 1821, essentially containing all fungi with pores. Almost all of these fungi grow in symbiotic association with trees, forming mycorrhizas that aid the uptake of mineral nutrients from soil (Binder and Hibbett, 2006).

The antimicrobial potential of methanolic extract of *Boletus aereus* (Fig. 5.3) has been investigated by the microdilution method. The extract possessed in vitro antimicrobial activities against a panel of pathogenic bacteria and microfungi, and successively inhibited the growth of meat contaminant bacteria, both at 25 and 4°C, after 7 days of inoculation (Stojković et al., 2015). The evaluation of methanol and water extracts from *Boletus edulis* suggested that water extracts had good antimicrobial potential. The metanolic extract of *Boletus lupinus* showed selective antibacterial properties, but it did not possess antifungal activity.

Antioxidant potential of methanolic extract of *B. aereus*, revealed a good reducing power (EC_{50} 0.75 mg/mL), scavenging of DPPH radicals (EC_{50} 3.29 mg/mL), inhibition

of β-carotene bleaching (EC_{50} 2.89 mg/mL), and lipid peroxidation inhibition (EC_{50} 0.33 mg/mL) (Stojković et al., 2015). The antioxidant capacity of ethanolic, methanolic, cold water, and hot water extracts, and the identification of compounds from the fruiting bodies *B. edulis* were investigated by Vamanu and Nita (2013). The researchers showed that the ethanolic extract of Romanian wild mushroom *B. edulis* represents a natural source of functional compounds. The antioxidant activities of three crude polysaccharides from *B. edulis* were investigated in vitro systems. Among these three polysaccharides, one fraction showed more significant reducing power and chelating activity; and highest inhibitory effects on superoxide radical and hydroxyl radical (Zhang et al., 2011). Luo et al. (2012) purified three major polysaccharide fractions from *B. edulis*. All showed in vitro and in vivo antioxidant activities. Kosanić et al. (2012) reported the acetone extract of the highly valued and endangered species *B. edulis*, originating from Serbia, reporting that it was more effective than α-tocopherol, BHA, and BHT. The polysaccharide (BSFP-1) with a molecular weight of 1.33×10^4 Da was isolated from the fruiting bodies of *Boletus speciosus.* Good antioxidant potential of BSFP-1 was observed in the study of Ding et al. (2012).

The effect in vitro cytotoxic activity of three nonisoprenoid botryane sesquiterpenoids, boledulins A–C, isolated from the cultures of *B. edulis,* were investigated by Feng et al. (2011). Boledulin A exhibited moderate inhibitory activity against five human cancer cell lines, while compounds Boledulin B and C were inactive against all the tested cell lines with IC_{50} values of more than 40 μM.

Wang et al. (2014b) purified a polysaccharide (BEP, Mw = 113,432 Da) from *B. edulis* and demonstrated that BEP possesses potential immunomodulatory activity and might be employed as effective therapeutic agents for the prevention of renal caner.

The water and methanol extracts from *B. edulis* possessed antiviral properties against herpes simplex virus type 1 (HSV-1) (Santoyo et al., 2012).

7 Cordyceps *spp.*

Cordyceps species are an entomopathogenic fungi widely used in traditional Chinese medicine. Das et al. (2010) reported that different constituents from *Cordyceps* species possessed antioxidant/antiaging, antimicrobial, immunomodulatory, antiinflammatory, and antitumor effects. Cordycepin as the major constituent of *C. militaris,* detected from fruiting bodies, is actually a derivative of the nucleoside adenosine (Fig. 5.4). These days this molecule could be produced industrially because of its antibacterial, insecticidal, and antitumor activities. Among other products isolated from *C. militaris* are polysaccharides and ergosterol with a number of biological effects: antioxidant, antiinflammatory, antimetastatic, antitumor, immunomodulatory, steroidogenic, hypoglycaemic, and hypolipidaemic. Polysaccharides extracted from fruiting bodies of *C. militaris* showed immunostimulating



Figure 5.4: ***Cordyceps militaris*.**

properties and stopped growth of melanoma cells tested in vivo on mouse model (Lee and Hong, 2011). Rao et al. (2010) also proved that different extracts and compounds of *C. militaris* possessed antiinflammatory, antiproliferative, and antiangiogenic activity. Polysaccharidic extracts are responsible for the in vitro antioxidant capacity of *C. militaris* (Chen et al., 2013; Wang et al., 2012a,b).

Another bioactive compound from *C. militaris* is cordymin. This peptide was studied for its antifungal properties, and found to inhibit mycelial growth of *Bipolaris maydis*, *Mycosphaerella arachidicola*, *Rhizoctonia solani* and *Candida albicans*. Cordymin also displayed antiproliferative activity toward breast cancer cells (MCF-7) (Wang et al., 2012a,b). Mannitol and trehalose were found in *C. militaris* by Reis et al. (2013). These compounds showed good diuretic, free-radical activities and antitussive effects (Das et al., 2010). Citric acid was detected in higher percentage (Reis et al., 2013) in this mushroom. This compound plays an important role in human metabolism especially for structure of bones (Hu et al., 2010). *p*-Hydroxybenzoic acid was the only phenolic acid compound presented in the extract of *C. militaris* (Reis et al., 2013). Hydroxybenzoic and hydroxycinnamic acids are the basic structure of phenolic acids. These compounds exhibited antioxidant activity because of their possibilities to be good free radical scavengers, on peroxyl and hydroxyl radicals, peroxynitrites, and superoxide anions (Carocho and Ferreira, 2013). Stearic acid, palmitic acid, linoleic acid, and oleic acid were detected as major fatty acids in this species. Polyunsaturated fatty acids were dominant with 68.87%, whereas saturated fatty acids were presented with 23.40%, and 7.73% was monounsaturated fatty acids (linoleic acid 68.00%) (Reis et al., 2013). The importance of unsaturated fatty acids is that they could protect against cardiovascular disease and decrease blood lipids. Moreover *C. militaris* is a great source of nonfat compounds. Reis et al. (2013) found that δ-tocopherol is the only form of tocopherols isolated from this species. This work represents the first result considering to organic and phenolic acids, and vitamin E isolated from *C.*

militaris. Authors showed that this extract possessed a number of biological activities: reducing power inhibition, lipid peroxidation inhibition, scavenges free radicals, and high antimicrobial effects. Extract of *C. militaris* showed much better antibacterial activity than commercial antibiotics, and antifungal activity was better than mycotics against *Penicillium ochrochloron*, *P. funiculosum,* and *Trichoderma viride*. This study proved that extracts of *C. militaris* could be used as a good alternative to synthetic antimicrobial agents in prevention and treatment of different plant, animal, human pathogenic species, and food-borne pathogens. It could be applied as a good preservative in food production and in mushroom and plant cultivation. Methanolic extract of *C. militaris* tested in previous studies exhibited an inhibitory effect on cell growth against a few human tumor cell lines, but did not affect tumor porcine liver primary cells. Methanolic extracts of *C. militaris*, mycelia, and fruiting body also confirmed this effect in other human tumor cell lines (Liu et al., 2014; Reis et al., 2013). Cordycepin isolated from *C. militaris* inhibited growth of human leukemia cell by inducing apoptosis (Jeong et al., 2011). Authors concluded that this effect is probably due to the generation of reactive oxygen species, activation of caspases, mitochondrial dysfunction, and cleavage of poly(ADP-ribose) polymerase protein. Apoptosis of human leukemia cells induced by cordycepin is caused by a cascade involving an ROS-mediated caspase pathway. Two acidic polysaccharide fractions, were extracted in fruiting bodies of *C. militaris,* cultivated one, and evaluated for proliferation of mouse splenocyte activity in vitro (Wu et al., 2012). Both fractions possessed dose-dependent mitogenic effects on mouse splenocytes, and could synergistically promote murine T- and B-lymphocytes induced by Con A and LPS. These results are valauble for the explanation of the connection between polysaccharide structures and their biological activities. Bizarro et al. (2015) tested the mechanism of action of *C. militaris* methanolic extract on lung cancer cell line (NCI-H460). The extract decreased cellular proliferation, induced cell cycle arrest at G0/G1, and increased apoptosis, and increased the levels of p53 and p21. An increase in p-H2A.X and 53BP1 levels, the number of 53BP1 foci/cell (all indicative of DNA damage), were also observed after treatment with the extract. This extract affected NCI-H460 cellular viability through a mechanism involving DNA damage and p53 activation, which supports the using use of extract as a source of bioactive compounds, which may be used in anticancer strategies.

8 Ganoderma *spp.*

Genus *Ganoderma* was established in 1881, when mycologist Karsten found it. It is one of the most numerous genus in the order Aphyllophorales, consisting of nearly 300 species, that mostly grows throughout tropical regions. The species is not edible, which seems obvious, considering that the surface of the mushroom is covered with thick and smooth skin, and fruiting body is not fleshy, but rather corky and tough (Ćilerdžić, 2011). This species is one of the most tested mushroom, especially in Chinese traditional medicine (Baby et al., 2015). Nearly 23 species of genus *Ganoderma* have been intensively studied in the last couple of



Figure 5.5: ***Ganoderma lucidum*.**

years due to a variety of biological activities, including *G. lucidum* (Fig. 5.5), *G. applanatum*, *G. cappense*, *G. austral,* and many others (Hakkim et al., 2016; Peng et al., 2016).

The literature survey since 2010 concerning the chemical characterization of bioactive metabolites isolated from *Ganoderma* sp., indicates the presence of the following compounds: benzopyran-4-one derivatives, benzofurans, couple of alkaloids, steroid compounds, C15 sesquiterpenoids, farnesyl hydroquinones (meroterpenoids), meroterpenoids, C30 pentacyclic triterpenes, C24, C25 lanostanes, C27 lanostanes (alcohols, lactones, esters), C27 lanostanes (lucidenic acids), as well as many other esters, lactones, and volatile compounds (Baby et al., 2015; Ferreira et al., 2015). Ultimately it can be concluded that the number of chemically characterized compounds is close to 430, and that a large number of these compounds showed potent biological activity (Baby et al., 2015). In a review study on *G. lucidum* (Ferreira et al., 2015), it was emphasized that polysaccharides originating from different parts of the mushroom have been subject of numerous studies on potential biological activity. These molecules showed potent activities including antioxidant, antitumor, antibacterial (in both in vitro and in vivo systems), and so forth (Ćilerdžić et al., 2016; Tel-Çayan et al., 2015; Young et al., 2016). Various activities of the polysaccharides may be attributed to their huge structural diversity (Ferreira et al., 2015). Experimental data on the mushroom's fruiting body, as well as mycelium and broth showed their significant biological activities. Investigation of biological activities of ethanol mycelium extracts of selected *Ganoderma* strains (Ćilerdžić et al., 2016) exhibited low to moderate scavenging activity, depending on the species. This activity positively correlated with total phenolic content, as it is usually the case. From the study of antimicrobial activity, it may be concluded that it has been species and type of protocol-dependent (disc-diffusion or microdilution). Tested species showed very good microbicidal effects (Ćilerdžić et al., 2016). In addition to scientifically confirmed antimicrobial activity of crude extracts *of G. lucidum*, isolated compounds as sulfated (1,3)-β-D-glucans from mycelium showed antimicrobial and

cytotoxic activity as well. Antimicrobial activity of the glucans was not as expressive, whereas results of cytotoxic activities showed that the compound is potentially safe for use, since it did not show cytotoxic effects on the PN2TA cell line (normal prostate epithelium cells) (Young et al., 2016). Polysaccharides isolated from a spent compost of mushroom *G. lucidum* yielded two fractions of polysaccharides, which showed reducing power in vitro, DPPH scavenging activity, and lipid peroxidation effect in vivo systems. Along with this result of dealing with isolated compounds, the most recent study deals with the isolation and identification of oxygenated lanostane-type triterpenoid compounds from chloroform extract of *G. lucidum* fruiting body (Zhao et al., 2016). Estimates by the World Health Organization from the year 2000 indicate that nearly 171 milion people are affected by potentially deadly illness diabetes mellitus and that by the year 2030. This number will increase to 366 million affected patients. Natural-sourced hypoglycemic activity has been scientifically confirmed more than once, as it is the case with *G. lucidum*. In a study by Ma et al. (2015) more than one compound was involved in antidiabetic activity of *G. lucidum*—namely, the compounds polysaccharides, proteoglycans, triterpenoids, and proteins. Along with the fact that bioactive molecules isolated from *G. lucidum* possessed hypoglycemic effects (decreasing the sugar level in the blood), they also affect other aspects of the disease. Since diabetes is considered as an autoimmune disease, and compounds isolated from the mushroom are able to regulate immune response, correlation between these two is obvious, but not simple. In addition, these compounds have an overall positive effect on the symptoms of the diabetic (retinopathy, nephropathy, and neuropathy), so they make it manageable and somewhat easier on a daily basis (Ma et al., 2015). *G. lucidum* was found to possess antiinflammatory activity against autoimmune diseases, such as lupus erythematosus (Cai et al., 2016). Symptoms of the disease include inflammation of joints, proteinuria, edema, and palpitations of the heart. Mushroom proved to be very effective in improving clinical effects of lupus in treated experimental animals (Cai et al., 2016). Extraction of another species named *G. capense* has led to isolation and complete elucidation of structure of eight aromatic meroterpenoids, which possessed potent radical scavenging activity (DPPH) (Peng et al., 2016). Another *Ganoderma* species, named *G. atrum*, proved to be a rich source of significant antioxidative and immunostimulating activities. With the use of ion exchange chromatography, two fractions were obtained, one of which showed good antioxidant activity, and the other (polysaccharidic) proved to stimulate macrophage cell (Zhang et al., 2016). This species showed neuroprotective and antioxidant activity, as well as in another study where it was attributed to the isolated lanostanoid triterpenes. The remarkable feature of the study is the fact that values that indicate mushroom's potent activities are in the range of commercially available synthetic compounds (Qiu et al., 2016). The first report on antioxidant and cholinesterase-inhibitory activities together with the total phenolic contents was determined in a study by (Tel-Çayan et al., 2015). Low-molecular-weight compounds isolated from *G. adspersum* proved to possess prominent activity. An interesting research considers the possibility of encapsulating *G. lucidum* polysaccharide into liposome, which

will be inoculated into experimental animals afterwards. This polysaccharide will, as a consequence, be more effective when delivered to the immune system. Mice immunized with this polysaccharide exhibited higher antigen-specific IgG antibodies, better splenocyte proliferation, higher cytokine secretion by splenocytes, and significant activation of $CD3^+CD4^+$ and $CD3^+CD^+$ T cells. The results indicate the polysaccharides possibility to act as a vaccine delivery system, enhancing immune response (Liu et al., 2016). Scientific data on activity on *Ganoderma* species are numerous, and although still in the spotlight of academic circles, they present an inexhaustible source of disease treatment possibilities.

9 Hericium *spp.*

Hericium presents genus of edible mushrooms in the Hericiaceae family. This genus was previously placed within the order Aphyllophorales, but recent molecular studies now place it in the Russulales (Hallenberg et al., 2013). *Hericium* means *hedgehog* in Latin. This species often fruits high on living tree trunks in Asia, Europe, and North America and generally throughout the North temperate latitudes. The fruit bodies typically have short stalks. Mature specimens are easily identified by drooping spines, which hang down; the spines may be arranged in clusters or, more usually, in rows. Positive identification of immature specimens can be more difficult as they often begin as a single clump, developing their branches as they age. The phylogenetic relationships of many *Hericium* species were analyzed by comparing the rDNA internal transcribed tracer (ITS) sequences. This genus is easily cultivated, particularly *H. abietis* and *H. erinaceus* (Fig. 5.6).



Figure 5.6: *Hericium erinaceus*.

In China and Japan, *Hericium* are highly valued for their medicinal properties specially *H. erinaceus* and it has been also called Lion's Mane Mushroom in Western countries, Monkey Head Mushroom in China and Yamabushitake in Japan. Fruiting is usually seen from late August to December and only rarely is there two "flushes" of fruiting from a single location on a tree within a year. The fungus is characterized by intermittent fruiting on occupied trees over several decades, and this often is prolonged for many years after the branch or trunk falls to the ground.

Health benefits of *H. erinaceus* have been known for hundreds of years but numerous studies began in the late 1990s to early 2000s. Scientists reported the potential medicinal values of fruit bodies and mycelium of *H. erinaceus* mushroom and their chemical extracts.

Khan et al. (2013) summarized advances in information on antioxidant activity in mycelium extract of *H. erinaceus,* which is rich in phenolic content and possesses antioxidant power. Extract of fresh fruit body also showed strong DPPH radical scavenging activity, whereas oven-dried fruit body extract was excellent in reducing the extent of β-carotene bleaching. In a recent study by Han et al. (2013) *H. erinaceus* polysaccharides showed significant antioxidant activity against ischemia reperfusion-induced renal oxidative injury damage in experimental animals.

Researchers found that various *H. erinaceus* extracts inhibited the growth of multiple Gram-positive and Gram-negative pathogenic bacteria. These extracts, bioactive erinacines and polysaccharides have been shown to be potent anti-MRSA activity, with minimum inhibitory concentrations (MIC) values ranging from 62.5 to 500 μM (Friedman, 2015). A report by Kim et al. (2012) on the inhibition of adverse effects of *Salmonella* in mice via stimulation of the immune system by mushroom extracts suggests that *H. erinaceus* mushrooms or extracts could be used to improve microbial food safety and infectious diseases.

The anticancer potential of different extracts of mycelium *H. erinaceus* was also demonstrated in animal systems. The hot water extract of mycelia, consisting primarily of polysaccharides, was shown to have antihepatocarcinoma activity. A protective effect for the methanol (MeOH) extract of mycelia on carbon tetrachloride (CCl4)-induced hepatic damage also reported (Wang et al., 2014a). The hot water and 50% EtOH extracts significantly suppressed growth of tumors induced by the colon carcinoma cell line CT-26 by daily intraperitoneal injections on mice.

Among the compounds isolated from *H. erinaceum* fruiting bodies and cultured mycelia, most interesting are the low-molecular-weight compounds belonging to a group of cyathin diterpenoids "hericenones" and "erinacines," molecules stimulating the differentiation and re-myelination of neurons. A study by Mori et al. (2011) examined the effect of oral administration of *H. erinaceus* powder added to the mouse diet over a 23-day experimental period on the amyloid β(25−35) peptide-induced learning and memory deficits (cognitive

dysfunctions) in mice. A review by Wong et al. (2012) on the treatment of peripheral nerve injury by *H. erinaceus,* concludes that the neuroprotective effects of *H. erinaceus* in rodents needs to be confirmed in human studies.

Lion's Mane Mushroom is a precious resource compound with gastroprotective activity (Wong et al., 2013), wound healing property (Khan et al., 2013) and different immune modulating activities (Friedman, 2015) that is rarely found in nature. Besides reports on *H. erinaceus* fruiting bodies cultivation and production of mycelial biomass as raw materials for culinary and medicinal use and polysaccharides by submerged culture, reports on biomass cultivation in bioreactors, particularly in solid-state reactors, are rare (Gerbec et al., 2015). The consumers at large do not know the potential of this unique mushroom to biosynthesize about 70 different secondary metabolites.

10 Laetiporus *spp.*

Genus *Laetiporus* was first described in the beginning of the twentieth century as a brown-rot mushroom, from Aphyllophorales family. It is readily recognizable, due to a strikingly yellow color of the bracket shaped fruiting bodies. *L. sulphureus* grows in form of clusters comprised of 5–50 fleshy fruiting bodies (Fig. 5.7), up to 40 cm wide, which are usually found on hardwoods and conifers throughout mild climate zones of Europe, Asia, and North America (Grienke et al., 2014).

Common names for the mushroom include: sulfur polypore, sulfur shelf and chicken of the woods. Fresh fruiting bodies have a pleasant odor and are used as culinary delicacy (Grienke et al., 2014). Since previous studies indicated that *L. sulphureus* is a rich natural source of bioactive compounds, an attempt was made in pharmacognostic standardization of *Laetiporus* specimens from different localities (Acharya et al., 2016; Petrović et al., 2013). Different organoleptic properties, such as scent, taste, and nature of the powdered samples



Figure 5.7: ***Laetiporus sulphureus*.**

were evaluated, as well as physicochemical properties (ash content, loss on drying, hydration property, etc.) and fluorescence analysis. Results suggest reliable data that may perhaps make possible introduction of unique criteria for standardization of the mushroom sample (Acharya et al., 2016). In a study by Grienke et al. (2014) it has been indicated that significant efforts were made in order to chemically characterize polypore's primary and secondary metabolites, such as polysaccharides, proteins, pigments, nucleic acids, terpenoids, and so on. Likewise, testing the potential biological activity was the subject of numerous studies. Various extracts of *L. sulphureus* proved to have very potent antioxidative, antimicrobial, acetyl-cholinesterase inhibitory activities, as well as hypoglycemic effect. The latter has a great significance, since diabetes presents a major metabolic disorder in both adults and children, causing serious health problems. Results of the study indicate that if orally applied, polysaccharides fraction extracted from the mycelia of *L. sulphureus* strongly alleviate plasma glucose level (up to 43.5%) in experimental animals. Possible mechanisms of the mushroom's action include enhancement of insulin secretory activity, alleviation of oxidative stress, and preservation of β-cell integrity; even though these assumptions are not entirely reliable, the true mechanism remains to be discovered (Hwang and Yun, 2010). Results showed that mushrooms extracts possessed antioxidant activity. This activity has been observed not only for the wild mushrooms, but for the cultivated ones as well (Karaman et al., 2010). In a study by Olennikov et al. (2011) antioxidant activity, including other potential health benefits of *L. sulphureus* may be attributed to the detected phenolic compounds. The following compounds were cultivated for the first time from fruiting bodies: *p*-coumaric acid, quercetin, kaempferol, caffeic acid, catechin, gallic acid, and 5-caffeyl quinic acid. All the compounds exhibited antioxidant activity to a different extent. Results concerning antimicrobial activity of *L. sulphureus* are numerous and include antimicrobial potential of different extracts (Petrović et al., 2014a,b; Sinanoglou et al., 2015). Some results indicate low activity with a high phenolic acid compounds profile, which indicates that these compounds are not involved in antimicrobial activity. Other results provide an overview of antimicrobial activity of volatile components, lipid compounds, and so forth. Analyses of methanol, acetone, and dichloromethane extracts of *L. sulphureus* showed differences between their volatile components, which may indicate that these components can be a reliable parameter for chemotaxonomic studies of the species (Petrović et al., 2013). As for the chemical characterization of compounds, hydrocarbons predominated in all the extracts, whereas 56 compounds were identified. The aforementioned extracts showed better antibacterial than antifungal activity, and considerably lower activity than the commercial antibiotics and mycotics. Nevertheless, since mushroom extracts are mixture of compounds, pathogenic microorganisms are less likely to develop resistance to their activity. Employment of three extraction methodologies (classical, ultrasound assisted, and high-energy techniques) provided information of dependence between chemical components and biological activity, and the type of extraction. The applied extraction technique had a narrow effect on the antioxidant properties of the mushroom extracts, except for their total phenolic content, which

highly increased the ultrasound-assisted extracts. As for the antimicrobial activity, extracts possessed good antimicrobial activity regardless of the type of extraction methodology (Petrović et al., 2014a). Chemical profiling of the *L. sulphureus* fruiting body showed a series of biologically active compounds, as well as nutritionally beneficial compounds (polyphenols, vitamins, unsaturated fatty acids, sugars, etc.). In addition, *L. sulphureus* proved to be a very successful biocontroling agent in in situ food systems (tomato paste and chicken patè), having dose-dependent and time-dependent influence on food-borne pathogen *Aspergillus flavus* (Petrović et al., 2013, 2014b). Antimicrobial activity of the mushroom's lipid fraction indicates that other compounds besides phenolic may possess this activity. Results of the study showed that extraction methodology and solvent type have influence on the fatty acid profile, but the activity is solely attributed to the type of solvent and the applied dose. Hexane extracts possessed better antifungal and slightly better antibacterial activity as compared to chloroform extracts. Neutral lipids (NL), which predominated in all extracts, mainly consisted of triglycerides (TG) followed by sterols. As for the polar lipids (PL), phosphatidylcholine (PC) seemed to have the highest content followed by phosphatidylethanolamine (PE) (Sinanoglou et al., 2015). The results of cytotoxic activity indicate that methanolic and polysaccharidic extracts were not toxic for porcine liver cells (GI50 $>$ 400 μg/mL) (Petrović et al., 2014a). A great biotechnological breakthrough is the development of a successful method for a large-scale cultivation of both edible and medicinal mushroom *L. sulphureus.* In a study by Pleszczyzska et al. (2013) it has been emphasized that the variation of cultivation conditions affected both the properties of carpophores and the time of fructification. Organic additive and moisture content of the substrate were the most influential factors in forming carpophores. Low organic supplementation and high water content were negatively correlated with the time of fructification (they formed later in comparison to high organic supplementation and lower water content substrates). This may be of great importance since it may contribute to constant yield of uniformly characterized fruiting bodies, which may have, in the end, considerable biotechnological implementation. Concerning isolation of pure compounds from *L. sulphureus*, one compound has been characterized and named Sulphureuine B (Zhang et al., 2015). It has been described as drimane type sesquiterpenoid; experimental data indicate that this compound induces apoptosis, mediated through endoplasmic reticulum stress, mitochondrial, and death receptor signaling pathways (Zhang et al., 2015). From a literature survey it can be concluded that the chemical characterization of the mushroom is still ongoing. Obtained data contribute to a better understanding of the mushroom and to its better applicability (utilization) in biotechnological and medicinal studies.

11 Lentinula *spp.*

Lentinula is a small genus of wood-dwelling fingi in the family Marasmiaceae, order Agaricales. *Lentinula* fungi feed saprobically on the deadwood of broad-leaved trees in the tropical and subtropical regions of North and South America, Asia, and Australia. The fruiting



Figure 5.8: ***Phellinus linteus*.**

bodies of these fungi are generally light-coloured to reddish brown or black, with a convex to flat cap supported by a fibrous stalk. The pileus can be 2–25 cm in diameter, depending on the species, and features white gills on the underside. *Lentinula* species characteristically produce white spores.

L. edodes species (shiitake, maitake, and reishi) is the most famous and is one of the world's second largest cultivated mushroom (Li and Hu, 2014) (Fig. 5.8). It has been referred to as a "medicinal mushroom" due to its long history of medical use, particularly in oriental medicine traditions (Bisen et al., 2010). Shiitake mushrooms are a wide variety of conventional nutrients for microelements, vitamins, and dietary fiber. *L. edodes* is the most studied species and has shown to present medicinal compounds, including polysaccharides, terpenoids, sterols, and lipids. Each of these compounds has been isolated from fruiting bodies and mycelia and provides *L. edodes* with many therapeutic applications.

Several studies have demonstrated the antioxidant properties of *L. edodes* for aqueous extract of the fruiting body, exudates obtained from the mycelium, and crude extract of polysaccharide. All reports have shown antioxidant activity with high phenolic content (Finimundy et al., 2013).

L. edodes extract showed antimicrobial action against both Gram-positive and Gram-negative bacteria, fungi, and viruses. Inhibitory antibacterial activity of reishi ethyl acetate extract against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus,* and *S. epidermidis*. Aqueous extract demonstrated good activity against MRSA. *Streptococcus pyogenes* was very sensitive to the *L. edodes*; chloroform extract. *L. edodes* extracts showed a strong bactericidal effect against *Streptococcus mutans* (Alves et al., 2012). Rincão et al. (2012) presented antiviral

activity of aqueous and ethanol extracts and polysaccharide from *L. edodes* in the replication of poliovirus type 1 and bovine herpes virus type 1.

The literature has reported the presence of various compounds, such as fractionated extract from mycelium *L. edodes* (LEM) and the aqueous precipitate extract extracted from the mycelium (LAP). These extracts contain various sugars and have revealed a strong antitumor activity in vivo (Bisen et al., 2010).

One of the most medically significant compounds isolated from the shiitake mushroom is a polysaccharide lentinan. This compound activates macrophage T-lymphocytes and other immune cells that in turn modulate the release of cytokines. Lentinan have applications as antitumor agents. This compound is used in clinical assays as adjuvant in tumor therapy and specifically in radiotherapy and chemotherapy. Yamaguchi et al. (2011) suggests that extracts of *L. edodes* mycelia have a synergistic action with chemotherapy and the possibility of improving the quality of patient life. Another compound derived from the extract of *L. edodes*, the active hexose correlated compound (AHCC), also proved effective as an adjunctive therapy in patients with cancer (Shah et al., 2011). A recent clinical study Ina et al. (2013) showed that chemo-immunotherapy using lentinan prolongs the survival of patients with advanced gastric cancer, as compared to chemotherapy alone. Researchers demonstrated the efficacy of administration of lentinan in the treatment of advanced colorectal cancer using β-glucans to deliver nanoparticles containing chemotherapeutic agents to the site of the colon cancer and, thus, improving the therapeutic efficacy. Good results can also be seen in advanced pancreatic cancer, as in the administration of lentinan in patients with hepatocellular carcinoma (Finimundy et al., 2013)

Other compounds with biological activity are lentinacin and lentysine, which each have been reported to show hypocholesterolemic and hypoglycaemic effects.

Various studies have shown that shiitake mushrooms lower both blood pressure and free cholesterol in plasma and accelerate lipid accumulation in the liver by removing them from circulation. In research Yoon et al. (2011) found a diet containing 5% *L. edodes* fruiting bodies given to hypercholesterolemic rats reduced plasma total cholesterol, triglyceride, low-density lipoprotein (LDL), total lipid, phospholipids, and the LDL/high-density lipoprotein ratio by 34.33, 53.21, 75.00, 34.66, 25.73, and 71.43%, respectively.

Another edible mushroom from the same genus is *L. polychrous*, which is traditionally used as medicine. Fruit bodies can be found in northern and northeastern Thailand. The methanolic extract and crude polysaccharides have antioxidative activity and inhibitory effect on ell proliferations of breast cancer (Thetsrimuang et al., 2011). Fungal sterols, mycosterols, have been found to be abundant in mushroom, yeast, and fungi, which are rich sources of bioactive metabolites. Based on the structural similarity between mycosterols and oestrogens,

Fangkrathok et al. (2013) postulated that these sterols might possess oestrogenic or antioestrogenic activity on oestrogen-mediated cancer-cell proliferation. Research has shown that mycelial extracts from *L. polychrous* have antiestrogenic activity, resulting from a new polyhydroxyoctane and several ergostanoids.

L. edodes and *L. polychrous* have shown to present a great potential for the production of useful bioactive metabolites that serve as a rich resource for drugs.

12 Phellinus *spp.*

Genus *Phellinus* sp. is one of the most numerous in phyla Basidiomycota, and consists of around 220 species. In China alone, nearly 70 species of genus *Phellinus* sp. are described; out of them 26 possess medicinal properties. Approximately 20 medicinal functions have been described empirically in traditional Chinese medicine and attributed to the members of *Phellinus* sp. (Dai et al., 2010). They include, among others, alleviation of septic shock, managing of cardiovascular diseases, antimicrobial, antiinflammatory, antioxidant, antitumor, hemostatic, immuno-enhancing activities, and many others (Hwang et al., 2012; Lin et al., 2014, 2016; Wu et al., 2013). However, even though chemical composition of many species has been defined, in most of the cases it is difficult to attribute the mechanism of their active compounds in the living organisms, especially since most of the research is based on the results in in vitro conditions. Limitation factor (as in many cases) in the mass consumption of the mushrooms is the timeframe they require for the full development of fruiting bodies. Still, for some species, a successful method of in vitro cultivation has been developed, while maintaining comparable biological activity of the extracted metabolites to the wild ones. *P. gilvus* has a proven bioactivity, even though compounds that are responsible for the activity are not quite defined (Chang et al., 2011). The choice of solvent for the extraction almost inevitably has an impact on the biological activity; this has been evaluated on several occasions and in the case of ethyl acetate extract proved to enhance the activity in comparison with other tested extracts. Ethyl-acetate extract yielded six compounds that were identified, whereas one of them, named protocatehualdehyde, was presumed and proved liable for DPPH antioxidant activity (Chang et al., 2011). Species *P. baumii* is an edible mushroom that possesses various biological activities, including hypoglycemic, antioxidant, and so forth. Ethanolic extract of this mushroom yielded five bioactive compounds, which were spectroscopically identified as hispidin, hypholomine B, inoscavin A, davallialactone, and phelligridin D. Compounds were found to exhibit antiviral activities in a dose-dependent manner against viruses that cause serious symptoms in humans (including H1N1, H5N1, and H3N2) (Hwang et al., 2015). The case of *P. linteus* (Fig. 5.9) immunomodulatory in in vivo systems is one of the few in which a certain effect on living organisms was attributed to the mixture of compounds. Mycelial water-soluble extract of the mushroom showed activity on atopic dermatitis presented in experimental animals (Hwang et al., 2012). The mechanism of



Figure 5.9: ***Trametes versicolor*.**

extracts immunomodulatory activity was estimated by IgE production by human myeloma U266B1, and measured by ELISA. Mushroom extract was topically applied onto the ears of mice presented with atopic symptoms on a daily basis within a timeframe of two weeks. Progression of the disease was measured by using a few parameters. The extract reduced IgE production, as well as alleviated persistent symptoms of atopic dermatitis, including erythema, and ear swelling (Hwang et al., 2012).

Extract of *P. pini* mycelium yielded two polysaccharides after gel filtration (Jiang et al., 2015). Even though phenolic compounds are usually correlated with high antioxidant activity, this study indicates that these two polysaccharidic compounds (MW of 22 and 38 kDa) possess scavenge radical, hydroxyl radical, and chelate ferrous ion, and reduce ferric ion activities, and are promising natural antioxidant substances. One of the most common metabolic disorders of present time is diabetes mellitus, which is life-threatening disease making those diseased in constant need of medicines on a daily basis. Synthetic compounds facilitate ailment symptoms making it manageable. Empirically, *P. linteus* has a long tradition of hypoglycemic use in Chinese traditional medicine, without supportive scientific data. In the study by (Kim et al. 2010), scientifically confirmed data on *P. linteus* antidiabetic activity are obtained. *P. linteus* polysaccharide efficiently delayed the beginning and the progression of induced diabetes mellitus in experimental animals. The prevention of diabetes development is achieved through changes in cytokine network (Kim et al., 2010). Yet in another study (Zhao et al., 2014), this hypoglycemic activity was confirmed in a different set of conditions, indicating that a hot water crude polysaccharide from *P. linteus* mycelium significantly reduce blood glucose level when orally administered. With the help

of ion-exchange chromatography and gel filtration, the pure compound was isolated from the extract and spectroscopically characterized (FT-IR and NMR) afterward. It turned out to be a branched type glycan with α- and β-linkages and a pyranoid sugar ring conformation (Zhao et al., 2014). A detailed literature survey on the activity of *P. linteus* over the last six years, indicates that various extracts of the mushroom's fruiting body and mycelia possessed a distinct biological activity and potential health benefits, including activity on benign prostatic hyperplasia, β-glucosidase inhibitory activity, antiviral, antioxidant and antiinflammatory activity of mushroom's culture broth, antitumour activity (on colon cancer cell lines HCT-116 and SW-480), imunomodulatory activity, and many others (Griensven and Verhoeven, 2013; Kim et al., 2013; Lee et al., 2011, 2015; Lin et al., 2016; Seklic et al., 2016; Wu et al., 2013; Zheng et al., 2011). Nutritional value and chemical composition of the mushroom was evaluated as well on several occasions. In a study by (Reis et al. 2014a), the most abundant compounds were carbohydrates, followed by protein and ash in lower amounts. This composition was confirmed on another *Phellinus* sample in a study by (Su et al., 2016). Saturated fatty acids predominated over unsaturated, which is not of great importance on fat level in consumer fluids, since overall presence of fat was low in the mushroom sample. Presence of two isomers of tocopherol was noticeable as well. In the aforementioned study, the mushroom proved to have significant activities: cytotoxic (ethanolic extract), antimicrobial (methanolic for antibacterial, glucan, and triterpenoid fractions had the best antifungal activity), and antioxidant (methanolic). This may indicate that all of these activities act by complementing each other in crude extracts (Reis et al., 2014a). Two polysaccharidic fractions from *P. pini* were obtained by gel filtration method and evaluated for their antioxidant activity (Jiang et al., 2015). In addition, polysaccharide fraction, MW of 17 kDa was isolated and purified from *P. baumii* (Xue et al., 2011). It possessed antiproliferative activity on of HepG2 cells by mechanisms of cell cycle arrest at S phase, which consequently led to apoptosis. Significant progress of studies concerning data of the *Phellinus* sp. activity has been achieved, since scientific data are increasingly coming from *in vivo* experiments along with in vitro results. Reliable data of *in vivo* activity was obtained from a study where *P. linteus* was orally supplemented within healthy young men; it exhibited significant effect on inhibition of inflammatory markers (Lee et al., 2010). As for the culture broth of *P. linteus*, it was extracted with ethyl acetate, and spawned six different compounds with a dose dependent antioxidant activity. Spectroscopic methods identified their structures as (1) caffeic acid, (2) inotilone, (3) 4-(3,4-dihydroxyphenyl)-3-buten-2-one, (4) phellilane H, (5) (2E,4E)-(+)-4′-hydroxy-γ-ionylideneacetic acid, and (6) (2E,4E)-γ-ionylideneacetic acid (Lee et al., 2015). Another member of the genus *Phellinus*, namely *P. igniarus,* exhibited antiinfluenza virus effect (Lee et al., 2013). Species *Phellinus* exhibit following activities: antitumor activity, antioxidant, SOD-like activity, antimutagenic activity, and neuroprotective (Lee et al., 2013; Li et al., 2015; Lung et al., 2010; Song et al., 2014; Suabjakyong et al., 2015; Yang et al., 2015; Zhang et al., 2014b).

13 Trametes *spp.*

Genus *Trametes* was established by Fries in 1835. It belongs to medically acclaimed family Polyporaceae and includes nearly 50 species. Members of the genus are widely distributed in various biotas throughout the world and have a tradition of use in China medicine for thousands of years (Knežević, 2015). The most studied species of the genus is *T. versicolor,* but recent studies include other species of the genus. The mushroom is considered useful in many aspects of healing, improvement of organ function, energy increase, and so forth. Among the confirmed biological activities, antioxidant, antimicrobial, and antiinflammatory are the most often mentioned (Yamaç and Bilgili, 2006). Kamiyama et al. (2013), examined various extracts of *T. versicolor* for their antioxidative and antiinflammatory activities. Acetone extract of the mushroom exhibited very high antioxidant capacity (50.9%), followed by methanol, n-hexane and chloroform extracts (33.9, 29.5, and 15.2%, respectively) (Fig. 5.10).

Potent acetone extract showed lipooxygenase inhibitory activity as well. Chemical analysis of the extract identified compounds, with main constituents in the following order: hexadecanoic acid (18.11 mg/kg), 5-hydroxy-2-pentanone (17.33 mg/kg), lactic acid (3.25 mg/kg), and acetic acid (3.21 mg/kg) (Kamiyama et al., 2013). Compounds responsible for potent



Figure 5.10: ***Trametes versicolor.***

antioxidative activity of the extract were identified: furfural (1.48 mg/kg), γ-butyrolactone (0.51 mg/kg), furfuryl alcohol (0.49 mg/kg), 2-methoxy-4-vinylphenol (0.49 mg/kg), 2,6-dimetoxy-4-vinylphenol (0.33 mg/kg), and benzaldehyde (0.15 mg/kg). As for the antiinflammatory activity, dichloromethane extract showed prominent lypoxygenase activity, followed by acetone and aqueous extract. In a study by (Knežević et al., 2015), three *Trametes* isolates (*T. versicolor*, *T. hirsuta*, *T. gibbosa*) were evaluated for their antigenotoxic activity against H_2O_2-induced DNA damage. Overall, a significant dose-dependent decrease of total DNA damage has been determined. The result indicates that the tested *Trametes* extracts showed strong, but different capacity as antigenotoxic agents. Mushroom mycelium did not exhibit this activity. Antigenotoxic effects of natural sourced substances are still insufficiently familiar, and presumably involve more than one mechanism. As for the antioxidant activity and total phenolic and flavonoid compounds, results of different *Trametes* isolates showed clear species-dependent activity, which was, in all cases, positively correlated to higher phenolic content. Total phenolic and flavonoid contents were higher for extracts derived from fruiting bodies, rather than mycelium. Antioxidant activity of different *Trametes* isolates may be presented in the following descending order: *T. versicolor*, *T. hirsuta*, *T. gibbosa*. *T. versicolor* basidiocarp and mycelium extract proved to be the richest source of phenols and flavonoids, which correlated with antioxidant activity. In a study by Yamaç and Bilgili (2006) antimicrobial activity of *T. versicolor* was evaluated. Out of wide panel of tested microorganisms, mushroom's extract exhibited inhibitory effect on *Enterobacter aerogenes*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Methanolic and hexane extracts of the species *T. ochracea* were evaluated for their chemical characterization and in vitro biological activities (Melappa et al., 2015). Chemical constituents of the methanolic extracts included the following compounds: proteins, carbohydrates, resins, saponins, flavonoids, alkaloids, steroids, phenols, and tannins in significant amounts. Hexane extract lacked saponins and flavonoids, and was presented with significantly lower amounts of the aforementioned compounds. Consequently, methanolic extracts possessed better antioxidant activity. *T. ochracea* also exhibited antilipoxygenase and antiinflammatory activities and proved to be a powerful tool in cytotoxicity assays. The result clearly indicates that *T. ochracea* methanolic extract is a potent bioactive agent. On the other hand, hexane extract of the same mushroom possessed lower activity than methanolic in all tested assays. Extracts of *T. hirsuta* fruiting bodies (aqueous and methanolic) were evaluated for their antimicrobial potential using well-diffusion assay (Sivaprakasam et al., 2011). The most susceptible bacteria to the activity of mushroom methanolic extract proved to be *S. aureus*, whereas *A. flavus* was the most susceptible micromycete. As expected, results indicate dose-dependents antimicrobial activity. The assessment of *T. hirsuta* mycelia as an antioxidant, antifungal, and cytotoxic agent was evaluated in a study by Milovanović et al. (2015). The effect of selenium on these activities was further investigated. In addition, *T. hirsuta* extracts showed low cytotoxic activity against HeLa and LS174 cells, whereas those enriched with selenium, possessed better potential. Extract of *T. hirsuta* did not exhibit potent antioxidant

activity, whereas a direct correlation between total phenolic compound content and antioxidant activity (DPPH) was observed. Ethanol extracts exhibited fungistatic but not fungicidal activity against a range of micromycetes, and mycelium enrichment with selenium inhibited this effect. Bioremediation potential of *T. versicolor* was evaluated, in terms of removal of common emerging contaminants and related estrogenic activity from wastewater effluent (Shreve et al., 2016). The mushroom did not remove every contaminant of the mixture, but it did remove the usual contributors of pollution. Since extracts are complex mixtures of compounds, in most cases it is difficult to define the underlying mechanism of their action. However, in some cases, this information can be provided. Homogenous polysaccharide of estimated size 8.7×10^4 Da was extracted from the fruiting bodies of *T. robiniophila* (TRP). In the study by (Zhao et al., 2015) it was demonstrated that the polysaccharide has inhibitory effects on the cell proliferation and induction of apoptosis if U-2 OS cells, using MTT assay. In a dose-dependent manner, TRP potently inhibited U-2 OS cell viability and caused morphological changes on the cells. The underlying mechanism of cell death caused by TRP in U-2 OS cells was presumed apoptosis, and flow-cytometric analysis confirmed this assumption. This is of great significance since apoptosis as a programmed cell-suicide process is necessary for the removal of damaged cells without the accompanying negative effects onto the surrounding cellular structures.

14 Miscellaneous Mushrooms

Mushroom species already mentioned are most commonly used as delicious food or as health-promoting food supplement, as a drug in limited geographic regions. However, some other species of mushrooms should be also reported as a good source of therapeutic food.

Petrović et al. (2014c) reported that the methanolic extract of *A. aegerita* exhibited high antimicrobial and antioxidant activities and demonstrated reduction of virulence factors in quorum sensing (QS) test, such as pyocyanin production, twitching, and swimming motility, as well as biofilm formation on *Pseudomonas aeruginosa*. Some protein components from *A. aegerita* possessed tumor-rejection activity (Gao et al., 2010), such as two antitumor lectins, AAL and AAL-2 (Feng et al., 2010).

Sarikurkcu et al. (2010) reported antioxidant potentials and total phenolics of *Amanita caesarea*, *Clitocybe geotropa* and *Leucoagaricus pudicus*. *L. pudicus* showed the highest potential in β-carotene/linoleic acid test and total phenolics. In DPPH system, *A. caesarea* showed 79.4% scavenging ability. The strongest super-oxide anion scavenger was *A. caesarea*.

Methanolic extract of cultivated and wild samples of *C. comatus* was tested for its antioxidant, antifungal, antibacterial potential and toxicity for liver cells by Stojković et al. (2013a). The cultivated species revealed the highest antioxidant potential and antimicrobial

activity. Both extracts are considered safe since they did not show toxicity in liver primary cell culture.

Heleno et al. (2012, 2013, 2014) studied the antioxidant activity of phenolic and polysaccharide fractions of five mushroom species, *Coprinopsis atramentaria*, *Lactarius bertilloni*, *Lactarius vellereus*, *Rhodotus palmatus,* and *Xerocomus chrysenteron*. *C. atramentaria* polysaccharide and phenolic extracts gave the highest antioxidant activity (lowest EC_{50} values), total phenolics (33.58 mg GAE/g extract) and total polysaccharides content (16.72 mg PE/g extract). The methanolic extract of *C. atramentaria* showed antimicrobial activities against all the tested microorganisms, as well as demelanizing activities (Osuji et al., 2013; Heleno et al., 2014).

A fungal immunomodulatory protein (FIP-fve) from *F. velutipes*, has shown antitumor effect on oral administration in murine hepatoma model (Chang et al., 2010). The sesquiterpenes, enokipodim F, G, and I, isolated from *F. velutipes* mycelium presented low antifungal activity against *Aspergillus fumigatus* (Wang et al., 2012a,b).

A water-soluble polysaccharide was isolated from the mushroom *Inonotus obliquus* and exhibited an inhibitory activity against α-glucosidase, whereas it had no effective inhibition on α-amylase (Chen et al., 2010). Inotodiol and trametenolic acid from ethyl acetate extract of *I. obliquus* were found to have an inhibitory effect on α-amylase activity (Lu et al., 2010). An alkaline-soluble polysaccharide isolated from *I. obliquus* (rhamnose, xylose, manose, galactose, glucose, and galacturonic acid), showed excellent activity against solid tumor sarcoma formation in mice, and the exerted activity was associated with potent immunostimulating effect of this polysaccharide (Fan et al., 2012). Another heteropolysaccharide was extracted and purified from *I. obliquus*, but was water-soluble and consisted of rhamnose, mannose, and glucose possessed in vitro cytotoxic effect, but exerted significant antitumor effect in human gastric carcinoma-bearing nude mice. Similar to other polysaccharides, authors suggested possible mechanisms related to cancer-prevention, immuno-enhancement, and direct tumor inhibition (Fan et al., 2012). Debnath et al. (2013) reported antioxidant activity of mycelia of *I. obliquus* the scavenging activity of water, ethanolic, and methanolic extracts on DPPH's were found. Glamočlija et al. (2015b) reported antioxidant, antimicrobial, anti-QS, and antitumor activities of water and ethanolic extracts of *I. obliquus*, wild samples obtained from Russia, Finland, and Thailand. All the tested extracts possessed high antioxidant and antimicrobial potential, and showed twitching and swarming motility of *P. aeruginosa*, and the amount of produced pyocyanin as QS parameters. Also, all the extracts demonstrated cytotoxic effect on four tumor cell lines and not on primary porcine liver cells PLP2.

One of the polysaccharide fractions isolated from fruiting bodies of *Tricholoma matsutake*, unlike other purified fractions of this mushroom, was found to consist of glucose, galactose, and mannose with exerted strong antiproliferative activity on HepG2 and A549 cell lines in MTT test (You et al., 2013).

The polysaccharides extracted from *Lactarius flavidulus* possessed anticancer properties when administered intraperitoneally into white mice at a dosage of 300 mg/kg inhibited the growth of sarcoma 180 by 100%. The lectin from dried *L. flavidulus* suppressed proliferation of HepG2 and L1210 cells (Wu et al., 2011).

The metanolic extracts of *Morchella esculenta* (morel) from Portugal and Serbia showed antioxidant and antimicrobial activity (antibacterial and demelanizing properties) (Heleno et al., 2013). The sample from Portugal possessed higher radical scavenging activity and reducing power, whereas sample from Serbia showed higher lipid peroxidation inhibition. Both samples exhibited antibacterial activity against five bacteria (in some cases even better than standard antibiotics) and demelanizing activity against four micromycetes. Also, the extracts of *M. esculenta* was studied for their antimutagenic properties and compared to protocatechuic acid (Stojković et al., 2013a). A sample from Serbia possessed slightly higher antimutagenic properties. Using the *Allium cepa* L. assay also assessed the genotoxic effect, regarding the mitotic index and the chromosomal aberration score. Protocatechuic acid showed the most significant decrease in mitotic index, as well as decrease in chromosomal aberration score. Vieira et al. (2016) reported the antioxidant and antimicrobial potential, as well as confirmation of nontoxicity tested in a primary cell culture from porcine liver cells of metanolic extracts from wild mushroom *Morchella conica* from Portugal and Serbia. The methanolic extract of the Portuguese sample showed the highest antioxidant potential, whereas the Serbian one possessed the highest antimicrobial activity. Both extracts revealed no toxicity toward porcine liver cells.

The characterization and antitumor activity in vitro of mycelial polysaccharides (PDP, PDP-1, PDP-2, and PDP-3) from *Pholiota dinghuensis* were investigated by Gan et al. (2011). The highest antiproliferative activity against human gastric cancer BGC-823 cells in vitro exhibited PDP-3

The bioactive properties (antioxidant, antimicrobial, and cytotoxicity) of metanolic extracts of *Polyporus squamosus* from two different origins, Portugal and Serbia, were tested by Fernandes et al. (2016). The extract from Portugal gave the highest antioxidant activity, but both extracts exhibited antibacterial activity against all the tested organisms, as well as without toxicity for liver cells. The samples from Serbia gave higher overall antibacterial activity and showed excellent antibiofilm activity (88.30%). Also, the antioxidant potential of different extracts from *P. squamosus* was analyzed by other researchers. Extracts from *P. squamosus* possessed good antimicrobial and antiradical activities (Akata et al., 2012; Dimitrijevic et al., 2015).

Plectasin peptide, obtained from *Pseudoplectania nigrella*, is the isolated compound with the highest antimicrobial activity against Gram-positive bacteria, whereas 2-aminoquinoline, isolated from *Leucopaxillus albissimus*, presents the highest antimicrobial activity against Gram-negative bacteria (Alves et al., 2012). Novel butenolides, ramariolides A–D, isolated

from the fruiting bodies of the coral mushroom *Ramaria cystidiophora*, showed in vitro antimicrobial activity against *Mycobacterium smegmatis* and *M. tuberculosis* (Centko et al., 2012).

Reis et al. (2014b) studied the antioxidant properties, as well as the antimicrobial activity of methanolic extracts of the wild mushrooms *Suillus granulatus* from Serbia and Portugal. The Serbian sample showed the highest reducing power and better antimicrobial potential then samples from Portugal.

The methanolic extract of *Tirmania pinoyi* showed in vitro antioxidant, antibacterial activities, and in situ control of the development of the bacteria *Staphylococcus aureus* in chicken soup (Stojković et al., 2013b). The methanolic extract possessed antioxidant activity including reducing power, free radical scavenging activity, and lipid peroxidation inhibition, as well as successfully inhibiting the growth of *S. aureus* in chicken soup.

The aqueous extracts from mycelium of 11 basidial fungi (*Daedaleopsis confragosa, Datronia mollis, Ischnoderma benzoinum, Trametes gibbosa, T. versicolor, Laricifomes officinalis*, and *Lenzites betulina*) possessed antiviral activity against type A influenza virus of birds (H5N1) and humans (H3N2) (Teplyakova et al., 2012).

The range of detected biological activities of mushrooms is very broad. Increasing knowledge in cultivation, chemistry, biotechnology, and molecular biology of mushrooms, as well as an improvement of screening methods, is necessary for a rapid increase in the application of mushrooms as a therapeutic food.

15 Conclusions

Medicinal mushrooms represent a new and growing source of pharmaceutical industry today. Keeping in mind that they have a long history of use across the world, they have currently become a focus of reasonable scientific investigation. New data concerning the isolation and chemical characterization of bioactive compounds of the medicinal mushrooms prove their use as a source of functional food. It was demonstrated that mushrooms could be an excellent source of a wide range of interesting molecules with pharmacological activity (antioxidant, antibacterial, antifungal, and antiproliferative) properties or even with antitumor potential. Modern medicines may not always provide the remedies required and mushrooms and their nutriceuticals are likely to prove extremely useful in both alleviating and preventing human disease. Disease prevention is particularly desirable, not only in having positive financial and social impacts, but also in maintaining/improving the quality of life and human dignity. Edible and medicinal mushrooms represent promising and relatively untapped sources of materials with potential nutritional and medicinal applications. However, many of the medicinal properties attributed to mushroom nutriceuticals are based on data obtained from in vitro and animal-based experiments. Mushroom extracts, nutriceuticals, compared with other

drugs, as noted, show a very low toxicity when regularly consumed, even in high dosages. Therefore, mushrooms become a valuable health food and also a dietary supplement in modern society.

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CHAPTER 6

Oregano: A Feed Additive With Functional Properties

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1 Introduction

Oregano is one of the world's commercially most valued spices. Its name, Oregano, derived from the Greek ancient words "*Oros*" and "*Ganos*" that means the beauty of the mountains. Oregano is the common name for a general aroma and flavor, typical for a plant self-grown worldwide. Oregano is primarily derived from more than 60 plant species used all over the world as a spice. The majority of them belong mainly to the *Lamiaceae* family. European oregano is used as a flavoring in meat and meat type products, such as sausages, as well as in, salads, stews, sauces, and soups. The essential oil (EO) and leaves are also used in food products, cosmetics, and alcoholic liqueurs. Mexican and Indian oreganos are also considered as medicinal plants and are mainly used as food flavorings (Franz et al., 2010; Kintzios, 2002).

The European genus of *Origanum* includes 39 species widely distributed in the Mediterranean region (Vokou and Kokkini, 1993). The species *Origanum vulgare* predominate in occurrence, while *O. dictamnus* is endemic of the Island of Crete in southern Greece. The oregano plants are perennial herbs spontaneously growing in calcareous substrates. *Origanum* plants have numerous glands covering the aerial vegetative organs (stems, leaves, bracts), whereas their density becomes reduced on the reproductive organs (calyces, corollas). These glands secrete an EO with a characteristic odor, mainly due to the major components of the oil, the monoterpenes carvacrol, and thymol. On the stamens and the gynoecium of the flowers, no glandular hairs have been reported to occur (Padulosi, 1997).

The *Origanum* species are subshrubs or perennial herbs with several stems, ascending or erect, subsessile or petiolate leaves and flowers aggregated in dense or loose spikes that are arranged in a paniculate or corymbiform inflorescence. The knowledge of the large morphological and chemical diversity of the genus *Origanum* and its native distribution of all the different taxa will allow the full exploitation of this most promising crop. Aromatic plants,

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also known as herbs and spices, such as oregano contain many bioactive compounds having strong antibacterial, antiparasitic, cytotoxic, and antioxidant activity (Christaki et al., 2012; Giannenas et al., 2013a; Sivropoulou et al., 1996). They have great economic importance, which is not only limited to their use as a spice but it is also related to animal and human nutrition, as well.

2 Antimicrobial Growth Promoters

Antibiotic-based growth promoters have been commonly used as supplements in animal feeds for many years because they are well known to help growing animals to digest their food more efficiently and to grow strong and healthy individuals. Today, pressure to reduce antimicrobial drug usage in pig and poultry production is encouraging researchers to find environmentally friendly natural alternatives that may exert therapeutic effects similar to in-feed antimicrobials (Sika and Bilkei, 2003). In recent years, animal production in many countries has faced several constraints on the use of antibiotics as growth enhancers and, for this reason, novel alternative efficient tools are being sought to support health of productive animals and improve their growth performance. Aromatic plants and their EOs could be seen as a potential source of novel ingredients, since their traditional use as effective conservatives or health promoters is only partly explained on the basis of their strong antimicrobial and other biological activities (Christaki et al., 2012; Giannenas et al., 2013a). The EOs of oregano have been found to develop very potent antibacterial, antiparasitic, antioxidant, insecticidal, and genotoxic/cytotoxic properties both in vitro and in vivo (Sivropoulou et al., 1996).

For the aforementioned reasons, during the last 2 decades, products containing EOs derived from several spices and herbs have been used as feed additives as growth promoters in animal nutrition. These phytogenic additives may have more than one mode of action, for example, by improving feed intake and flavor; stimulating the secretion of digestive enzymes; increasing gastric and intestinal motility; endocrine stimulation; antimicrobial, antiviral, anthelminthic, and coccidiostat activities; immune stimulation; and antiinflammatory and antioxidative activity. Nowadays, there is an increasing interest in the use of natural growth promoters, such as direct-fed microbials, oligosaccharides, symbiotics, and aromatic (or so called medicinal) plants as feed additives in poultry diets to promote body weight gain and feed utilization of broiler chickens (Christaki et al., 2012; Bozkurt et al., 2013; Giannenas et al., 2013a).

Aromatic plants and their extracts are now garnering huge scientific and commercial interest and are being common tools as health promoters, as well as therapeutic, prophylactic and growth supplements. As concerns about food safety have risen, it has become a prerequisite to exploit nonchemotherapeutic, rather than chemotherapeutic methods, with the use of vaccines, probiotics, prebiotics, organic acids, immunostimulants, and EOs of aromatic plants.

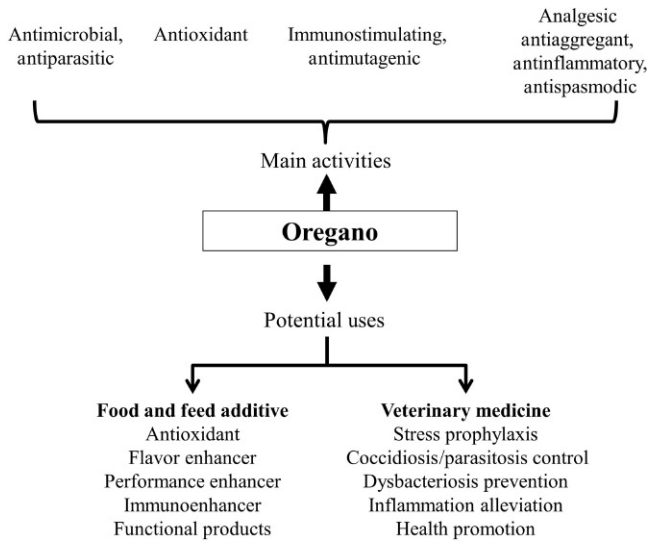


Figure 6.1: Oregano as a Functional Ingredient of Animal Diets.

Natural products, containing a pure plant extract, or the EO, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Besides their use as antimicrobials, several plants are being used in different areas of human health, such as folk medicine, and the production of functional foods or dietary supplements. As healthcare trends move toward disease prevention, there will be higher market for these products, as consumers are choosing foods over pharmaceuticals for their well being (GBA, 2010; Negi, 2012; Sloan, 2000, 2007).

The aim of this chapter is to provide credible data on the effects of oregano as an alternative natural dietary growth enhancer and to quantify its effects on productive animals of different categories. The current review chapter presents a summary of global trials conducted with a variety of animals and diet formulations. This literature review of the efficacy of the oregano or oregano EO in animal nutrition is part of a comprehensive research quantifying and comparing the efficacious results, as well as future research needs of the oregano as a replacer candidate for antibiotics. Furthermore, this review chapter synopsizes the experimental knowledge on efficacy, potential mechanisms of efficacy, and several aspects of infield application of oregano EOs as feed additive with functional properties. Today, as well as in the near future, oregano can be a principal functional ingredient both in the food and feed industry, as well as in veterinary medicine (Fig. 6.1).

3 Oregano Essential Oil

EOs or volatile oils are aromatic oily liquids extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, and roots (Miguel, 2010). They are water steam-volatile distilled or organic-solvent (ethanol, methanol, toluene, or other organic

solvents) extracts and have been used habitually for millennia in several areas worldwide. They are considered to have pleasant flavor and aroma, as well as preservative properties. Some specific components of EOs can be extracted from plant parts or can be synthetically manufactured. Oregano EO usually contains a rich amount of different compounds, such as terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters (Negi, 2012). These substances can exert a protective role against bacterial, fungal, or insect attacks. Accordingly, EOs can be considered as safe mixtures of natural compound oils (Brenes and Roura, 2010).

EOs are named according to the aromatic characteristics of plant materials from which they can be isolated (Oyen and Dung, 1999). The term "essential" was adapted from the theory of "quinta essential." This theory was first introduced by Paracelsus von Hohenheim who predicated this quintessence as an effective element in a medical preparation (Oyen and Dung, 1999) as early as 500 years ago. The term "essential oil" is considered as an incomplete definition. For this reason, even in the years of medieval pharmacy, the term "volatile oil" has been proposed as a substitute (Hay and Waterman, 1993). However, the former term "essential oil" is used more frequently even in the published literature.

The composition of EOs in individual components is variable. For example, the EO of oregano that is extracted of *O. vulgare* ssp. *hirtum* plants by water steam-distillation exhibit a great variation. Not only does it contain more than 30 different compounds, their inclusion level in the total oil is not constant (1.8–8.2 mL/100 g dry weight) (Adam et al., 1998; Economou et al., 1991; Sivropoulou et al., 1996). The major compounds are carvacrol and thymol that make up for about 80%–85% of the total oil (Adam et al., 1998). In oregano oil, carvacrol should be the richest compound; however, in rare cases, only traces of carvacrol are determined (Sivropoulou et al., 1996). The other main components also show variable concentrations. For example, γ-terpinene and *p*-cymene being the two main monoterpenes usually constitute about 10% of the total oil. Accordingly, the biological activities following the dietary use of oregano EOs use can have a vast variability and the benefits of its usage as a dietary promoting agent could be inconsistent due to the variability of the oil composition or because it depends on the synergistic effect of all the different compounds (Adam et al., 1998; Deans and Waterman, 1993; Janssen et al., 1989; Schilcer, 1985). In addition, it is possible that cultivation techniques with the use of arbuscular mycorrhizal fungi can effectively improve the production of oregano EO (Karagiannidis et al., 2011).

The EO content, as well as the ratio of carvacrol to thymol to the total oil determined in the different Greek populations, were studied already 3 decades ago by Kokkini and Vokou (1989). An extremely high yield of EO (>7 mL/100 g dry weight) was found on the islands of Crete and Amorgos, as well as in Gythion and Athos Peninsula. The highest yields correspond to plants growing at low altitudes, in Mediterranean ecosystems. Therefore, the highest EO yields among any of the oregano plants were reported. It is indeed common for

the whole family of *Labiatae* plants. Quantitative and qualitative EO analyses have shown that the major constituents are carvacrol and/or thymol, accompanied by *p*-cymene and γ-terpinene (Vokou and Kokkini, 1993). In some cases the EO consists of a high quantity of carvacrol, as in the South Peloponnese—more than 90% of the total oil—or in other cases the predominant phenol is thymol, as in self-sown oregano plants on the island of Corfu or other islands. In these cases, carvacrol, the compound that should be found at higher quantity in order to characterize that a plant is belonging to the oregano type, is a minor component. The dominance of thymol suggests that these should belong to the group of plants named as thyme.

O. vulgare subsp. *hirtum* is the most commonly used spice in Greece, with its EOs being rich in carvacrol, γ-terpinene, and *p*-cymene. The biological properties of these compounds may justify some of the reported traditional uses. Many oregano species are found growing only in the wild, but many others, used as medicinal, culinary herbs, and garden plants, are also found as cultivated plants. The main production sites of cultivated oregano in Greece are presented in Fig. 6.2. The chemical analysis of the different components of EO of cultivated Greek oregano is presented in Table 6.1. Photographs of cultivated oregano plants in the area of Kilkis are presented in Fig. 6.3, whereas self-sown oregano plants in Lemnos island are presented in Fig. 6.4.

EOs nowadays are used as food flavorings or functional components in pharmaceutical products (Corbo et al., 2009; Negi, 2012), while their potential use as natural antimicrobial agents has been explored for over a decade (Burt, 2004). Most EOs are classified as GRAS (generally recognized as safe) substances, but their use in food as preservatives can be limited if their flavor impairs the normal flavor of the food (Brenes and Roura, 2010; Lambert et al., 2001).

4 Modes of Action of Oregano

4.1 Antimicrobial Activity of Oregano

The antimicrobial properties of the great variety of the chemical components found in EOs are not the result of one specific mode of action, but it is possibly the synergistic action of many different compounds on several different targets in different parts of the cell (Burt, 2004). The effectiveness of the EOs is attributed on several factors, such as pH, chemical structure, lipophilic/hydrophilic properties, concentration, or the individual bioactive compounds, along with the population and types of affected microorganisms. Oregano is found to be one of the most potent inhibitory spices tested on broad spectra of bacteria. The antibacterial activity of EOs of *O. vulgare* and *O. majorana* have been investigated for their activity against many different bacteria, such as *Acinetobacter baumanii, Aeromonas veronii* biogroup sobria*, Streptococcus faecalis, Escherichia coli,*

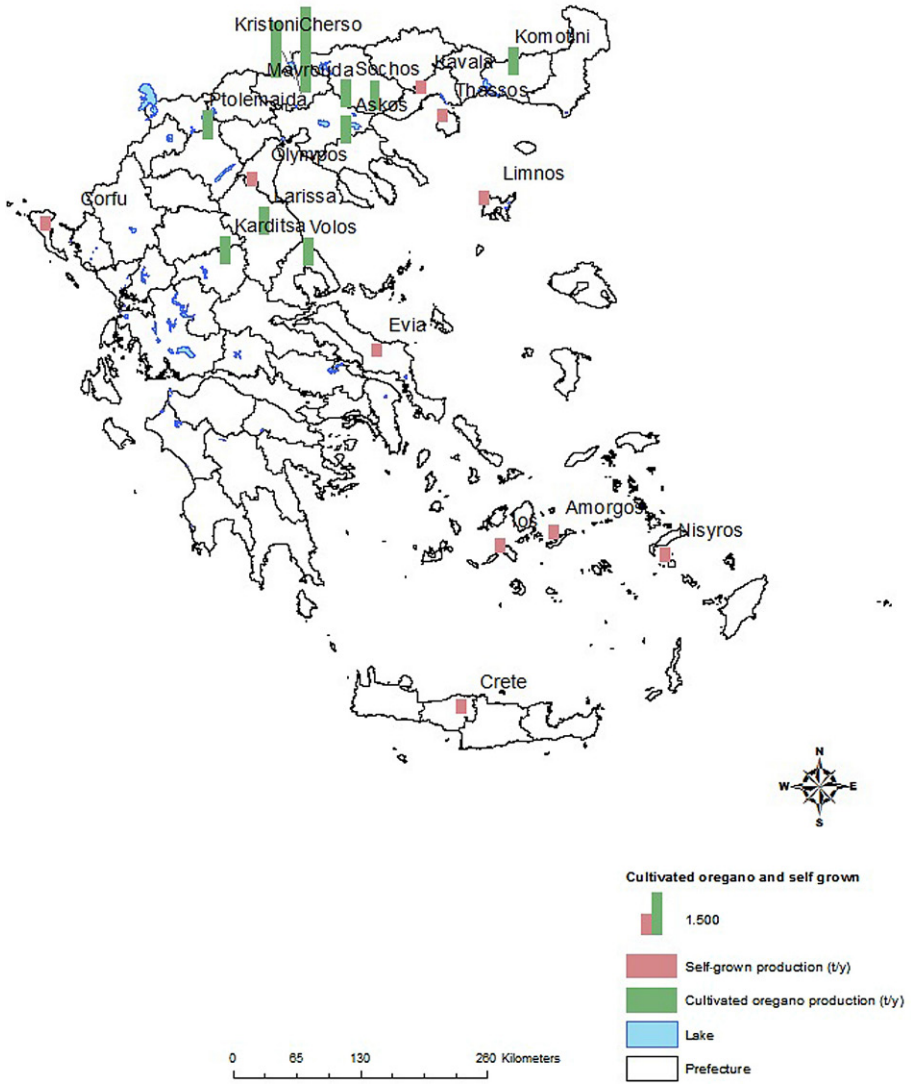


Figure 6.2: Main Sites of Cultivated and Self-Grown Oregano in Greece.

Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enterica subsp. *enterica* serotype Typhimurium, *Serratia marcescens, and Staphylococcus aureus*. It has been reported that *O. vulgare* originating from Australia yielded one of the most potent antibacterial EOs, which significantly inhibited the growth of the examined microorganisms. The lowest minimum inhibitory concentration of *O. vulgare* EO was 0.12% (v/v) and 0.25% (v/v) of *O. majorana*. The most resistant bacteria found among the examined ones was *P. aeruginosa*. Its growth was inhibited by *O. vulgare* EO at 2% (v/v), but was not affected by *O. majorana* oil (Hammer et al., 1999).

Table 6.1: Composition of the oregano EO compounds from cultivated *Origanum vulgare* plants.

Compound	%
Carvacrol	77.9
p-Cymene	5.4
γ-Terpinene	4.6
Thymol	3.0
β-Caryophyllene	1.6
β-Myrcene	1.2
β-Bisabolene	1.1
α-Terpinene	1.0
α-Thujene	0.8
α-Pinene	0.6
Terpinen-4-ol	0.5
β-Phellandrene	0.4
Carvacrol methyl ether	0.3
1-Octen-3-ol	0.3
Borneol	0.3
Cis-sabinene hydrate	0.2
α-Humulene	0.2
Camphene	0.1
α-Phellandrene	0.1
β-Pinene	0.1



(A) (B)

Figure 6.3: Photos of cultivated oregano plants in the area of Kilkis, in Greece (A–B).

An evaluation of the antibacterial properties of *Origanum* EO *against* several different tested organisms, such as *S. aureus, Bacillus anthracis, E. coli, K. pneumonia, Helicobacter pylori,* and *Mycobacterium terrae* revealed a bactericidal activity to all tested organisms. However, oregano EO was found to possess only bacteriostatic activity against *B. anthracis* (Preuss et al., 2005).



(A) (B)

Figure 6.4: Photos of self-sown oregano plants in the area of Lemnos island, in Greece (A–B).

As the pH of a food system declines, the EOs antibacterial effect increases, due to the hydrophobic character of the oils. The low pH increases their partition ability to dissolve in the bacteria's cell or its membrane (Burt, 2004). Other partition coefficients of the EOs may also affect their ability diffusion rate through the bacterial cell membranes (Raybaudi-Massilia et al., 2009).

The main oregano EO compounds, carvacrol, and thymol, also show a great variety of other antibacterial mechanisms responsible for the disruption of the cellular membrane, such as inhibition of ATPase activity, release of intracellular ATP and other constituents of microbes resulting in cell death (Burt, 2004; Gill and Holley, 2006; Lambert et al., 2001; Oussalah et al., 2006; Raybaudi-Massilia et al., 2009). The oregano phenolic compounds can enter into the phospholipid bilayer and get aligned between the fatty acid chains of the microorganisms (Ultee et al., 2002b), where they impair cell membrane permeability (Lambert et al., 2001). This membrane modification causes the expansion and the destabilization of the membrane, membrane fluidity increases, which in turn imposes a higher rate of passive membrane permeability (Negi, 2012 ; Ultee et al., 2002a,b). Moreover, carvacrol and thymol due to their hydrophobic character can bind to the proteins of the bacterial membrane and change the membrane permeability characteristics (Juven et al., 1994).

4.2 Antiparasitic Activity of Oregano

Currently, the coccidia have developed resistance to all commonly used coccidiostats. This resistance represents a serious health problem in poultry industry. Several plants and their EOs have been reported to have antiparasitic properties. However, only ethnoveterinary reports are the main sources for the widely held perception of their antiparasitic properties, and there is a lack of scientific quantitative evidence of the antiparasitic effects of numerous aromatic plants and their EOs. Such scientific evidence is necessary for the incorporation

of the plants in parasite control strategies in animal production either in ruminants or in monogastric animals (Athanasiadou and Kyriazakis, 2004). Also, it is often found that evidence can be contradictory (Githiori et al., 2003; Ketzis et al., 2002). Nevertheless, EOs have been used for centuries to treat parasite infections, especially in small ruminants.

In poultry, it is known that coccidial infections may appear either with acute clinical form or with a subclinical one. However, even the subclinical infections with *Eimeria* species can lower broiler performance (Bozkurt et al., 2013; Giannenas et al., 2004) and considerably impair their economic profitability. Birds once infected with coccidia show slower grow rate than noninfected ones, even at similar feed intakes. The main oregano oil compounds, such as carvacrol and thymol, have anticoccidial action against *E. tenella* (Giannenas et al., 2003; Williams, 1997), *E. acervulina* (Ibrir et al., 2009), and mixed *Eimeria* spp. infection (Oviedo-Rondon et al., 2006; Saini et al., 2003). However, it must be noted that the protective effect and recovery rate of chickens with dietary carvacrol or thymol was lower, compared to that of chemical anticoccidials.

In the literature, several in vivo and in vitro studies have both reported a high oocysticide effect of phenols, especially against *E. tenella* (Allen et al., 1997; Williams, 1997). It has been asserted that the major components of the oregano EOs carvacrol and thymol may have a toxic effect on the upper layer of mature enterocytes of the intestinal mucosa (Giannenas et al., 2003) due to the hydrophobic character of carvacrol (Sikkema et al., 1995). Similarly, Christaki et al. (2004) have reported several facts about the toxicity and the side effects of aromatic or medicinal plants or their extracts. Therefore, the dietary supplementation levels of use, their mechanism of action, and the possible clinical effects should be extensively studied in experimental dose titrating studies, before the widespread use in animal nutrition. For example, the carvacrol content of the EO blend (19 mg/kg of feed) reported by Küçükyıllmaz et al. (2012) was much lower than that declared by Giannenas et al. (2003) and Ibrir et al. (2009) where the inclusion levels were 300 and 250 mg/kg of feed, respectively. This low inclusion level of phenols could stand for the overall poor performance of chickens. Also, this low inclusion EOs could justify why the lacking anticoccidial effect was found in those birds, contrasting with the previous works where birds were receiving almost ten times or even higher the therapeutic agents. This can also be attributed to the fact that EOs vary considerably with respect to their phenolic composition (Faleiro et al., 2003; Marino et al., 1999), and this variation can negatively affect the animal metabolism, performance, or immunity of the broilers (Brenes and Roura, 2010; Lee et al., 2004). Mortality also depends on the infection level or the resistance of the chickens. Accordingly, mortality was relatively high in the cases of coccidial infection described by previous studies (Christaki et al., 2004; Giannenas et al., 2003), and considerably lower in the investigation by Küçükyıllmaz et al. (2012) in which the infection level was markedly lower. However, it must be noted that, contrary to the general expectations, monensin, a widely used ionophore anticoccidial, did not limit oocyst output at any examined age in contrast with untreated control chickens. Oregano

EO can be regarded as a possible alternative to coccidiostatic drugs, since several studies have proven that extracts and EOs of selected herbs can have a positive effect on the reduction of fecal oocyst shedding, among which oregano has the first place (Bozkurt et al., 2013; Giannenas et al., 2003; Ibrir et al., 2009; Lee et al., 2004). Also, it has been reported that a mixture of herbal extracts of different plants containing *O. vulgare, Allium sativum*, *Salvia officinalis*, *Echinacea purpurea,* and *Thymus vulgaris* strongly alleviated the negative impacts of *Eimeria* experimental infection (*E. acervulina, E. tenella, E. maxima,* and *E. necatrix*) in broiler chickens (Arczewska-Wlosek and Swiatkiewicz, 2012). Despite the fact that the direct connection between the number of oocysts in feces and bird performance is not always clear (Giannenas et al., 2003; Küçükyıllmaz et al., 2012), oocyst shedding is an important diagnostic tool for coccidiosis. The combined use of vaccination and addition of specific blends of plant EOs in the diet of chickens might be used as a tool to combat mixed coccidial infections. Since the dietary use of oregano EOs is an effective tool to limit the oocyst shedding, it can provide poultry industry with new strategies for the prevention of coccidiosis.

Waldenstedt (2003) also investigated the possibility of rearing broilers without growth promoters and coccidiostats by incorporating oregano EO in their diet. In this innovative experiment, however, oregano oil was not examined as an anticoccidial compound, but mainly as an antibacterial agent against intestinal colonization by *Clostridium perfringens*, where it decreased caecal numbers of *C. perfringens* at 31 days but not at 52 days of age. Another field study conducted by Köhler (1997) with a commercial preparation of EOs showed a reduction of colony forming units of *C. perfringens* as compared to the positive control diet containing zinc bacitracin. This preparation was also reported to slightly reduce ileal ATP concentrations (Veldman and Enting, 1996), which is an indicator of microbial activity in broilers. Similarly, another promising study reported that another blend of the plant extracts of capsicum, cinnamaldehyde, and carvacrol, lowered the number of *E. coli* and *C. perfringens* in caecum (Jamroz and Kamel, 2002). Mitsch et al. (2004) showed that an EO mixture containing thymol, carvacrol, and eugenol could control the proliferation of *C. perfrigens* in the intestine of broiler chickens and could possibly reduce the potential negative effects of necrotic enteritis outbreak associated with coccidiosis.

Giannenas et al. (2004) showed that diet supplementation with ground oregano plant could alleviate the negative effects imposed by *E. tenella* infection, as judged by the increased body weights and improved feed-to-gain values as compared to the challenged control group. At the age of 35 days, the oregano-supplemented broiler chickens at 5.0 and 7.5 g/kg, as well as the group of chickens, received the anticoccidial lasalocid at 75 mg/kg presented higher mean body weights than the oregano groups at 2.5 and 10.0 g/kg. The recorded feed-to-gain ratio values, and the extent of bloody diarrhea, mortality, and intestinal lesion scores suggested that diet supplementation with oregano at 5.0 and 7.5 g/kg of feed was most effective against the infection with *E. tenella*, suggesting that oregano could be administered to broiler chickens as an alternative to ionophore antibiotics for protection against caecal coccidiosis.

Tsinas et al. (2011) published a study to investigate the effect of dietary oregano EO supplementation on the performance of broiler chickens infected with coccidian oocysts of *E. acervulina* and *E. maxima* at 14 days of age. The results showed that dietary oregano oil supplementation in both oregano supplemented groups at levels of 30 and 60 mg/kg, managed to attain body weight gains and feed-to-gain ratio values similar to those of the nonchallenged group of chickens or the group of chickens that received the approved anticoccidial salinomycin. The challenged control presented lower performance than those of the other groups for the next weeks after the challenge. Evaluation of these performance parameters together with the extent of bloody diarrhea, survival rate, lesion score, and oocyst shedding, indicated that oregano EO exerted a comparable anticoccidial effect with salinomycin against an *E. acervulina* and *E. maxima* challenge.

The aforementioned studies from different institutes and laboratories worldwide clearly showed the anticoccidial effect of oregano herb or EO against *Eimeria* coccidian oocysts. The use of oregano in rotation programs for coccidiosis control is a suggestion that needs further investigation.

4.3 Antioxidant Activity of Oregano

The antioxidative effects of *O. vulgare* have been studied in the light of both direct use of *O. vulgare* as stabilizer of fat and, indirectly, as feed additive in order to improve both the oxidative stability and shelf-life of meat and fat-containing foods obtained from animals dietary treated with *O. vulgare* (Vichi et al., 2001). In contrast with the noteworthy antioxidative and stabilizing effects of oregano extracts in lard measured by photochemiluminescence, no effect on the quality or shelf life of the fat obtained from animals fed with oregano additives was observed (Vichi et al., 2001). Antioxidant properties were also demonstrated for another hydroalcoholic extract of *O. vulgare* leaves and stems that exerted an iron-reducing oxidative activity in vitro (Yoshino et al., 2006).

The antioxidant mechanisms of plant EOs are based on both their ability to donate a hydrogen or an electron to free radicals and their ability to delocalize the unpaired electron within the aromatic structure of the phenolic substances (Fernandez-Panchon et al., 2008), exerting a protective effect against lipid oxidation. A plethora of different methodologies have been applied for the in vitro or the in vivo examination of the antioxidant properties of phenolic compounds (Antolovich et al., 2002; Sanchez-Moreno, 2002). It has been proposed that it is necessary to obtain values from different methods in order to make a ranking order of the antioxidant capacity of the aromatic phenolic substances (Fernandez-Panchon et al., 2008).

Several phenolic compounds have been tested for their antioxidant activity in vitro (Frankel et al., 1993, 1995; Sato et al., 1996; Villano et al., 2005). It was stated that phenolics can be more powerful antioxidants than vitamins E and C or carotenoids (Rice-Evans et al., 1997).

The capacity of phenolics to protect lipids from oxidation relies on the number and organization of their hydroxyl groups (Cao et al., 1997; Sang et al., 2002), or phenolic acids, the free -OH groups in the ring; the larger TEAC (Trolox Equivalent Antioxidant Capacity) value is measured with ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant tests (Lien et al., 1999; Villano et al., 2005).

Regarding the oxidative stability of chicken and turkey meat, the effect of oregano EO has been thoroughly investigated. Supplementation of turkey diets with oregano EO at the level of 200 mg/kg decreased lipid oxidation of cooked and fresh meat during refrigerated storage for 1 week. Also, oregano EO preserved the quality of chicken meat under refrigerated or frozen storage alone or in combination with extracts of other aromatic plants of the same family, showing a strong antioxidant activity (Botsoglou et al., 2002, 2003a,b; Giannenas et al., 2016a,b,c). In similar studies with chickens and turkeys it was reported that herbs and spices extracts in combination with the antioxidant vitamins C and E showed higher capacity to decrease lipid oxidation in poultry meat or internal tissues, such as liver (Papageorgiou et al., 2003; Young et al., 2003).

In a study with fattening pigs, Zhang et al. (2016) showed that oregano EO can alleviate stress and increase antioxidative capacity after the transportation of pigs. It was reported that serum cortisol and norepinephrine concentrations of transported pigs were reduced in oregano-EO-fed pigs. Reactive oxygen species and malondialdehyde were increased in the serum and liver of pigs. Serum glutathione peroxidase was markedly raised in dietary treatment. These results indicated that oregano EO was beneficial in alleviating transportation stress and improving antioxidative activity in pigs.

The use of oregano EOs as the main dietary antioxidant or as a direct food additive to prevent the increase of the antioxidative capacity of foods has been effective in various different animal models in at different food systems, storage time or conditions. It is possible that the dietary use of oregano will increase in the future because a positive appreciation of consumers is anticipated, as well as its effectiveness.

4.4 Analgesic, Antiaggregant, Antiinflammatory, and Antispasmodic Activity of Oregano

Several studies in literature have shown that oregano could be a rich source of potent bioactive compounds. In those studies, various species of oregano were investigated and it was found that these plants exhibited antiinflammatory activities both in vitro and in vivo, and identified the active agents to be the rosmarinic acid, oleanolic acid, and ursolic acid (Shen et al., 2010). These authors further concluded that rosmarinic acid, oleanolic acid, and ursolic acid are the major nonvolatile secondary compounds present in the EO of oregano. In other similar studies, the antiinflammatory activities of different plant EOs have been extensively investigated. However, further research is necessary using in vitro techniques or different

animal models with both healthy and challenged animals under various disease models in order to elucidate the underlying mechanism and their effectiveness.

Carvacrol-rich EO of *O. onites*, showed a marked dose-dependent analgesic activity as assessed by the tail-flick method in male albino mice (Aydin et al., 1996; Okazaki et al., 1998). Findings obtained in the study strongly suggested that the analgesic activity of the EO is related to its carvacrol content (Aydin et al., 1996). The antiaggregant effect of a methanol extract of *O. majorana* on human platelets has been studied by Okazaki et al. (1998). *O. majorana* extract inhibited platelet aggregation induced by collagen, ADP, arachidonic acid, or thrombin, depending on its concentration (Okazaki et al., 1998).

Only a few reports on the topical antiinflammatory effects of *O. vulgare* refer to oregano–herbal mixtures or their decoctions, used in the treatment of inflammation as supporting material (Deryabin, 1990). The hydroalcoholic extract of *O. vulgare* leaves found to possess strong antiinflammatory activity after oral administration to mice. The mice did not suffer from gastritis, although they were inflammation-induced by cold-resistant stress, whereas the cutaneous oregano application prevented contact hypersensitivity induced by oxazolone (Yoshino et al., 2006).

While analyzing the antispasmodic effects of oregano, it was found that aquous extracts considerably inhibited smooth-muscle response induced by any of the tested spasmogens (acetylcholine, histamine, serotonine, $BaCl_2$, nicotine) in the guinea-pig ileum. Moreover, in the pharmacological inhibition of smooth muscular activity, nonspecific and noncompetitive mechanism of action was attributed to thymol and carvacrol; they caused both direct musculotropic (muscle-relaxant activity), an indirect neurotropic (inhibition of the nerve action potential) action on the smooth muscle (Kintzios, 2002). The same results were obtained by testing the antispasmodic effects of pure active components, that is, thymol and carvacrol. It was concluded that both phenols act as noncompetitive Ca^{2+} antagonists, which block nerve fiber conduction and induce musculotropic and eurotropic spasmolyse (Kintzios, 2002). Oregano EO contains phenolic compounds that are known to possess strong antiinflammatory properties, such as the terpenoids and flavonoids. These phenolic substances may control the metabolism of inflammatory prostaglandins (Craig, 2001).

4.5 Immunostimulating, Antimutagenic, and Protective Effects of Oregano

Oregano extracts or herbal mixtures with *Origamun* spp. possess in vitro antiviral activity or have immune-stimulating effects both in vitro and in vivo. However, little knowledge has been attained so far on the mechanism of immune-modulating activity or underlying active compounds. A strong and dose-dependent capacity of inactivating dietary mutagen Trp-P-1 in the *S. typhimurium* TA 98 assay was observed for *O. vulgare* water extracts that exhibited significant antimutagenic effects in vitro (Kintzios, 2002).

A study evaluated the effect of oral administration of *O. majorana* (volatile oil, alcoholic, and aqueous extracts) on the toxicity induced by oral administration of lead acetate in the

diet of mice for 1 month. Mice treated with the three different preparations of *O. majorana*, 1 month before and maintained with lead acetate administration, showed a decrease in serum transaminases, alkaline phosphatase, urea, and creatinine, and improved the liver and kidney histology in comparison with lead acetate treated group. Alcoholic extract reduced the rate of micronucleus, the number of aberrant cells, and different kinds of chromosomal aberrations. Volatile oil extract reduced the rate of micronucleus and chromosomal fragments. Aqueous extract and volatile oil also reduced the number of gaps, ring chromosomes, and stickiness. The study demonstrated that *O. majorana* plays a notable role in ameliorating liver and kidney functions and genotoxicity induced by lead acetate (El-Ashmawy et al., 2005). Nowadays, a central research field is the control of lipid oxidation and the prevention of biological damages caused by free radicals. The effective natural antioxidants, such as oregano EO and its compounds can offer health benefits against oxidative stress in animals and also, in the food industry by increasing its antioxidative capacity in order to prevent food deterioration.

Several research groups in several countries have investigated the immunomodulating potential of various EOs, such as oregano EOs. Szigeti et al. (1998) reported that the incorporation of a plant-based product in chicken feeding diets supported their antibody production against *S. enteritidis*, *Leptospira pomona,* and *Pasteurella multocida*.

Significant productions of specific immunoglobulins—IgA, IgM, and IgG—were detected in the intestinal mucosa of chickens with *E. tenella* infection (Girard et al., 1997). Küçükyıllmaz et al. (2012) reported that coccidial infections interfere with the immune responses for a mixed infection with *Eimeria* spp. inducing a onefold decrease in serum antibody titer against the infectious bursal disease vaccine. However, dietary supplementation with a blend of oregano EO accelerated the development of humoral immunity following vaccination of broilers with live coccidian vaccine and subsequent challenge with multiple coccidian species.

However, the literature review shows a great variability on the effectiveness of different methodologies or different plant preparations or storage time. A possible explanation lies in the different experimental designs and the variable level of dietary supplementation. New and widly acceptable experimental designs should be followed in order to differentiate of the immune function or their affecting parameters to be under control (Hanieh et al., 2010). The effectiveness of immuno-modulating properties of oregano EOs should be further tested on different animal models and should be further investigated with both healthy and disease-challenged animals.

5 Use of Oregano in Animal Nutrition

Oregano can be added in the feed as dried plant, as EO (alone or in mixture with other plant EOs), or used as a source of carvacrol and thymol. Depending on the examined farm-animal species, the main examined performance parameters were final body weight, body-weight

gain, feed consumption, mortality, and feed-to-gain ratio. Also, egg production, in trials with layer hens, or milk production, in trials with ruminants, have also been investigated. In this vast examined literature, there was a wide variety in experimental protocols, examining from 20 to 6300 animals, under diverse commercial or experimental conditions (Giannenas et al., 2013b). Also, in some cases, the chickens were under challenge (for example inoculated with coccidia) thus dietary oregano was also examined as a possible healing agent. Oregano inclusion levels in the feeds ranged from 1.25–30.00 g/kg for the dried plant parts, 15–1000 mg/kg for the EOs and 24–1500 mg/kg for mixtures or combinations of oregano EO with other EOs of several plants. The reported significant findings for the main examined parameters were: final body weight (−6.7% to +23%), weight gain (up to +14.1%), feed intake (−15.1% to +9.2%), and daily milk yield (up to +35.4%) (Giannenas et al., 2013b).

5.1 Effects of Dietary Oregano in Poultry

A dietary supplementation of oregano ground herb exerted a growth-promoting effect and potent antioxidant activity (Giannenas et al., 2005a). Feed supplementation with oregano decreased lipid peroxidation as expressed by malondialdehyde values in meat samples as compared with the control. The dietary supplementation of oregano EO improved production efficiency in broiler chickens and lowered the feed intake (Symeon et al., 2010). In another study, broiler chickens were fed 1 g EO (oregano/clove) or 1 g EO (oregano/cinnamon) per kilogram body weight and after 35 days the final body weight did not differ, but the feed utilization was improved (Halle et al., 2001).

Besides to the meat, the poultry industry provides eggs for human consumption. The egg is an excellent food with important inherited mechanisms of protection. However, nowadays, the production of eggs enriched with ω3-fatty acids is growing and there is a need to support the antioxidant capacity of the egg. For the egg quality characteristics to be further improved, however, it is a prerequisite to follow appropriate guide in nutrition, management, and environmental practices (Khan et al., 2012; Mahmoud et al., 2010). In the literature, only a few published papers provided evidence on the beneficial effects of oregano on egg-production rate and egg quality. The effects of dietary supplementation of oregano EO on the antioxidative capacity of shell eggs or egg yolk were examined in comparison to those of dietary α–tocopherol. It was reported that the extent of lipid oxidation in shell eggs, presented significantly different values among the control diet and the oregano supplemented diet. However, the effect of storage time, as measured by malondialdehyde formation, was not altered. In the stored egg yolks, the lipid peroxidation values showed significantly different values at all measured time points among the different dietary treatments. Considering that the egg yolks from the oregano groups exhibited increased antioxidant resistance in comparison to the control diet, it could be hypothesized that oregano oil components, such as the phenolics can be absorbed in the intestine and enter the egg yolk through blood circulation of the hen, providing their superior antioxidant properties to the

egg (Giannenas et al., 2005b). The transfer rate of phenolic compounds into the egg can be guaranteed, if a critical quantity of phenolic compounds is added into the diet.

The substitution of antibacterial growth promoters with other efficient, safe, and natural substances is a crucial point for the poultry industry, especially in broiler-meat production. In the literature, several positive reports have been announced about the use of natural products or plant EOs as alternative growth promoters. Typical performance parameters for poultry rearing are body weight, weight gain, feed intake, mortality, and feed-to-gain ratio values. It has been stated that the dietary inclusion of oregano EOs at 50 and 100 mg/kg of feed, in conventional commercial broiler chicken diets had no significant effect on overall body weight and feed-to-gain ratio values. Oregano supplemented chickens gave similar performance values as compared to control chickens or chickens whose diets were supplemented with 200 mg α-tocopheryl acetate (Lee et al., 2004). An experimental trial with broiler chickens showed that feed supplementation with two commercial phytogenic products containing two mixtures of three components—the first one oregano, pepper, and cinnamon, and the second sage, rosemary, and thyme—at the level of 200 mg/kg of feed and 5000 mg/kg of feed did not improve body weight gain, feed consumption, or feed-to-gain ratio as compared to chickens fed the control diet or compared to chickens fed a diet containing avilamycin (Hernandez et al., 2004). In another study by Jamroz et al. (2005), chickens were fed a diet that contained 100 mg/kg of a plant extract rich in carvacrol, capsicum oleoresin, and cinnamaldehyde, and showed considerably better feed-to-gain ratio up to 4%. Similarly, it has been found that feed enriched with 500 mg/kg rosemary powder in broiler chickens resulted in greater body-weight gain as compared to chickens fed the control diets (Spernakova et al., 2007). Other researchers (Cabuk et al., 2006) reported that chickens fed a diet containing a mixture of oregano, sage, anise, laurel, and citrus EOs showed a superior feed-to-gain ratio value as compared to chickens with unsupplemented diets. Moreover, in another study with chickens fed diets enriched with mixtures of oregano, cinnamon, cayenne pepper, thyme, and citrus EOs or a mixture of plant extracts and organic acids showed higher body-weight gain and higher feed intake as compared to chickens fed the control diet or a diet containing the antibiotic avilamycin. Lippens et al. (2005) also tested the efficacy of a mixture of oregano, cinnamon, cayenne pepper, thyme, and citrus EOs extracts, and a mixture of plant extracts and organic acids in comparison to growth-promoter avilamycin in broiler chickens. The authors reported an improved feed-to-gain ratio compared to the chickens fed with avilamycin-supplemented diet, as well as compared to the chickens that received the control diet. However, the authors did not find any synergistic effect among the plant extracts, organic acids, and EOs in terms of growth, feed intake, or feed-to-gain ratio.

Several reasons can stand for variability of the aforementioned results; different mixtures of plant extracts, differences in levels of diet supplementations, single or combined use, duration of the period that chickens fed with supplemented diets, and differences in processing of the medicinal plants and EOs, along with differences in management and husbandry conditions

are only some of those. Khan et al. (2012) reported that different commercial products containing different plant materials should not be regarded as products with equal possible efficacy, even when their claims for composition are similar.

There are several modes of action that may affect the intestinal microflora or modulate functionality of the gastrointestinal tract to support both the animal health and its growth performance. In an extensive review, some of them, such as feed transit time, augmentation of digestive secretions, and greater activity of digestive enzyme activities, or the combination of all these effects that can improve nutrient digestibility have been essentially highlighted (Puvaca et al., 2013). In the same way, Lee et al. (2004) suggested that that plant extracts in chicken diets might enhance the intestinal activities of major digestion enzymes, such as trypsin, lipase, and amylase. Jamroz et al. (2005) reached the conclusion that diet supplementation of plant EOs or extracts in broiler chickens substantially improved lipase activity by almost 50%. In a similar study, Jamroz et al. (2006) reported a significant impact of plant EOs on intestinal architecture. However, in another study, chickens that received through their diet a commercial preparation containing EOs of cinnamaldehyde and thyme at the level of 100 mg/kg diet showed no effect on apparent ileal digestibility either of crude protein or starch. Also, no differences were noted for the total tract fat digestibility at the age of 21 or 40 days of age (Lee et al., 2004). In contrast, a study in which male chickens fed a diet containing two different plant-based products consisted of three-components showed a significant effect on ileal digestibility of dry matter, starch, and total apparent digestibility of crude protein and fat compared to the birds that received a diet without plant extracts (Hernandez et al., 2004). Another product based on plant extracts and EOs containing carvacrol, capsicum oleoresin, and cinnamaldehyde when given to male broiler chickens through their diet did not change substantially the apparent ileal digestibility of nutrients compared to chickens that consumed the control diet. In a recent study, Cross et al. (2007) investigated five different plants and their respective EOs. Despite the effects of dietary plant extracts on growth performance, no change was noticed on the apparent digestibility of metabolizable energy or the total tract apparent digestibility coefficients of dry and organic matter. On the other hand, Theron and Lues (2007) found that the dietary addition of EOs blend made up from oregano, citrus, and anise given at the level of 125 mg/kg of diet improved ileal apparent fat digestibility in broiler chickens.

Another aspect concerning the meat industry is the effect of aromatic plants, herbal extracts, and EOs on meat quality. It has been found that plant EOs, when given to chickens through their diet, can lead to beneficial effects on stored meat quality. One possible effect is the potent antioxidant properties of their phenolic compounds. These plant antioxidants can delay lipid peroxidation in the chicken meat or tissues (Puvaca et al., 2013). Sage and rosemary EOs (Lopez-Bote et al., 1998), ground oregano plant, oregano oil (Young et al., 2003), ground rosemary leaves (Govaris et al., 2007), and rosemary in the form of powder (Spernakova et al., 2007) have been investigated for their antioxidant properties with positive results.

However, diet supplementation with anise oil led to a proportional increase on breast muscle of the eviscerated carcass by only 1% compared to chickens given the control diet, whereas anise-seed supplementation had no effect on carcass dressing percentage (Jamroz et al., 2005; Soltan et al., 2008).

The addition of EOs in chicken diet not only can improve their antioxidant status but it can also contribute to an increased food safety by improving its microbiological status and the overall quality during refrigerated storage of either raw or cooked meat products (Soltan et al., 2008). Recently, it has been reported that even microminerals in certain amounts of diet may positively affect meat quality or carcass meat safety because their concentration may either affect antioxidant meat status or inhibit the growth of spoilage surface bacteria (Stanacev and Puvaca, 2011).

5.2 Effects of Dietary Oregano in Pigs

The pig industry is also interested in supporting both the growth performance and meat quality of slaughtered animals. Several experimental reports support the theory that dietary inclusion of plant extracts or EOs may improve both performance and meat quality of pigs. One notable effect of plant EOs is that they may improve feed palatability and enhance appetite and digestion. Indeed, Janz et al. (2007) showed that that pigs preferably consumed feeds supplemented with rosemary and garlic compared to the feed supplemented with ginger or oregano. Also, Jugl-Chizzola et al. (2006) showed that weaned pigs consumed less feed when it was supplemented with oregano or even thyme compared to the feed of control animals. On the other hand, cinnamon, laurel, clove, cardamom, and mint are plants known to possess strong appetite-stimulating effects (Loo and Richard, 1992).

As early as almost two decades ago, a number of studies in pigs or piglets showed beneficial growth-promoting effects of dietary oregano extract (Kyriakis et al., 1998; Tsinas et al., 1998) on young piglets and fattening pigs. Dietary carvacrol, capsicum oleoresin, and cinnamaldehyde given in combination beneficially modulated the intestinal microflora to a more favorable one in weaned piglets (Manzanilla et al., 2004). This mixture was also tested in vitro, providing positive effects on blood lymphocytes of pigs against oxidation impairment after dietary incorporation at the level of 270 mg/kg of diet. This effect was similar to that of α-tocopherol at inclusion level of 90 mg/kg of diet. On the other hand, this mixture was not effective in delaying lipid oxidation, during the consumption of high amounts of oxidized polyunsaturated fatty acids.

Allan and Bilkei (2005) investigated the dietary addition of 1000 mg/kg of dried oregano leaves and flowers or 500 mg/kg oregano EO and found positive effects, especially on mortality rate. He also noticed less culling during lactation periods, shorter service intervals, more live born and fewer stillborn piglets. According to these findings, herbs, spices, plant extracts, and their EOs can be regarded as appetite enhancers, digestion stimulators,

growth promoters, and effective supporters of animal health or welfare. In another study by Bauer et al. (2001) raw belly bacon produced from animals fed with oregano demonstrated improved stability compared to that produced by the control group, after 34 weeks of storage.

Oregano and its extract, as was already mentioned, hold a strong antioxidant activity. Oregano can be used as fat stabilizer, to improve shelf-life meat quality, or antioxidant status of fat-containing food. However, on some studies, oregano failed to sustain meat quality and shelf life of fat obtained from pigs given dietary oregano EO (Vichi et al., 2001). Another study showed a positive effect of an oregano-rich phytogenic feed additive in combination with high levels of vitamin E on average daily weight gain when compared with pigs fed a diet without phytogenic feed additives. However, no differences were noted on feed-to-gain values among the experimental groups (Bilkei and Gertenbach, 2001).

Other researchers investigated the effect of dietary oregano EO as nonspecific immune-modulator in pigs that showed retarded growth presenting low final weight at growing-finishing period. A group of pigs were fed with commercial fattening diet supplemented extra with a commercial feed additive containing oregano at the level of 3 g per kg. This product was made by composing dried leaf and flowers of *O. vulgare*, enriched with 500 g/kg cold-pressed oregano EO, containing 60 g carvacrol and 55 g thymol per kilogram of its EO. It was interesting to find that dietary supplementation with oregano extract and oregano oil improved daily growth rate in growth-retarded growing-finishing pigs and had positive immune stimulating effects on porcine immune cells supporting the function of nonspecific immunity (Walter and Bilkei, 2004).

In another study, the effect of phytogenic feed additive containing 1 g of dried oregano powder per kg on vaccination was investigated on piglets after weaning. Mortality postweaning due to *E. coli* was reduced as piglets received the vaccination with purified VT2e toxoid along with dietary oregano extract supplementation, losses due to postweaning *E. coli* complex in weaned pigs were prevented and their growth performance was improved (Ken and Bilkei, 2003).

In a study with sows and weaned animals in Switzerland, a phytogenic fed additive containing 1000 mg oregano EO per kilogram of feed improved the performance of sows after their vaccination against *Haemophilus parasuis* serovar 5 (HPS 5) and *E. coli*. The average daily weight gain, feed efficiency, and mortality of weaned pigs was observed from day 35 and was improved on the groups of weaners fed the oregano supplement compared to the groups fed without the supplement (Sads and Bilkei, 2003).

The dietary EO of oregano in pigs was again tested and found to increase daily weight gain and decrease feed conversion ratio of the fattening animals. The pigs fed the diet with the oregano EO also had higher carcass weight, dressing percentage, and carcass length than those of pigs given the basal or an antibiotic-supplemented diet. In the pigs that consumed the oregano diet, lower subcutaneous fat thickness was noticed. Moreover, lean meat and portions

of ham portions were found to be higher in the oregano supplemented pigs. Therefore, these results could support the hypothesis that the use of *Origanum* EO as feed additives can improve the growth performance of pigs and has more pronounced positive effects on carcass composition than antibiotic growth promoters (Onibala et al., 2001).

The oregano EO was also tested in the diet of weaning pigs as alternative for antimicrobial growth promoters. In this trial, a liquid product containing 10% oregano oil was added to the drinking water. The oregano oil supplemented pigs showed higher growth performance during the first two weeks after weaning. These authors reported that oregano EO has a proven efficacy on the performance of the piglets and it should be tested in prestarter diets to further improve performance of these animals (Van Krimpen and Beinnendijk, 2001).

In a similar study, the aim was to test whether oregano oil supplementation to the diet, could affect nutrient digestibility, N-balance, as well as several parameters characterizing the microflora and its properties in the gastrointestinal tract of weaned pigs. In contrast to previous findings, all the apparent digestibility values of nutrients except for fiber and the N-balance of the weaned piglets were not affected by feeding piglets restrictively with feed additive and the determination of bacterial microflora and its activity was not influenced by oregano feed additive (Moller, 2001).

A study compared the resistance patterns of *E. coli* isolated from pig herds receiving antibiotics or oregano based feed additive, as preventive feed additives. Oregano-fed pig herds demonstrated lower MICs (minimum inhibitory concentrations; μg/mL) for oxytetracycline, ampicilline, doxycycline, gentamicin, enrofloxacin, and sulfamethazine against *E. coli* compared to herds with prophylactic use of antibiotics. Resistance to ceftiofur revealed important differences between the antibiotic and the nonantibiotic oregano-treated farm units (Docic and Bilkei, 2003).

With the increase in antibiotic-resistant bacteria and the lack of new antibiotics being brought onto the market, alternative strategies need to be found to cope with infections resulting from drug-resistant bacteria. A possible solution may be to combine existing antibiotics with phytochemicals to enhance the efficacy of antibiotics. Many reported assays show additivity or moderate synergism, indicating that EOs may offer possibilities for reducing antibiotic use (Langeveld et al., 2014).

In another similar study with sows during lactation or until the first standing oestrus, the sows were given an oregano oil–supplemented diet (*O. vulgare*), chlortetracycline, or no supplement. It was found that weaning-to-oestrus interval was shorter in sows supplemented with oregano compared to other treatments. Farrowing rate was greater in sows supplemented with either chlortetracycline or oregano compared to untreated sows (Kis and Bilkei, 2003).

Another study also reported that dietary oregano supplementation improved the sows reproductive performance. Farrowing sows receiving 1000 ppm of an oregano based

product with their lactation diet showed a lower mortality rate, a lower culling rate during lactation, an increased farrowing rate, more born piglets, and fewer stillbirth piglets per litter compared to females that received a diet without the oregano-based supplement (Kovac and Bilkei, 2003). In similar trials in farrowing groups of sows during lactation, from parturition throughout lactation and from weaning to mating, it was found that sows received the diet without oregano showed higher annual mortality rate, increased culling rate during lactation, increased farrowing rate, fewer born piglets per litter and more stillbirths. These trials in piglets, pigs or sows showed that oregano can be regarded as an efficient feed additive of natural origin (Mauch and Bilkei, 2004).

5.3 Effects of Dietary Oregano in Ruminants

Nutritionists have proposed novel methods to improve feed efficiency and productivity in ruminants. Microbiologists have been searching alternative methods to manipulate rumen functionality (Benchaar et al., 2009). Plant EOs have proven antimicrobial activity that enables them to be potential alternatives to antibiotics to improve the rumen microbial activity. In the last decade, a vast number of research studies have investigated the effects of EOs, or their active components, on rumen fermentation characteristics (Benchaar et al., 2008, 2009). Although, most of these studies were in vitro, they provide significant evidence that EOs and their individual components have the potential to improve nitrogen and/or energy utilization in ruminants. New research insight on the effects of oregano EOs or its main compounds on ruminal N metabolism is more likely mediated by their impact on hyper-ammonia producing bacteria causing lower deamination of amino acids and less production of ammonia. On the other hand, it must be noted that these responses are only reported by high doses of EOs, which also can impair the process of ruminal fermentation as reflected by a decline in total volume of volatile fatty acids production. Other reports concerning the effects of oregano EOs on methane production are inconsistent, although, evidence to date indicates that there is a potential to select EOs, or active components, that could selectively inhibit ruminal methanogenesis, this has not been fully achieved yet (Benchaar et al., 2008). Long-term in vivo research studies with in vitro continuous culture studies showed that rumen microflora populations could adapt to dietary EOs. This may account for the lack of an effect of EOs on ruminal metabolism and animal performance in long-term trials. These findings have prompted animal scientists to further investigate their capability to reduce ruminal methanogenesis (Benchaar et al., 2008; McIntosh et al., 2003; Mohammed et al., 2004), limiting the production of this gas that mostly promotes greenhouse phenomenon. In vivo research using novel EOs compounds in ruminant nutrition may provide the potential to support animal production and health, as well as to benefit the environment.

Oregano EOs can be used as feed additives in ruminant nutrition to improve performance of dairy or meat animals, increase feed efficiency, and support health. On the other hand,

detailed characterization of EOs, or their active components, that can favorably alter rumen fermentation without resulting in broad overall inhibition of rumen fermentation, remains a major challenge for animal scientists or nutritionists. Hristov et al. (2013) showed that oregano leaf material decreased rumen methane production when fed at a level of 250–750 g within 8 h after feeding. Also, oregano decreased rumen ammonia concentration but it did not affect microbial diversity of either milk composition or milk fatty acid profile. In another study, oregano plants did not affect methane production; however, milk flavor was improved by low dietary supplementation at the level of 0.2 and 1.0 g oregano EO per kg of feed in dry matter (Lejonklev et al., 2016).

Evidence of long-term in vivo antimicrobial activity of aromatic plant EOs has been confirmed in several ruminant cases, although plant extracts have been used to manipulate ruminal metabolism in order to improve feed efficiency and animal productivity in the last decades (Benchaar et al., 2009; Greathead, 2003). However, it has been reported that in vitro batch culture studies provide evidence that EOs or their components have the potential to improve nitrogen and/or energy utilization in ruminants by altering microbial populations (Benchaar et al., 2008). Eight dual-flow continuous culture fermenters were used in four consecutive periods of 10 days to study the effects of several plant-based products on ruminal fermentation profile and rumen protein degradation rate. Fermenters were fed a 60:40, Lucerne hay:concentrate diet (DM). One of the treatments was 7.5 mg/kg DM of extracts of *O. vulgare*, which increased the acetate and decreased the propionate proportion during the first 6 days of fermentation, in comparison to controls untreated with the plant extract. However, these differences disappeared after 1 week, suggesting that rumen microbes were adapted to the additives within 1 week and that data from short-term in vitro fermentation studies may lead to erroneous conclusions, and should be interpreted with caution (Carnozo et al., 2004).

5.4 Effects of Dietary Oregano in Rabbits

Oregano feed additives may also be used in rabbit diets as an alternative to antibiotics and other substances, such as chemical antioxidants and anticoccidials. Oregano compounds may also function as activators of the immune system. The published information on the dietary use of aromatic plants or especially oregano oil in rabbit nutrition is limited. According to Ayala et al. (2011) dried oregano plants after dietary supplementation improved rabbit growth performance, while Pogany-Simonova et al. (2010) have found that oregano and sage EOs or their extracts improved health of rabbits and altered their carcass composition. However, other researchers (Botsoglou et al., 2004; Soultos et al., 2009) found that oregano EO had no effect on performance parameters of rabbits but increased the antioxidant activity of raw and cooked carcass meat parts, such as *Longissimus dorsii* muscle tissue parts, during refrigerated storage for 7 days. Additionally, dietary oregano EO was also very effective in delaying microbial growth on rabbit carcass parts during refrigerated storage for 7 days

(Soultos et al., 2009). Moreover, dietary oregano EOs supplementation greatly improved carcass quality that exhibited considerably less slime and off-odors than the control animals. It is possible that oregano EO components entered the circulatory system after digestion, and were distributed and retained in tissues through metabolism, and thus was responsible for the paramount antioxidative and antimicrobial activities. However, the bioavailability of these components is not easy to be monitored because there are not available analytical methods capable of identifying and quantifying them at trace levels in tissues. For this reason, further research is necessary in order novel analytical methods to be fully developed (Soultos et al., 2009).

6 Conclusions

A plethora of literature reports proposes that oregano EOs and their main compounds have proved their in vitro efficacy as antimicrobial, antiparasitic, immunomodulating, antiinflammatory, and antioxidant, agents. These phytogenic compounds deserve a significant position as dietary additives that are generally considered as safe. The oregano EOs and their main derivative compounds could be used for those situations where difficult problems of bacteria, parasite, or protozoa resistance development has occurred or unwanted side-effects of the available approved drugs or chemical substances, such as synthetic antioxidants or chemical antimicrobials, are to be expected. The variability of oregano EOs and their composition may urge future research to select products with constant natural composition or enriched with pure compounds—that is, carvacrol, thymol, terpenes—for establishing their possible role as alternatives to antibiotic growth promoters in animal diets. Based on the available in vivo data, it can be concluded that oregano EOs and their compounds can be used as natural nonantibiotic growth promoters in animal nutrition. Nevertheless, there are still some research topics that need further light to be shed to provide evidence of the positive effects, such as nutrient digestibility and functionality in the gastrointestinal tract of poultry, pigs, and ruminants. The efficacy of oregano EOs on animal performance relies on many factors. The main concern is the differences in composition of the active components, dietary inclusion levels, animal genetics, and the diet composition. The progression of knowledge of the complexities of the gastrointestinal ecosystem of the different animal models is necessary in order for the mechanisms of action of oregano EOs to be fully investigated. This will provide significant data for designing further research trials of the highly efficacious oregano EOs based products. The vast literature review confirms several beneficial responses to dietary oregano in animal production parameters. They also indicate a need to clarify oregano dose–response relationships in order to maximize efficient use in praxis and to facilitate cogent comparisons with other additives, including other nonantibiotic feed additives as growth promoters for use in poultry, pig, or ruminant livestock, such as organic acids, prebiotics and probiotics. However, a methodological investigation for evaluating the

growth efficacy and the safety of oregano EOs or the products containing oregano is necessary, as feed additives still need further clarification. According to this review, oregano EO can be used as a novel functional ingredient in the animal feed and medical industry, although further research is needed to confirm and standardize all the positive beneficial properties.

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CHAPTER 7

*Potential of Chokeberry (*Aronia Melanocarpa *L.) as a Therapeutic Food*

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1 Introduction

Regular consumption of fruits, vegetables, and other foods rich in antioxidants is often associated with improvement in overall health and lower incidences of cardiovascular and other chronic diseases. The positive health effects of plant foods are partly attributed to the presence of vitamins, minerals, and dietary fibers, but mostly to the nonnutritive, biologically active compounds. These compounds, known as phytochemicals, can exert protective effects against development of cardiovascular diseases (CVD) by acting independently or in synergism with each other. Since it has been shown that oxidative stress can contribute to the pathogenesis of CVD, the intake of dietary antioxidants should be recommended for their prevention. Dietary antioxidants are defined as constituents of foods, which can prevent or ameliorate deleterious effects of reactive oxygen species and free radicals on normal physiological functions. Among dietary antioxidants, phenolic compounds are of special interest, due to their high content in plants, high dietary intake, and strong antioxidant activity. Polyphenols are secondary plant metabolites with unique chemical structures, containing at least one aromatic ring and one or more hydroxyl groups. In plants, polyphenols have a role in protection from herbivores, infection, and UV radiation, but also in attracting pollinators (Del Rio et al., 2010). More than 8000 phenolic compounds have been identified and most often they are classified as flavonoids and nonflavonoids. The first group includes flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavan-3-ols, while the second one is composed of phenolic acids, stilbenes, and lignans. According to some studies, the average intake of polyphenols in Europe is around 1 g/day (Scalbert and Williamson, 2000; Zujko et al., 2012). Potential health impacts of polyphenols are broadly investigated and their beneficial effects are claimed in subjects with cardiovascular risk factors, such as diabetics, metabolic syndrome subjects and dialysis patients, but in healthy subjects as well. At the same time, people are becoming more aware of the importance of polyphenol-rich foods, as do the small, medium, and industrial enterprises. Still, European Food Safety Authority (EFSA) has approved only a

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few health claims related to polyphenols and polyphenol-rich foods. The reasons for this may be the lack of strong evidence of a cause–effect relationship, insufficient description of food or food matrix, and unclear mechanisms of action (Konić-Ristic et al., 2015). In addition, many of clinical trials suggest that the beneficial effects of polyphenols are not adequately designed, randomized, or controlled. The biggest issue can be the absence of placebos in these studies, because it is difficult to mimic complex food items, especially when the compounds tested for their activity contribute to sensory properties as well. Still, numerous epidemiological, as well as in vitro studies, have confirmed polyphenols' health-promoting effects. Polyphenols are considered to be the most important dietary antioxidants, with the activity higher than vitamin C or E. They can exhibit strong protective effects against cellular oxidative damage, and act both directly and indirectly against oxidative stress. Direct effects include scavenging electrons from free radicals and converting them to more stable forms, while indirect effects involve regulation of endogenous antioxidant enzymes' activities (Mitjavila and Moreno, 2012; Rice-Evans et al., 1997; Scalbert et al., 2005). Additionally, recent investigations suggest that polyphenols can protect biological membranes against lipid peroxidation by interacting with the phospholipid bilayer and incorporating in its outer hydrophilic part. Among various plant foods, chokeberry (*Aronia melanocarpa*) is of special interest due to its high content of polyphenols, which results in promising health effects.

2 *Chokeberry (*Aronia melanocarpa*)*

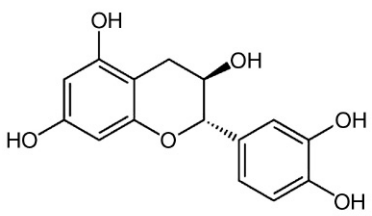
Genus *Aronia* is a member of the Rosaceae family and *Maloideae* subfamily and includes two species of shrubs originating from eastern part of North America: *A. melanocarpa* and *Aronia arbutifoila*, black and red chokeberry, respectively. *A. prunifolia* is a third entity, but it is differently classified, a variety of each of the other two or a hybrid (Kulling and Rawel, 2008). Comparing these three, black chokeberry is the most valuable as food ingredient. It is also named (black) aronia, or just chokeberry, meaning "bitter berry." Accordingly, it is most often cultivated and used for the fruits production and related goods, such as juices, jam, wines, and food colorants. Chokeberry is naturally grown in Canada and southern part of the USA, and it is often used as an ornamental plant in gardens, since it can be adjusted to the conditions not favorable for other fruits, such as acidic, poor, or humid soils. Since its strong resistance to cold, chokeberry can be grown not only in milder climate conditions, but also at temperatures below −35°C. Nowdays, it is often grown in Russia and Baltic countries, but also in Poland and Germany, where industrial processing of fruits has been developed. Chokeberry products are traded in Europe, as well as in USA, mostly in form of fresh, dried fruits, or juice.

2.1 Polyphenolsas Active Compounds of Chokeberry

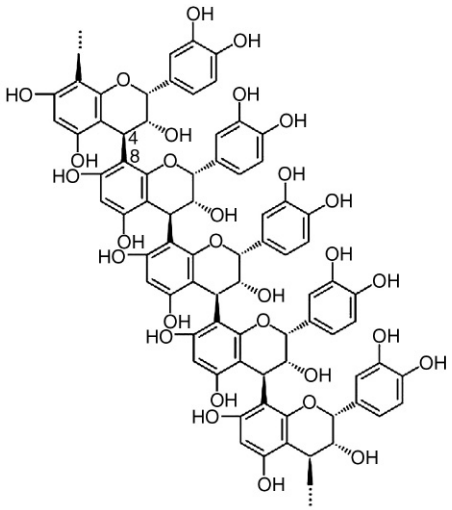
Different plant materials and plant parts have been analyzed as a potential dietary source of polyphenols. It is believed that berry fruits have the highest content of polyphenols, with a wide range of phenolic subclasses, mainly flavonoids and phenolic acids (Oszmiański

and Wojdylo, 2005). In comparison to the extracts of 92 edible and nonedible plants, berry fruits were characterized with the highest antioxidant activity (Kähkönen et al., 1999). Antioxidant potential of berry fruits depends not only on the total phenolic content, but also on the structure of the present phenolic compounds. Chokeberry has the highest content of polyphenols as compared with the other berry fruits and polyphenol sources in general. For example, chokeberry juice has up to fourfold higher antioxidant activity than red wine, blueberry or black current juice (Gil et al., 2000; Seeram et al., 2008).

The content of polyphenols in chokeberry fruits varies and depends on the environmental conditions, habitat, cultivar, maturation, harvest time, and other factors (Kulling and Rawel, 2008). Accordingly, the total phenolic content ranges from 3.44 g up to 7.85 g/100 g of dry weight. Besides the earlier mentioned factors, obtained values depend on the applied methodology as well (Kulling and Rawel, 2008). Based on the literature data, the main polyphenolic subclasses of chokeberry fruits are: procyanidins, anthocyanins, and phenolic acids. Polymeric procyanidins are the major subclass with up to 66% of total polyphenols; at the same time, they are the main contributors to the specific astringent, tart taste of chokeberry fruits, and their products (Oszmiański and Wojdylo, 2005). Procyanidins are predominately composed of (–)-epicatechin (flavan-3-ol) subunits connected mainly with C4—C6 and C4—C8 bonds (B-type bonds), with the degree of polymerization from 2 to 23 units in the fruits (Fig. 7.1) (Kokotkiewicz et al., 2010). Free flavan-3-ol epicatechin also appears in black chokeberries, but compared with polymeric procyanidins, in much lower concentration. In comparison with other berries, chokeberry is the best dietary source of anthocyanins with 5,000–10,000 mg per kg of fresh fruits (Neveu et al., 2010). Anthocyanins



(–)-Epicatechin


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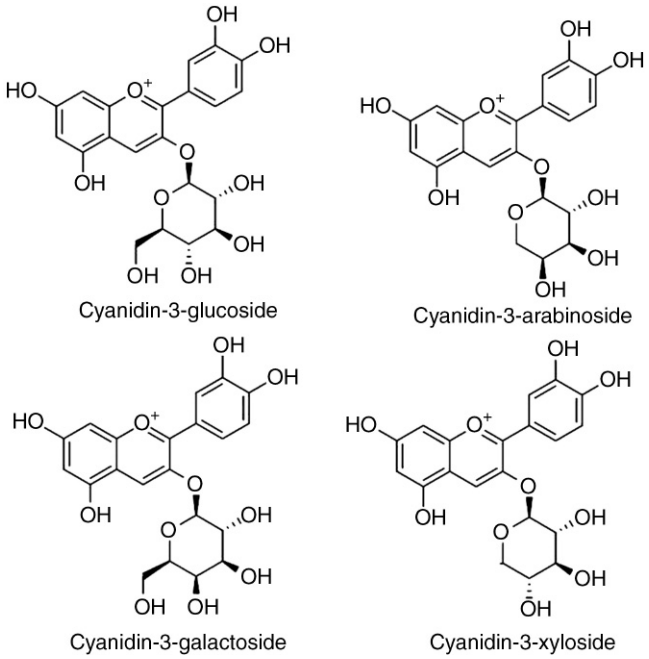


Figure 7.2: Structures of the Main Anthocyanins.

make up approximately 25% of total phenolic compounds present (Fig. 7.2). They consist mainly of cyanidin glycosides: glucoside, galactoside, arabinoside, and xyloside, with cyanidin arabinoside and galactoside accounting for 60%–75% of the total anthocyanins (Oszmiański and Wojdylo, 2005). Due to their high content, anthocyanins are important factors of the antioxidant defense contributing with 33 and 42% to the total antioxidant capacity of chokeberry fruits and juice, respectively (Zheng and Wang, 2003). Phenolic acids, mainly hydroycinnamic acids: chlorogenic and neochlorogenic, are the third highest abundant phenolic subclass present in chokeberry fruits. Among berry fruits, chokeberry is the best source of phenolic acids, with 96 mg/100 g of fresh fruits (Mattila et al., 2006). Smaller amounts of flavonols, mainly quercetin glycosides, such as galactoside, glucoside, and rutinoside, are also found in chokeberry juice (Kardum et al., 2014a).

2.2 Antioxidant Potential of Chokeberry

Intake of dietary antioxidants, as part of the optimal diet, is recommended in order to boost antioxidant defense of the body and prevent development of cardiovascular and other chronic diseases (Arts and Hollman, 2005; Hooper et al., 2008). Polyphenols represent the main compounds contributing to the antioxidant capacity of plant foods. Due to substantial content of polyphenols, chokeberry is believed to be one of the strongest dietary antioxidants. The evaluation of the total phenolic content and antioxidant potential of different berry

fruits revealed significantly higher total antioxidant capacity (expressed as oxygen radical absorbance capacity, ORAC) in chokeberry, as compared with other berries, such as: cranberry, blueberry, and lingonberry. Accordingly, chokeberry had significantly higher anthocyanin and total phenolic content than other berry fruits (Zheng and Wang, 2003). Measurement of ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]) radicals confirmed high antioxidant activity of chokeberry, but also revealed notable variation between activities of fresh fruits, juice, and pomace (Oszmiański and Wojdylo, 2005).

Except for chemically based assays, antioxidant activity of chokeberry is confirmed in in vitro studies as well. For instance, a study that tested the effect of chokeberry extract on oxidative stress in homocysteine-treated human plasma, revealed an increase in total antioxidant capacity as a result of chokeberry extract addition (Malinowska et al., 2012). Additionally, Kedzierska et al. (2012) showed inhibitory effects of chokeberry extract on the superoxide anion radicals' production in platelets obtained from both breast cancer patients and healthy subjects. Furthermore, another study investigated cellular antioxidant activity of protocatechuic acid (PCA), which is considered to be one of the main human metabolites of cyanidin glycosides, compounds highly present in chokeberry. The cellular antioxidant activity was determined as an ability to protect erythrocytes being exposed to hydrogen peroxide. As a result, PCA decreases levels of intracellular reactive oxygen species' production with inverse dose-response correlation. This finding suggests antioxidant potential of PCA, which is probably not mediated by its direct antioxidant action (Konić-Ristić et al., 2013b). Chokeberry anthocyanin extract showed protective effects on pancreatic β-cells (βTC3) against hydrogen peroxide and high glucose–induced oxidative stress and cytotoxicity. In addition, activities of antioxidant enzymes were increased in pancreatic β-cells pretreated with chokeberry extract as compared to the cells exposed only to the prooxidant agents (Rugină et al., 2015).

2.2.1 Antioxidant effects in humans

Because lipids are assumed to be the major targets of free radical attack, indicators of lipid peroxidation are the most commonly used indices of oxidative stress level (Dotan et al., 2004). Besides usually measured products of lipid peroxidation, such as TBARS and isoprostanes, relative concentration of membrane polyunsaturated fatty acids (PUFAs) is suggested as an indirect marker of lipid peroxidation (Solans et al., 2000; Suárez et al., 1999). Lipid peroxidation is a result of complex long-chain reactions that leads to the formation of different products: peroxyl radicals and hydro peroxides in the first place. These reactions start by free radicals taking over hydrogen atoms from the carbons in the fatty acids side chains: more precisely, from the carbons linked with unsaturated bonds in fatty acid chains (Oszmiański and Wojdylo, 2005). Formed lipid radicals further react with each other in a process that could convert even several hundred fatty acids into hydro peroxides. PUFAs

are the most prone to the lipid peroxidation and oxidative damage, because they have many double bonds, which are the main site of free radicals attack. PUFAs are usually classified as $n-3$ and $n-6$ according to the position of the first double bond counted from the methyl end of side chain. Considering biological membranes as the first line of contact between cells and extracellular substances, including free radicals (Cyboran et al., 2012), content of PUFAs in the phospholipids bilayer of membranes can be an indicator of cell oxidative damage. Uncontrolled lipid peroxidation can be one of the main causes of cell damage leading to the inflammation, CVD, and other pathological processes. Oxidative damage of fatty acids leads to the changes in phospholipids structure and biophysical properties of cell membranes, such as its fluidity (Rice-Evans et al., 1997). Red blood cells are often used as a model of biological membranes, because of their high content of PUFAs and high permeability, which makes them susceptible to the free radicals attack. At the same time, erythrocytes are the most numerous blood cells and the main source of antioxidant enzymes. A recent study, conducted in healthy female volunteers (Kardum et al., 2014a), showed the protective effects of chokeberry juice consumption on membrane lipids, manifested as an increase in levels of total PUFAs in erythrocytes phospholipids. In the same study, unsaturation index, which represents an average degree of fatty acids unsaturation, also was increased. Similarly, glucomannan-enriched chokeberry juice affected relative concentration of membrane omega-3 PUFAs and unsaturation index in obese postmenopausal women (Kardum et al., 2014c). Besides a positive impact on fatty acid status, both of the studies revealed stimulatory effects of chokeberry juice on cellar antioxidant enzymes, as above stated. In addition, study on healthy females (Kardum1) reported a significant correlation between increase in GPx activity and changes in status of some membrane fatty acids. More precisely, bigger stimulation of GPx activity was followed by a higher increase in content of total and $n-6$ PUFAs, as well as an unsaturation index. Besides affecting the content of total PUFAs, chokeberry juice induced an increase in levels of $n-3$ PUFAs in both mentioned studies. In addition, the relative amount of docosahexaenoic fatty acid, $n-3$ fatty acid with six double bonds was increased, while $n-6/n-3$ ratio significantly dropped.

In addition to the in vitro findings, chokeberry products affected the oxidative status of humans as well. This mostly includes the reduction in levels of thiobarbituric acid-reactive substances (TBARS) and stimulation of antioxidant enzymes activities. TBARS are indicators of lipid peroxidation and are often used as markers of oxidative stress and damage in general. Chronic intake of chokeberry extract induced significant decrease in TBARS production in metabolic syndrome subjects. These subjects consumed extract, composed mainly of cyanidin glycosides, 3 times per day, in total amount of 300 mg, during the course of 8 weeks (Broncel et al., 2010). Levels of TBARS were also decreased in sportsmen performing ergometer test before and after 1-month-long daily intake of chokeberry juice (Pilaczynska-Szczesniak et al., 2005). Furthermore, the TBARS level was reduced in healthy female volunteers after 12 weeks of regular consumption of chokeberry juice (100 mL),

containing 386 mg of total polyphenols (expressed as gallic acid equivalents, GAE) (Kardum et al., 2014b). The same study detected improvement in prooxidant–antioxidant balance and an increase in activity of serum paraoxonase-1 (PON1) toward diazoxon. As stated earlier, polyphenols can exert indirect antioxidant effects through the regulation of antioxidant enzymes activities (Scalbert et al., 2005). The suggested mechanisms include modulation of cell signaling pathways and gene expression. Several human studies reported stimulation of red blood cells antioxidant enzymes after intake of chokeberry juice or extracts. Activities of erythrocytes antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) significantly increased after chokeberry juice consumption in metabolic syndrome subjects (Broncel et al., 2010). Intake of 240 mg of chokeberry anthocyanin-rich extract during 30 days similarly affected GPx and catalase (CAT) activity in men with hypercholesterolemia (Kowalczyk et al., 2005). In addition, dietary supplement—composed of chokeberry juice enriched with stable glucomannan fibers—caused an increase in GPx activity in postmenopausal women with abdominal obesity, defined as increased waist circumference, that is, ≥88 cm (Kardum et al., 2014c). During 4 weeks, these subjects consumed 100 mL of supplement containing 586.7 mg of total phenolics (expressed as GAE). Chokeberry juice positively affected activity of antioxidant enzymes not only in subjects at higher cardiometabolic risk, but in apparently healthy subjects as well. Three months long chokeberry juice intake significantly increased activities of both SOD and GPx in healthy female volunteers (Kardum et al., 2014a).

2.2.2 Antioxidant effects in animals

Similar results were obtained in animal studies. The aim of this study (Ćujić et al., 2015a,b) were to investigate potential antihypertensive effect of polyphenol rich chokeberry extract (CE) in sponateneously hypertensive rats (SHR). Animals were divided in two groups; the first, SHR-CE (10 animals) were gavaged with freeze-dried CE (50 mg/kg daily, dissolved in 1 mL water), and the second, SHR-CE (9 animals) with 1 mL tap water, 1 month long. All hemodynamic parameters of anesthetized adult male SHR (35 mg/kg b.m. sodium pentobarbital, i.p.) were recorded on Cardiomax III. After pentobarbital anesthesia and hemodynamics measurements, rats were sacrificed and blood samples were collected for determination of total plasma cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), glucose, and plasma thiobarbituric acide reactive substances-pTBARS. Erythrocytes were prepared for the determination of eTBARS, SOD, CAT, and glutathione peroxidase activity.

Four weeks of CE treatment (prepared from dried chokeberry fruits using 50% ethanol maceration, ethanol was evaporated under vacuum and freeze dried) induced a significant decrease of sistolic blood preasure (SHR-C: 205.1 ± 20.5 vs. SHR-CE: 184.5 ± 10.09 mm Hg, $P < 0.05$) and pulse pressure ($P < 0.05$) compared to control group. Diastolic and mean arterial preasure, sistemic vascular resistance, and resistance through the renal and carotid artery

were unaffected with CE. Interestingly, the diuresis had been significantly elevated after CE (SHR-C: 9.25 ± 0.79 vs. SHR-CE: 12.89 ± 1.04 mL/24 h, $P < 0.01$). Chronic CE consumption also significantly reduced pTBARS (8.90 ± 1.28 nmol/mL vs. 4.11 ± 0.31 nmol/mL, $P < 0.001$), eTBARS (284.12 ± 13.41 nmol/g Hg vs. 170.13 ± 15.54 nmol/g Hg, $P < 0.001$), and SOD activity ($P < 0.001$) of SHR, without effects on CAT and GPx. Holesterol, HDL, TG, and glucose levels remained unchanged in normolipidemic SHR after chronic CE treatment.

Diuretic effect of CE in SHR, probably provided blood pressure–lowering effect without sistemic vasodilatation, and also polyphenol rich CE, as a natural antioxidant, could inhibit lipid peroxidation in hypertensive rats therefore protect them from oxidative stress.

2.3 Potential Health Effects of Chokeberry

Polyphenols found in chokeberry fruits, mainly anthocyanins and procyanidins, represent one of the most potent natural antioxidants; they exert a positive impact on overall health, but also on diseases and states associated with marked level of oxidative stress. These compounds beneficially affect cardiovascular health, and cancer cell proliferation; fight against viruses and bacteria, improve memory and digestion (Chrubasik et al., 2010). Based on literature data, consumption of chokeberry fruits, juice and extracts have no adverse or toxic effects (Valcheva-Kuzmanova and Belcheva, 2006).

2.3.1 Effects on cardiovascular health

CVDs are one of the most common chronic diseases, and great contributors to the mortality and morbidity worldwide. Oxidative stress is related to many risk predictors of CVD: hypertension, metabolic disorders, and obesity. As a result of their chronic consumption, chokeberry products can affect numerous risk factors for CVD development.

Hypertension is the major contributor to the cardiovascular and related disease development. This is confirmed in epidemiological studies conducted in both developed and developing countries (Catalán-Ramos et al., 2014; Mancia et al., 2007). Chokeberry juice induced significant decrease in the diastolic blood pressure (DBP) in men with mild hypercholesterolemia (Skoczynska et al., 2007), after 6 weeks of regular intake. Study subjects were drinking 250 mL of juice each day, which equaled to approximately 1000 mg of total phenolics. Another study evaluated effects of 4-weeks long chokeberry juice consumption (200 mL/day containing around 780 mg of total polyphenols) on ambulatory monitored blood pressure (Kardum et al., 2015). At the end of the intervention period, subjects with no pharmacologically treated high normal BP or grade I hypertension (Mancia et al., 2007), had significantly lower levels of both average 24-h systolic and diastolic blood pressure (SBP, DBP). Significant reductions in blood pressure were also recorded for the average daytime values. Additionally, 24-h pulse pressure and variability of daytime

pressure—expressed as standard deviation—were also significantly reduced. Besides the juice, chokeberry extract also affected blood pressure levels in human intervention trials. Both SBP and DBP significantly decreased in patients having myocardial infarction after 6-week-long regular intake of 255 mg of chokeberry flavonoid-rich extract (Naruszewicz et al., 2007). In addition, 2-month-long supplementation with chokeberry extract (300 mg/day) significantly decreased level of SBP in no pharmacologically treated subjects with metabolic syndrome (Sikora et al., 2014a). Another study in metabolic syndrome subjects reported significant effect on BP level after consumption of chokeberry extract for 2 months. The authors suggested a decrease in the level of serum endothelin-1 as a potential mechanism of anthocyanins blood pressure lowering effects (Broncel et al., 2010).

The positive impact of chokeberry juice and extract consumption on BP is most probably related to its substantial content of phenolic compounds. Polyphenols can affect hypertension and general cardiovascular health due to their ability to reduce vascular oxidative stress. Studies of rats with induced hypertension showed lower values of blood pressure in a group treated with chokeberries ethanol extract comparing with the control group of nontreated animals. The authors explained this drop in blood pressure with improvement in total antioxidant capacity (TAC), and decrease in lipid peroxidation—effects that they observed in the same study (Ciocoiu et al., 2013). The cardio-protective effects of polyphenols could also result from their impact on endothelial function. This mostly referred to an increase in endothelium synthesis of nitric oxide (NO) and an improvement in flow-mediated dilatation (FMD). Accordingly, a 6-week-long consumption of chokeberry juice (250 mL/day, containing 1065 mg of total polyphenols) improved both mentioned parameters, along with the brachial artery diameter (BAD) in subjects with mild hypercholesterolemia (Poreba et al., 2009). Phenolic compounds showed the potential to increase NO levels in vitro, accompanied with an enhanced activity of endothelial NO synthase (eNOS) (Appeldoorn et al., 2009). Among other tested compounds, procyanidins—compounds highly abundant in chokeberry fruits—increased NO level, although not significantly. Another in vitro testing confirmed polyphenols potential to increase FMD and thus reduced the blood pressure. Still, this was mainly referred to the compounds present in dark chocolate (Medina-Remón et al., 2013). Furthermore, inhibition of angiotensin-converting enzyme (ACE), responsible for the production of strong vasoconstrictor angiotensin II, is also a possible mechanism of phenolics blood pressure lowering effects. ACE inhibition is one of the main therapeutic targets in controlling elevated blood pressure. Some flavonoids, including cyanidin-glycosides are present in chokeberry-express promising ACE inhibiting activity (Balasuriya and Rupasinghe, 2012). However, data on this particular mechanism of action are not uniform. Hellström et al. (2010) reported low ACE inhibiting activity for chokeberry and its juice, extracts of single phenolic compounds, and combined phenolic extract. Despite this, the same authors revealed short-term blood pressure lowering effects of both chokeberry juice and polyphenol extract in in vivo study with spontaneously hypertensive rats. The authors

concluded that blood pressure lowering effects most likely originate from the enhanced endothelial nitric oxide production by an ACE-independent mechanism (Hellström et al., 2010). Besides polyphenols, other constituents of chokeberry juice could contribute to blood pressure lowering effects. Analyses of macronutrient and micronutrient composition of chokeberry juice suggest high levels of potassium in both fresh and pasteurized juice (Chrubasik et al., 2010). Metaanalysis of randomized clinical trials confirmed that intake of potassium-based supplements can decrease the levels of SBP and DBP in hypertensive subjects, but in people with blood pressure values in optimal reference range (Whelton et al., 1997).

Among polyphenols health effects, their enrollment in the regulation of lipid status has an important role. It is proposed that polyphenols, as antioxidants, could protect lipid from oxidation in both foods and in the body after their ingestion. This is especially important in case of foods rich in fats, mostly PUFAs, because these are most prone to the lipid peroxidation. The lipid peroxidation products can be absorbed and thus cause the damages to the body. Polyphenols located in the gastrointestinal tract (GIT) could ameliorate the level of lipid oxidation and reduce the negative effects of lipid peroxides (Gorelik et al., 2013; Kanner et al., 2012). In addition, some polyphenolic compounds affect the liver synthesis of lipids and/or their metabolism in the intestines. Thus, by various mechanisms, polphenols from food sources, including chokeberry, can beneficially influence our lipid status. Six-week-long supplements with chokeberry juice induced significant reduction in triglycerides, total and LDL cholesterol level in men with mild hypercholesterolemia (Poreba et al., 2009). Another study in hypercholesterolemic subjects reported an increase in HDL cholesterol level after chokeberry juice intake (Skoczynska et al., 2007). Furthermore, 4-week-long juice consumption reduced triglycerides level in subjects with high normal BP or grade I hypertension (Kardum et al., 2015). A positive impact on disturbed lipid status was also reported for the chokeberry extract intake. Thus, an intake of 300 mg of chokeberry extract per day during the course of 2 months significantly decreased the level of triglycerides, total and LDL cholesterol in metabolic syndrome subjects (Broncel et al., 2010). Moreover, another study with same dosage of extract, involving patients with hypercholesterolemia, observed significant decrease in cholesterol concentration in erythrocyte membranes (Duchnowicz et al., 2012). Similar to chokeberry, other polyphenol-rich sources and dietary antioxidants showed effects on lipid status as well. Some authors suggest that phenolic plant extracts can reduce triglycerides level, through their stimulatory effects on endothelium-bound lipoprotein lipase, which hydrolyzes triglycerides into fatty acids (Devi and Sharma, 2004). In addition, flavonoids can enhance activity of enzyme lecithin-cholesterol acyl transferase (LCAT), responsible for cholesterol removal from the blood stream and thus causes the reduction in total and LDL cholesterol level (Katare et al., 2014). Some phenolic compounds, such as resveratrol contribute to the reduced triglycerides synthesis in the liver, by suppressing the expression of genes encoding relevant enzymes (Gnoni and

Paglialonga, 2009). Finally, anthocyanins, as active compounds of chokeberry, express the potential to decrease postprandial blood lipids and glucose in rats, by the inhibition of pancreatic lipase activity (Takahashi et al., 2014). Among 31 tested fruit extracts, chokeberry was found to be the strongest inhibitor of pancreatic lipase activity. Since pancreatic lipase is the most important enzyme in absorpiton of dietary triglycerides, these findings suggest consumption of chokeberry fruits as a dietary option for reduced fat absorption (Sosnowska et al., 2015). Some studies reported that polyphenols could affect absorption of fatty acids and fats by changing the emulsification processes. Prior to the absorption, digestive products of dietary fats must be emulsified by bile acids and phospholipids in the intestinal lumen. By affecting emulsification of fats, polyphenols can decrease the activity of lipolytic enzymes and thus lower the fat absorption. It has been suggested that polyphenols from green and black tea can increase the size of lipid droplets, formed in the emulsification process, and decrease the specific area of contact with enzymes. Increase in lipid droplets can result in smaller absorption of dietary fats (Shishikura et al., 2006). Recent studies have revealed that chokeberry has a potential to attenuate the expression of genes involved in de novo lipogenesis in hepatocytes of mice having nealcoholic fatty liver disease. Actually, the administration of chokeberry powder regulated the expression levels of sterol regulatory element-binding protein (SREBP), acetyl-CoA carboxylase, and fatty acid synthase, and decreased the size of lipid droplets in liver (Park et al., 2016).

The positive impact on lipid status is important in the context of cardiovascular health also. Although hypertension is the most common CVD risk predictor, only a small number of subjects have high blood pressure alone (Thomas et al., 2001). Disruption in lipid status—dyslipidemia—is, among others, a risk factor included in the calculation of total cardiovascular risk (D'Agostino et al., 2001). Dyslipidemia is often defined as an increased level of triglycerides, total or LDL cholesterol, or decreased HDL cholesterol level. Considering this, the positive impact of chokeberry juice on membrane fatty acid profiles observed in both healthy and overweight subjects suggest its cardioprotective effects. It is well established that high blood pressure associates with the altered fatty acid profile, as well as that decreased content of $n-3$ fatty acids can promote inflammation. This could further lead to the alteration in membrane fluidity and disruption in membrane ionic channels, proteins, and receptors (Mozaffarian and Wu, 2011; Dart, 2010). On the contrary, the increase in the level of $n-3$ fatty acids could lead to antiinflammatory and antiarrhythmic effects. In addition, $n-3$ fatty acids serve as ligands of nuclear receptor family and affect lipid metabolism through the regulation of gene expression (Mozaffarian and Wu, 2011). The fatty acid profile of cell membranes is important for the estimation of overall cardiovascular risk. Thus, it has been shown that there is a negative association between the content of eicosapentaenoic and docosahexaenoic fatty acids and risk of acute coronary syndrome, angina pectoris and myocardial infarction (Block et al., 2008). In addition, some authors suggest "omega-3-index," defined as a sum of these two fatty acids, as a risk factor

of coronary heart disease (Harris and Von Schacky, 2004). A decrease in "omega-3-index" was reported in both healthy and obese women after consuming chokeberry juice for 12 and 4 weeks, respectively. Since being an interface between cells and extracellular compartment, membranes represent the first line of reactive species and free radicals attack. Some studies pointed out that polyphenols from polyphenol-rich extracts could incorporate in the lipid phase of erythrocytes membranes, more accurately in its outer parts (Bonarska-Kujawa et al., 2011). These interactions with lipids in membranes can be associated with protective effects against lipid peroxidation, observed for several plant extracts including chokeberry. In addition, treatment with polyphenol-rich extracts induced changes in the shape of red blood cells, which was detected by electronic microscope. Thus, the formation of echinocytes was observed, while chokeberry extract induced generations of the most diverse forms of echinocytes. Based on this, the authors concluded that polyphenols mainly concentrated on the outer lipid layer, without reaching the inner parts of membranes.

Excep for lipids, disturbed glucose metabolism is known as traditional risk factor of CVD development. There are some findings that indicate beneficial impact of chokeberries on blood glucose level. Bräunlich et al. (2013) suggested that different phenolic fractions from chokeberry can serve as potent α-glucosidase inhibitors; thus they may reduce the blood glucose level and prevent diabetes onset. It should be emphasized that compounds could act as α-glucosidase inhibitors in vivo even if they are poorly absorbed from the GIT, since this enzyme is a membrane-bound and it is placed in the intestine epithelium (Matsui et al., 1996). Long-term consumption of chokeberry juice also affected blood glucose level in subjects with risk factors for CVD. In men with mild hypercholesterolemia, juice intake induced significant decreases in fasting glucose levels, while in patients with noninsulin-dependent diabetes mellitus, this effect was accompanied with a decrease in levels of glycated hemoglobin (Simeonov et al., 2002; Skoczynska et al., 2007). These effects on blood glucose indicate that chokeberry juice could have an impact not only on initiation and progression of CVDs, but also of metabolic disorders, such as insulin resistance, metabolic syndrome, and diabetes. Metabolic syndrome is a cluster of several risk factors of CVD and diabetes development, including high blood pressure, disturbed lipid and glucose metabolism, and abdominal obesity. Often, it is called insulin resistance because hyperinsulinemia leads to its development. Abdominal obesity is also an important part of metabolic syndrome, and it is often associated with oxidative stress. The direct association between obesity and oxidative stress is based on macrophage infiltration in adipose tissue and production of reactive oxygen species. In addition, the expansion of fat mass leads to hypoxia, which triggers adipocytes apoptosis and further macrophage infiltration (Berkemeyer, 2010). Also, accumulated fat is accompanied with disturbed oxidative status: an increase in expression of NAD(P)H oxidase and a decrease in activity of antioxidant enzymes (Martínez, 2006). Abdominal or central obesity correlates with a lower TAC independently of other CVD risk factors, such as levels of blood glucose and lipids, blood pressure, and smoking (Furukawa et al., 2004). In addition,

it has been reported that the fatty acid profile alters the changes in body mass index (BMI): the obese women were found to have less amounts of $n-3$ PUFAs in their red blood cells in comparison with lean women. This was accompanied with higher ratio of $n-6$ and $n-3$ in phospholipids of cell membranes in obese subjects (Cazzola et al., 2004). Considering these facts, earlier stated positive influences of chokeberry juice consumption on fatty acid profile in women with abdominal obesity (Kardum et al., 2014c) indicates its potential impact on ameliorating or decreasing the incidence of obese-related diseases. However, it is important to mention that these subjects were receiving chokeberry juice that was enriched with glucomannan dietary fiber. The daily dose of juice (100 mL) contained 2 g of these fibers. In addition, significant reductions in waist circumference and BMI were observed after taking this supplement for 4 weeks. Relying on literature data, the observed effects on weight control could be related to both ingredients of supplement: chokeberry juice and glucomannan fibers. Still, European Food Safety Authority (2010) stated in its report that glucomannan could have an effect on weight loss but only in the context of a diet with restricted energy and with daily intake of 3 g. On the other side, the data on the possible effects of chokeberry juice on weight loss in human studies is limited. However, some authors suggest that polyphenol-rich foods could influence insulin sensitivity, adipogenesis, and inflammation and thus participate in weight management as well. Chokeberry extract showed suppressive effect on weight gain in rats fed with fructose-rich food (Qin and Anderson, 2012). In addition, anthocyanins-rich phytochemicals from chokeberry fruits showed inhibitory effects on visceral fat mass accumulation in rats fed with a high-fat diet. The same study observed significant reduction in blood glucose level in rats treated for 4 weeks with chokeberry in comparison with the control group (Takahashi et al., 2015). As already stated, the antioxidant effects of the mentioned supplement are most probably caused by the chokeberry polyphenols and hardly can be attributed to the presence of glucomannan. As far as the authors know, data on the effects of glucomannan intake on fatty acid profile or oxidative status in humans are lacking.

Platelet hyperactivation can also affect CVD development. This is based on platelets uncontrolled interactions with other platelets, monocytes, and neutrophils that can result in thrombosis and atherosclerosis. Altered platelet function is manifested as an increase in the number of circulating platelets, which have more adhesion molecules on their cell surface. This alteration in platelet function has been observed in hypertensive subjects, and also in people with: hypercholesterolemia, diabetes and metabolic syndrome (Angiolillo et al., 2007; Bröijersén et al., 1998; Lip, 2003; Santilli et al., 2012). In addition, consumption of meals rich in dietary fats, or in vitro treatment with high glucose levels, were found to have an impact on enhanced platelet activation (Bröijersén et al., 1998; Sudic et al., 2006). The European Food Safety Authority (2011) has confirmed the importance of optimal platelet function and accepted polyphenols effects on platelets as beneficial for cardiovascular health. It has been shown that different plant extracts—rich in polyphenols or other bioactives—can beneficially affect platelet function in terms of their aggregation with themselves or with the other blood cells

(Konić-Ristić et al., 2013a). In vitro studies demonstrated a positive impact of chokeberry juice and extracts on platelets from both: healthy and metabolic syndrome subjects. This refers to the decrease in platelet adhesion to collagen, fibrinogenor endothelial cells, and reduced platelet aggregation induced by thrombin (Konić-Ristić et al., 2013b; Malinowska et al., 2013; Sikora et al., 2014b). Another study reported that preincubation of human umbilical vein endothelial cells with chokeberry extract could decease the platelet aggregation and adhesion to these cells (Luzak et al., 2010). Furthermore, a preliminary study on healthy volunteers suggested that acute intake of chokeberry juice could induce significant decrease in the expression of platelet activation and aggregation markers, after the action of platelet agonists (Konić-Ristić, 2013).

2.3.2 Other health-promoting effects of chokeberry

Besides the positive impact on cardiovascular health, many in vitro and human studies investigated potential beneficial effects of chokeberries on inflammation, neurological health, urinary tract and cancer incidence. It is assumed that these effects are also associated with the high content of polyphenols and their activity.

Inflammation, especially when it becomes chronic, is associated with many conditions and diseases, such as adiposity, nonalcoholic fatty liver disease, and hypertension. A study that included members of Polish rowing team investigated whether 8 weeks of chokeberry juice consumption can modulate postexcercise antioxidant status and levels of proinflammatory factors and indicators of iron metabolism. As a result, supplemented subjects had lower plasma levels of TNF-alpha and higher TAC comparing to the controls that were consuming placebo drinks. The authors suggested that chokeberry juice consumption beneficially influences athletes undergoing maximal exercise and should be considered as part of their dietary regimen (Skarpańska-Stejnborn et al., 2014). Potential antiinflammatory activity of chokeberry has been investigated in in vitro studies as well. Appel et al. showed that concentrates made of chokeberry juice can inhibit release of proinflammatory factors, such as TNF-alpha, IL-6, and IL-8 by human monocytes and to suppress the activation of NFκB pathways by macrophage cells. Furthermore, this concentrate combined with selenium showed the same effects, along with the inhibition of prostaglandin PGE2 synthesis. Thus, it could be concluded chokeberry juice exerts some antiinflammatory effects—associated with NFκB pathway—that can be enhanced in the presence of micronutrient selenium (Collado et al., 2015). Another in vitro study examined commercial chokeberry extract and its effects on TNF-alpha-induced adhesion of mononuclear leukocytes to human aortic endothelial cells. The authors reported significant inhibition in expression of cell adhesion molecules (CAM) expression, such as VCAM-1 and ICAM-1. This was accompanied with the decrease in the intracellular production of reactive oxygen species and attenuation of NFκB-transcription factor activity that could lead to the lower expression of many proinflammatory mediators. These findings partly disclosed possible molecular mechanisms underlying the beneficial effects of chokeberry fruits on endothelial cells (Zapolska-Downar et al., 2012).

Numerous epidemiological studies investigated the relationship between cancer incidence and the intake of fruits and vegetables, while many in vitro experiments indicate that polyphenols can affect tumor development and carcinogenesis. Berry fruits and their products have often been mentioned as chemopreventive agents. Chokeberry and its anthocyanins are suggested to have antimutagenic activity based on their radical scavenging properties, but also on the inhibition of enzymes involved in promutagen activation (Kokotkiewicz et al., 2010). In addition, some studies suggest that polyphenols may decrease the risk of cancer based on the amelioration of DNA oxidative damage. Some authors suggested that chokeberry fruits and their extracts could exert antiproliferative or protective effects against colon cancer (Kulling and Rawel, 2008).

Polyphenols have also been studied for their potential to improve neurological health, while berries are believed to have an impact on memory and learning. These beneficial effects are based on different mechanisms; including influencing intracell neurological signaling and improving cerebrovascular blood flow (Del Rio et al., 2010). Besides the potential impact on cognitive functions, some authors have suggested that chokeberries could promote healthy aging. Beneficial effects of chokeberry juice on age-related processes are revealed in a recent animal study. Supplementation with chokeberry juice retarded changes in rats aortic wall and lowered the level of proatherogenic LDL cholesterol; thus, the authors suggested chokeberry juice as a promising prophylactic tool for healthy aging. They based their findings on antioxidant activity of chokeberry juice, because it is well known that free radicals and reactive species can contribute to the age-related damage of the cells and tissues (Daskalova et al., 2015). A recent study investigating the impact of 1-month-long chokeberry juice intake on behavioral characteristics of male rats, revealed some beneficial effects. The findings of this study demonstrate the reduction of anxiety-like and depression-like behaviors, as a result of juice consumption. The authors suggested MAO-A/MAO-B inhibition as a possible mechanism of the observed behavioral actions (Tomić et al., 2016).

Urinary infections are considered to be one of the most persistent infections, requiring severe treatment with antibiotics, especially in the elderly. A long-term placebo-controlled trial investigated effects of chokeberry juice consumption on the incidence of urinary tract infections in Norwegian nursing homes. The subjects were consuming both polyphenol-rich chokeberry juice (100 mL containing 715 mg GAE) and placebo drink for 3 months in a crossover trial. The results revealed a reduction in frequency of infections and consequent use of antibiotics; still, this effect was not an immediate significant change and was found in the 3-month period that followed juice consumption. The authors suggested delayed bacteriostatic effects of phenolic compounds and their metabolites as a potential mechanism of urinary infection retracting (Handeland et al., 2014).

The positive effects of regular chokeberry juice consumption were also investigated in healthy women with grade 2 cellulite, according to the Nurnberger–Muller scale. The study reported

a significant impact on all investigated parameters of epidermis, dermis, and subcutaneous tissue, such as edema, the length of subcutaneous tissue fascicles, and subcutaneous tissue thickness (Šavikin et al., 2014). Significant effects recorded by ultrasonography were observed after 45 and 90 days of regular chokeberry juice consumption, as compared to the baseline values. These findings can be explained with the potential role of polyphenols in enhancing blood flow and protecting endothelial cells. Still, a study that involved women with cold constitution reported a lack of significant impact on peripheral blood flow, after a 4-week-long supplementation with anthocynin-rich extract of chokeberry. Despite the poor effect on blood flow, the extract induced a significant increase in peripheral body temperature and beneficially affected subjective cold feelings in hands, feet, and hips. The study also reported an increase in plasma noradrenalin level, which could lead to the contraction of peripheral blood vessels. Because the blood flow remained the same, the authors concluded that the extract most likely expressed some compensatory mechanism that counteracted vasoconstriction (Sonoda et al., 2013).

3 Bioavailability and Metabolism of Polyphenols Present in Chokeberry

Polyphenols in general are recognized as bioactive compounds with low bioavailability and intensive metabolism; thus, their health effects should be attributed to their metabolites or compounds present in circulation after the consumption of polyphenol-rich foods. Additionally, the identification of metabolites and their levels in circulation is required for the proper understanding of the polyphenols health effects. Because recent absorption, distribution, metabolism, and elimination of different phenolic subclasses have been increasingly investigated. Unlike isoflavones, flavanones, and gallic acid, identified as the best absorbed polyphenols, compounds present in chokeberries—procyanidins and anthocyanins—are considered to be the ones with the lowest absorption (Manach et al., 2005). In general, the absorption of most, but not all, phenolic compounds mainly takes place in the small intestine. Since phenolic compounds are mostly present in form of glycosides in plants, absorption usually starts with cleavage of sugar moiety and further diffusion of aglycones to enterocytes. Before they enter the circulation, polyphenols can undergo the phase II of metabolism that includes formation of glucuronide-, sulfate-, and/or methyl derivates. Phenolic compounds resistant to the activities of hydrolytic enzymes pass unchanged to the colon, where they can be metabolized by the action of bacterial microflora. Even compounds metabolized in the upper parts of GIT can be partly degraded by colonic bacteria (Del Rio et al., 2010). Despite previous reports on low bioavailability of anthocyanins, recent studies have established that they are absorbed and metabolized to a greater extent. Unlike some earlier findings that anthocyanins can be found in blood only as glycosides, Kay et al. (2005) revealed the presence of conjugated metabolites in serum, after administration of cyanidin-3-glycosides in males. Investigation of isotopically ^{13}C labeled cynidin-3-glucoside intake and its recovery in urine, serum, and feces, revealed

substantial number of breakdown products and their conjugates (Czank et al., 2013; De Ferrars et al., 2014). However, the majority of compounds were in the form of lower mass metabolites of the parent anthocyanin. The cyanidin-3-glycoside, as parent compound, represented only 2% of the metabolites detected in the circulation, and was present for a short time. The other metabolites included conjugates of cyanidin-3-glucoside and cyanidin, degradation products, such as PCA and phloroglucinaldehyde, conjugates of PCA, phenylacetic, and phenilpropionic acids. Based on their findings, the authors concluded that a primary route of cyanidin anthocyanins metabolism and elimination includes degradation, followed with phase II conjugation of breakdown products. In addition, the recovery of the ingested initial dose was measured in urine samples and was found to be around 12%. Additionally, recent research has detected cyanidin glycosides in their unchanged form and in form of metabolites, after consumption of chokeberry juice that contained anthocyanins in their optimal daily doses. The presence of anthocyanins, their glucuronides and methyl derivatives was confirmed in both blood and urine samples in the first 24 h of ingesting the juice (Wiczkowski et al., 2010). Animal studies have also shown that anthocyanins can be rapidly absorbed in gut and small intestine, but that also have good tissue distribution (Felgines et al., 2009; Talavéra et al., 2004). The anthocyanins were detected in different organs, including heart and adipose tissue, which confirms their potential beneficial effects on cardiovascular, and metabolic diseases, as well as on health promotion.

In a study reported by Stanisavljević et al. (2015) chokeberry juice was subjected to in vitro gastric digestion in the presence of food matrix in order to determine the changes in polyphenol content and antioxidant and antiproliferative activity. Food addition decreased the total phenolic and anthocyanin content, DPPH scavenging activity, as well as total reducing power by 36, 90, 45, and 44%, respectively. The contents of individual anthocyanins were also affected by digestion and the addition of complex food matrix, and their content decreased by approximately 90%. Only ferrous chelating capacity exhibited higher values in digested fractions than in the initial juice, which could be explained by the release of peptides from the food matrix, which possess strong metal chelating features. Chokeberry juice digested in the presence of food matrix possessed antiproliferative capacity and markedly reduced proliferative rate of caco-2 cells by approximately 25%. These results showed that although a large proportion of chokeberry phenolics undergo transformation during digestion they are still potent as antioxidant and antiproliferative agents. Binding of phenolic compounds to the food matrix combined with prolonged exposure to mild acid environment during intestinal digestion could allow the transport intact phenolic compounds to the colon.

4 Chokeberry Products-Extracts

Due to low bioavailability of the health-promoting chokeberry compounds, it is necessary to reach a maximum yield and the input of polyphenolic compounds in order to achieve a positive impact on health. The main source of chokeberry fruit on the market originates

from plantations, and besides fresh and dried fruits various processed, products like juices, jams, jellies, as well as extracts and dietary supplements are available (Gonzales-Molina et al., 2008; Kokotkiewicz et al., 2010; Kulling and Rawel, 2008). Nowadays, there is an increasing interest for chokeberry extracts, which have very high amounts of biologically active phenolics. For the pharmaceutical, cosmetic, and food industry, the quality of the extracts is critical and the amount of biologically active phenolics in extracts is a challenge. Various extraction techniques could be used, from traditional (maceration, maceration assisted with stirring, and Soxhlet extraction) to the modern ones (ultrasound-assisted extraction, microwave-assisted extraction, sub- and supercritical fluid extraction, and accelerated solvent extraction) (Khoddami et al., 2013). Each technique has its own advantages and disadvantages, but the main goal of the chosen method is the achievement of complete compounds extraction and avoidance of their chemical modification. Extraction efficiency could be influenced by several factors, such as type and concentration of solvent, solid-solvent ratio, time, temperature, pH. The influence of different factors on extraction of chokeberry polyphenols using experimental design strategy was examined (Ćujić et al., 2016a). The industry demands production costs and it was important to investigate the optimal conditions for the efficient extraction of chokeberry polyphenols. The effects of four factors (time, solid-solvent ratio, solvent type, and particle size) on the extraction efficiency of polyphenols from chokeberry dried fruit were analyzed using factorial design in order to optimize experimental procedure using maceration as traditional techniques for the extraction compared with ultrasounic-assisted extraction as a new method (Ćujić et al., 2016a). Quantitative analysis of individual phenolic compounds by HPLC was performed. Various solvents (distilled water, 50, 70, and 96% mixture ethanol-water); 5 particle sizes (6, 3, 2, 1, 0.75 mm), according to Yugoslavian Pharmacopoeia (Yugoslavian Pharmacopeia, 2000); 3 different solid–solvent ratios (1:10, 1:20, and 1:30); and extraction times (15, 30, 60, 90 min) have been investigated as independent variables. To determine the optimal levels of each factor for the extraction of total phenolics (TP) and total anthocyanins (TA), a preliminary screening was performed. The purpose of this screening was to find the two most promising levels of each factor, which exhibit significant influence on the yield of TP and TA, which will be further included in full factorial design. The first 2^4 factorial designs showed that solvent, solid–solvent ratio, and particle size were significant factors, whereas time was not relevant in affecting the total phenolic and total anthocyanins contents. This factor was excluded from the optimization step and the optimum extraction time was fixed at 60 min. The second 2^3 full factorial designs determined the optimal values for the significant factors (solid–solvent ratio, solvent type, and particle size) on the yields of TP and TA, and optimized the evaluation of the interactions between these factors. The solvent concentration was the most relevant factor concerning the content of total phenolics. For the TA content, the results indicated that particle size was the most significant factor for their extraction, and increase of particle size would lead to decrease of TA content. Solid–solvent ratio was also significant factor for obtaining a high yield of TA.

The optimization showed that the best experimental conditions for obtaining high yields of total phenolics (27.82 mg GAE/g DW) and total anthocyanins (0.273%) were 50% of ethanol, solid–solvent ratio of 1:20, and particle size of 0.75 mm. The lowest content of TP (11.78 mg GAE/g DW) was recorded with 70% ethanol, 1:20 solid–solvent ratio and 0.75 mm particle size, whereas lowest yield of TA was obtained at 50% ethanol, 1:30 solid–solvent ratio and particle size of 1 mm (0.203%). Higher solid–solvent ratio generates a decrease in the consumption of plant material and decrease in the cost of extraction. Galvan D'Alessandro et al. (2012) also reported in their previous examination reported that use of medium concentration for ethanol/water mixture (50%) resulted in higher TP yield compared with water or other ratios of the ethanol/water mixture. Binary solvent systems containing hydroorganic solvents are superior to a monocomponent solvent system (pure water or ethanol) in the extraction of phenolic compounds (Cacace and Mazza, 2003; Ćujić et al., 2016a; Galvan D'Alessandro et al., 2012; Zhang et al., 2007). The mixture of water and ethanol as a solvent agent shows synergistic effect, which facilitates phenolic extraction from chokeberry fruit. The extraction efficiency regarding TP content showed improvement as the particle size decreased. The smallest particle size (0.75 mm) had the highest yield of TP and TA. These results are in accordance with previous reports where the extraction from grounded chokeberries was more efficient than the extraction from berries cut in half (Galvan D'Alessandro et al., 2012). Smaller particle sizes had a higher contact surface, which allows the increase of mass transfer.

The quantification of individual anthocyanins and flavonoids in chokeberry extracts afforded under different extraction conditions was carried out using an HPLC method and the main anthocyanin compound was cyanidin-galactoside, followed by cyanidin-arabinoside and cyanidin-glucoside. Among flavonoids, the dominant were quercetin derivatives, such as quercetin-glucoside (isoquercitrin), quercetin-galactoside (hyperoside), and quercetin-rutinoside (rutin). HPLC analysis showed that the same extraction conditions (50% ethanol, 1:20 solid–solvent ratio, and 0.75 mm particle size) provided the highest yield of individual anthocyanins and flavonoids.

In order to evaluate the effect of ultrasonic on the yields of TP and TA during extraction under selected optimal conditions determined by factorial design, extraction was performed in an ultrasonic bath for 30 and 60 min. Extracts obtained by maceration contained higher yield of TP (27.6 mg GAE/g) than both extracts obtained by ultrasonic-assisted extraction for 30 and 60 min (25.4 and 25.9 mg GAE/g, respectively). TA content was significantly higher in maceration extract (0.27%) than in ultrasonic extracts obtained after 30 and 60 min (0.23 and 0.24%, respectively). Maceration was a more effective, less complex in operation and inexpensive method for extraction of phenolic compounds from chokeberry fruit, although the extraction time was not shortened. Maceration should be the preferred extraction method based on the polyphenols extraction yields, production cost as a reduced consumption of energy.

5 New Technologies in Chokeberry Products

Chokeberry extracts and juices are useful in prevention and treatment of CVDs, diabetes, and many other chronic diseases, market demand for these products is growing steadily in the last decade (Galvan D'Alessandro et al., 2012) but extracts do not provide storage stability of polyphenols, especially anthocyanins, and the amount of bioactive compounds could be affected by the presence of oxygen, light, humidity, or other inappropriate storage conditions (Bakowska-Barczak and Kolodziejczyk, 2011). One of the methods to protect the beneficial properties of chokeberry extracts and to overcome the problem of polyphenols instability could be the microencapsulation technology. The main goal of microencapsulation are enhancement of stability of bioactive compounds during storage, protection of extracted compounds from harmful environmental conditions, product shelf-life extension, controlled delivery, covering unpleasant taste of bioactives (bitter taste of phenolic compounds), and circumvention of the damaging effects of GIT (Fang and Bhandari, 2010), which is very important in the food industry. Microencapsulation also represents a promising tool for bioavailability increment of bioactives (Bakowska-Barczak and Kolodziejczyk, 2011; Belščak-Cvitanović et al., 2011), which could be used as food additives and pharmaceuticals. Microencapsulation of chokeberry extract obtained by maceration within calcium alginate matrix was performed using electrostatic extrusion technique, which is a simple, precise, efficient, and economical method for obtaining particles with encapsulated bioactives. It has great potential for controlled preparation of microbeads with appropriate organoleptic and uniform size, which could be applied in the industry (Prüsse et al., 2008). In a very few studies, chokeberry polyphenols were encapsulated via inclusion complexation (Howard et al., 2013) or stabilized via different drying methods. Horswald et al. (2013) explored different drying methods, that is, spray, freeze, and vacuum drying, to preserve the chokeberry polyphenols. However, spray-drying methods demand high processing temperatures, around 100°C, when higher degradation of active compounds may occur, especially anthocynins (Leong and Oey, 2012; Stojanović et al., 2012).

During the optimization of the encapsulation process different types of alginate (low and medium viscosity), addition of inulin as filler, as well as different needle sizes (18, 20, and 22 gauges) were tested in order to obtain the best entrapment efficiency and release profiles Ćujić et al. (2016b). Utilization of the electrostatic extrusion technique for the microbeads production and encapsulation of chokeberry extract resulted in the formation of particles of high uniformity and spherical shape.

The size of the produced hydrogel particles ranged from 800.0 to 1340.0 μm. High viscous alginate-chokeberry solution, related to the high content of sugars in chokeberry extract (Kokotkiewicz et al., 2010; Kulling and Rawel, 2008) was the explanation for the relatively big particles obtained. The biggest needle diameter led to the formation of the biggest particles. The addition of inulin affected the microbeads size, because the addition of inulin

as filler in the alginate solution caused bead enlargement. Since the dry microbeads are a preferable form for the possible application as food additives, the effects of the drying process on the microbeads size was evaluated. After the freeze-drying process, particle size was reduced by 18%–24% when compared with initial hydrogel particle diameter, especially beads prepared with inulin. Formulation with medium alginate viscosity and addition of inulin showed the smallest size reduction, indicating that inulin has an impact on the drying process.

The difference in the amount of encapsulated polyphenols was also examined. The best encapsulation efficiency (0.24 mg GAE/g beads) was achieved with medium viscosity alginate and inulin formulation, using 20-needle size. Greater polyphenols amount was presented in particles obtained with inulin than without.

Differences in entrapment efficiency among hydrogel beads and freeze-dried beads were examined because dried microbeads are more important for the food industry than hydrogel forms.

Hydrogel microbeads contained 0.23–0.24 mg GAE/g, whereas freeze-dried contained 3.24–3.57 mg GAE/g of beads, depending on the water content, type of carrier (medium viscosity) with or without inulin inclusion, as well as the drying process. Freeze-dried particles had about 14–15 times higher amount of polyphenols compared with the hydrogel particles of the same type. These results showed that after drying, alginate or alginate/inulin beads became more concentrated with total phenolic entrapped.

The release of polyphenols from chokeberry encapsulated hydrogel microbeads, and the effects of certain factors (different carrier type, with or without inulin as filler and the influence of needle size), were also investigated. Previous studies have shown that kinetics of polyphenols release depends on the shape and size of the beads, type of polymer used, as well as the content of active compounds (Belščak-Cvitanović et al., 2011; Stojanović et al., 2012). Particles obtained with medium viscosity alginate carrier (1.5% w/v), using inulin as filler (5% w/v) and medium needle size (20 gauges) showed the best results in the release studies (0.21 mg GAE/g beads) in distilled water. The lowest polyphenol release was achieved with the smallest microbeads obtained with a needle diameter of 22 gauge (0.16 mg GAE/g beads). Bigger microbeads allowed much slower release, which is appropriate in the case when prolonged delivery is preferable. Release profile of encapsulated hydrogel microbeads was prolonged to 10 min and freeze-dried particles prolonged release to 40 min for both formulations.

The influence of different environmental pH conditions on the release of phenolics was examined comparing hydrogel and freeze-dried forms of microbeads. Comparing the results for phenolic release from the alginate and alginate/inulin particles in the water and in acidic environment, phenols liberation was better in water than in an acidic environment. This

could be important for the absorption and bioavailability in human organism indicating that microencapsulated chokeberry phenolics would be rather absorbed in the alkaline intestinal fluid than in gastric fluid, especially taking into account that Stanisavljević et al. (2015) demonstrated reduction of total phenolics, anthocyanins, and proanthocyanidins of chokeberry juice during simulated gastric digestion, although digested juice retained antioxidant and antiproliferative potential. pH sensitive characteristic makes alginate beads promising carriers for controlled release.

Fourier transform infrared analysis was used to analyze the functional groups of the polymers and chokeberry extract, as well as to examine the possible interactions between chokeberry extracts compounds and matrix.

FTIR spectra showed several relevant changes but did not show any significant modification in the spectrum of alginate or alginate/inulin systems with the encapsulated chokeberry extract in comparison with blank microbeads, which confirmed that the extract was successfully incorporated into the particles. It could be concluded that alginate hydrogel or alginate/inulin matrixes are a compatible and appropriate material for chokeberry polyphenols encapsulation.

SEM analysis has been given information about surface morphology and the shape of alginate and alginate/inulin microbeads (blank and with encapsulated extract).

The formulation medium viscosity alginate/inulin showed the most appropriate characteristics and uniform structure. The SEM micrographs showed that freeze-drying could be used as a promising method for preservation of hydrogel beads, where addition of inulin as filler improved the final properties of the microbeads. Such microbeads seem to be a promising food additive, which could be incorporated into dietary supplements, functional food, or pharmaceutical and cosmetic preparations.

6 Conclusions

Many of the previously mentioned studies showed and confirmed that chokeberries have health-promoting effects, especially in CVD, inflammation, neurological and urinary diseases, adiposity, in some types of cancer, cerebrovascular disease, edema, and also are recommended as chemopreventive and antiatherogenic agents. The main reason for these activities is the highest amount of dietary polyphenols among berries and other plant species. Polyphenols have documented beneficial health effects, especially antioxidant potential. Beside polyphenols, biological activity is highly connected to other main compounds presented in chokeberries, anthocyanins, proanthocyanidins, flavan-3-ol and flavonol glycosides, phenolic acids, which are responsible for antioxidant activity. Decrease of oxidative damage markers, antioxidant improvements, blood pressure level, triglycerides and glucose level decrease, platelet function, and antiobesity were described in many studies

where chokeberries were consumed. Nowadays, the chokeberry products mainly present on the market are fresh and dried fruits, juices, jams, jellies but there is also increased interest for extracts, especially from the food industry standpoint. The problem with chokeberry polyphenols are low stability, because none of aforementioned products provide long stability, resistance of the oxygen, light, and humidity presence. New microencapsulation techniques could be promising way to overcome problems with polyphenols instability, low bioavailability, and degradation.

Because chokeberry polyphenols are considered as the most important dietary antioxidants, which could exhibit strong protective effects against cellular oxidative damage, and act both directly and indirectly against oxidative stress, chokeberries are highly recommended as dietary and therapeutic food.

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CHAPTER 8

Beta Glucan as Therapeutic Food

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1 History

It is proverbial in the history of science, and for medicine in particular, that former knowledge and experiences are often forgotten for centuries, only to be rediscovered. This also applies to reunderstanding of the influence of nutrition on human health. In antiquity it was known that diet directly affected health. Hippocrates (5th century BC) had a completely modern attitude when he noted that "health consists of proper mixing of body juices of various kinds ... and medicine this is achieved by an appropriate diet..." (Thorwald, 1962). In this context, it should be recalled that "dietetic" was a doctrine about the right way of life (including hygiene and gymnastics) and "hé diaíta" meant that ancient nations did not thought only about food. In an essay "On the Old Medicine" (Peri archaiés iétrikés) it is noted that the development of medicine depended mainly on prescribing special diets. Aulus Cornelius Celsus (35 BC–50 AD) was of the same opinion. He recommended a salt-free diet in kidney diseases, prescribed liver for therapy of night blindness, and advised people to drink milk in poisoning. A physician of Marcus Aurelius emphatically pioneered the treatment of diseases by using the specific diets Galen of Pergamum (130–201 AC) (Ackerknecht, 1973).

For many centuries these recommendations were almost forgotten and even in the 17th and 18th centuries diets, and especially some of their composite components for the prevention of diseases and their treatment, were completely ignored. In 1754, however, J. Lind, an English ship's doctor considered the founder of "naval hygiene in England," in his "A Treatise of the Scurvy" noted that scurvy (a disease caused by vitamin C deficiency), killed more seamen than were killed in battle. On the basis of the 200-year-old knowledge of Dutch people about the benefits of citrus fruits to the health of the sailors on long voyages, J. Lind conducted one of the first large-scale clinical medical trials and in his next important paper "On the Most Effectual Means of Preserving the Health of Seamen" published 3 years later, proposed the eradication of scurvy from the British Navy by using fresh citrus fruit and juice prepared from them. In 1795, when his recommendation was adopted and implemented, scurvy definitively disappeared from the British fleet, as it is historically preserved, "magically." However, we have to remember that it took 70 years for the scientific community to accept that vitamin C cured scurvy.

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A similar situation was repeated almost 100 years later, this time with the eradication of beriberi (a disease caused by insufficient intake of vitamin B_1) in the Imperial Japanese Navy. T. Kanehira, the ship's doctor, noticed that the officers, who ate a varied diet, and not just rice, did not suffer from the disease.

2 New Discoveries

In 1810, J. F. Menkel described atrophy of the thymus, the central organ of immunity, in which the functional subpopulations of immunocompetent cells differentiate and maturate, as a result of inadequate nutrition and fasting (Beisel, 1992). About 35 years later, Simon (1845) presented a detailed study on the thymus, in which he considered thymus as a "barometer of malnutrition." In the first half of the 20th century, the importance of vitamins as essential nutrients, whose inadequate intake causes severe immunodeficiency, was gradually investigated and conclusively documented. Concurrently, the influence of exhaustion and malnutrition upon growth was studied (Jackson, 1925). Significant textbooks on nutritology issued in the late 1940s still contain only a brief note on the relationship of nutrition to infection, where it was stated that, with the possible exception of tuberculosis, there is no evidence that the interrelationships among infectious disease, nutrition, and immune status are confirmed (Jeliffe, 1950).

Nutritional immunology was intensively developed in the 1960s and 1970s on the rise of immunology as a separate medical discipline. This period is now traditionally known as the "Golden Age of Immunology." New discoveries in immunology were awarded by several Nobel prizes and also were accompanied by laypeople and specialists interested in the issue of the impact of individual components of nutrition on the immune status of the organism. The research focused mainly on determining the optimal composition of the nutrition that was used both in the treatment of various infectious diseases, so as to support the treatment of ongoing disease and in convalescence. Gradually, experts began to understand that every infectious disease is accompanied by a reduction of food intake, both of which culminates in the deterioration of the immune system.

In 1968, the World Health Organization published a monograph dealing with the relationship between nutrition and infection, first introducing the assertion that the relationship between malnutrition and infection is synergistic (Scrimshaw et al., 1968). In the early 1970s, it was definitively demonstrated that protein-calorie malnutrition is the cause of extreme involution of lymphoid tissues including the thymus. Therefore, it was known as nutritional thymectomy (Smythe et al., 1971). Its consequences are reflected in a reduction of the functional effectiveness of both branches of immunity, the cellular and humoral, for example, attenuation of reactions of the delayed hypersensitivity, decrease of the number of T cells, reduced production of the secretory IgA, and humoral antibodies, including their affinity, phagocytic dysfunction, and low activity of complement components (Chandra, 1999).

Nutrition and infections are intimately related, which is particularly important for young children who frequently suffer respiratory problems and diarrheal disease. The link between malnutrition and impaired immune functions is well described (Law et al., 1974).

In 2003, the World Health Organization defined nutritional recommendations, which should be applied in the prevention and possible treatment of the common communicable and noncommunicable diseases, including cancer. These recommendations were based on a row of extensive experimental and clinical studies, which proved that any change in the composition of nutrition like reduced content of unsaturated fatty acids, or increased amounts of fiber, and also the presence of substances called biological response modifiers (BRM), for example, muramyl peptides, transfer factor, and particularly beta glucans could support the functions of the immune system particularly if it is weakened for any reason (environmental stress, prolonged illness, radiation, chemotherapeutics, and application of immunosuppressant substances, fading immunity with aging) (Torrence, 1985; Vetvicka, 2013).

3 Nutrition and Immunity

Proper nutrition offers one of the most effective and least costly ways to decrease the burden of numerous diseases and their associated risk factors. Modern nutritional research significantly contributes to improving the health of both current and future generations. An important part of nutrition and health is immunonutrition, which can be defined as the effect of the provision of specific nutrients on immune function.

The potential to modulate the activity of the immune system by interventions with specific nutrients is the true concept of immunonutrition. This concept may be applied to any situation in which an altered supply of nutrients is used to modify inflammatory or immune responses. However, immunonutrition has lately become associated most closely with attempts to improve the clinical course of critically ill and surgical patients, who will often require an exogenous supply of nutrients through the parenteral or enteral routes. Unfortunately, this rather narrow focus ignores the fact that with optimal nutrition it is possible to modulate the health status of all individuals. Three potential targets exist for immunonutrition—mucosal barrier function, cellular defense, and local or systemic inflammation. Many trials used different compositions, but there are also trials that have shown some benefit from the single immunonutrient approach. For example, enteral provision of glutamine decreased the incidence of sepsis in premature neonates and the incidence of pneumonia, and severe sepsis in critically ill patients.

Many of the protective functions of immune cells depend on membrane fluidity, which adversely affects immune response. Therefore, the relevance of antioxidants is particularly critical for the functionality of the immune system. Nutritional status represents an important factor contributing to immunocompetence and to optimal cooperation among nutrition,

infection, and immunity. It is now clearly established that nutritional deficiency is closely related to an inadequate function of immune response, including phagocyte function, cytokine and antibody production, and antiinfection immunity. In addition, we have to remember that antioxidants represent our first line of defense and are crucial for maintaining optimal health; therefore we need an optimal intake of components with antioxidative properties. We also have to remember that the respective antioxidants play a role in the development of other diseases that are not related to the immune system. Several studies show that antioxidant constituents may be important in the development of eye disease and significantly lower glaucoma risk.

Interrelationships between nutrition and immunity are considered completely interconnected (Mainous and Deitch, 1994). It is shown that immunity does not work in malnutrition, poor nutrition, and during starvation. Also, the abundance of food damages the immune functions and reduces the capacity of the immune system to cope with infectious and other noncommunicable diseases (Chandra, 1992).

Deterioration of the immune system caused by malnutrition increases the susceptibility to infectious diseases. Trauma and stress accompanying the development of the disease converge into a catabolic state, which leads to further deepen malnutrition, which in turn worsens the course of infection. This creates a vicious circle that can interrupt treatment, along with applications appropriate nutritional composition. Most experts estimate that inadequate nutrition is the leading cause of up to 60% noninfectious and more than 75% of infectious diseases. Syndromes accompanying immune system dysfunction induced nutritionally are so similar to HIV-induced AIDS that have been identified as analogously NAIDS (nutritionally acquired immune deficient syndromes) (Beisel, 1992). Many people living in western countries do not realize that NAIDS affects the bulk of the population of all ages of the third world and is also the primary cause of morbidity and mortality.

Each disease, particularly infectious, and no matter what its nature and duration, has aversive effects on the immune system. It is obvious that effects described earlier depend not only on the previous nutritional condition of the person, but also on the nature and duration of the disease.

Without doubt, concomitant illness will subsequently worsen the nutritional status of an individual, and consequently also its immune capacity (Scrimshaw and SanGiovanni, 1997). There are numerous infectious diseases that are accompanied by malabsorption of nutrients. They are especially bacterial, viral, and protozoan enteritis, intestinal helminthiases, and febrile diseases, which lead to malabsorption of protein, vitamins, and trace elements. Virtually all infections, even if they are not accompanied by fever, cause catabolic loss. The first response to infection is caused by anorexia cytokines. Interleukins, such as IL-1, IL-6, and IL-8 and TNF-α can decrease food intake through the action of the CNS (Plata-Salaman, 2001). These cytokines simultaneously decrease the concentrations of

Zn, Fe, and Cu, which subsequently prevents their utilization by pathogenic microbes (Mackowiak, 2005). Under the influence of the increased production of IL-1 and significant changes of endocrine secretions, numerous amino acids are released from the skeletal muscle and used for liver gluconeogenesis (Keusch and Farthing, 1986). Anabolic losses are caused by redirection of the metabolic pathways of amino acids on the synthesis of immunoglobulins and other protein molecules necessary for a full immune response (Beisel, 1975).

Average protein loss in adults recalculated for normal infection are 0.6 g kg^{-1} per day, for the diseases accompanied by diarrhea 0.9 g kg^{-1} per day, and typhoid fever and severe infections to 1.2 g kg^{-1} per day (Powanda, 1977). Fever, on average, increases basal metabolic rate by 13% with an increase in body temperature every 1°C and a high fever by one-third, whereas nitrogen and amino acids are lost through sweating (DuBois, 1936).

In 1980, an extensive review was published, which summarized experimental and clinical studies on the effect of nutrition on immunity from the 1960s. From these very important and still valid data it can be concluded: protein and energy malnutrition determines the performance of immunity, with the severest impacts on phagocytosis and complement system. Cellular immunity is affected even more than humoral immunity. These effects deepen the deficiency of vitamins (especially A, B6, folate, riboflavin, and thiamine) and also the deficiency of trace elements, such as Fe, Zn, and especially Se. On the contrary, the excessive intake of fat (mainly polyunsaturated) has immunosuppressive effects (Tables 8.1 and 8.2).

The conclusions of the studies published from 1966 to 2006 concerning nutritional support of antiinfectious immunity by means of various vitamins and multivitamin preparations, trace elements, and yogurts with probiotics suggest that the therapeutic effects are not unambiguous and nutritional supplement (perhaps with the exception of glutamine supplementation) do not help in the treatment of certain infectious diseases already ongoing (Donabedian, 2006) (Table 8.3).

Table 8.1: Main consequences of malnutrition on the immunity.

Protein-Energy Malnutrition
Thymus atrophy
Decrease of lymphoid perivascular cells in spleen
Attenuation of delayed hypersensitivity reactions
Decrease of differentiated T cells (mainly CD4)
Decrease of cytotoxic CD8 cells
Decline of concentration of secretory immunoglobulins
Decline of functional capacity of macrophages to kill phagocyted bacteria
Decline of concentrations of C components (mainly C3 and C4)
Decrease of cytokine production (mainly IL-1 and IL-2)

Source: Adapted from Keith, M., Jeejeebhoy, K.N., 1997. Immunonutrition. Baillierés Clin. Endocrin. Metabol. 11, 709–738.

Table 8.2: Insufficient intake of nutritional components essential for immunity (*vitamins* and trace elements).

Thymus atrophy
Atrophy of lymphoid tissue
Lymphopenia
Attenuation of specific humoral immune response
Attenuation of delayed hypersensitivity reactions
Attenuation of mitogenic reaction
Delaying of allograft rejection

Table 8.3: The impact of nutritional interventions for the treatment of certain infectious diseases in nonhospitalized patients.

Supplements	Curative Effects
Zn	No effect on rhinitis
Zn	Reduction of infections in patients with sickle cell diseases
Zn + Se	Reduction of infections in general
Vitamins and minerals	Reduction of infections only in diabetes 2 and in elderly population
Multivitamins	No effect on infections including pnemumonia
Vitamin E	Reduction of infections of the kidneys and urinary tract, no effect on infections of lower respiratory tract
Vitamin C	No effect on rhinitis
Yogurt	No effect on diarrhea, reduction of diarrhea in patients with antibiotics
Lactobacilli	No effect on diarrhea in adults, reduction of diarrhea in children

Source: Adapted from Donabedian, H., 2006. Nutritonal therapy and infectious diseases: a two-edged sword. Nutr. J. 5, 21–30.

4 Why the Immune Responses are Dependent on Nutrition

Lymphoid tissues represent tissue basis in which various kinds of immunocompetent cells are produced. They are vectors of all immune functions and are characterized by a relatively high proliferation activity, which greatly increases after the encounter with the immunogen (antigen). This machinery puts extraordinary demands on energy intake (saccharides and fats) and also the high intake of basic building blocks for new cells: mainly proteins for building cell components and nucleotides for the synthesis and replication of nucleic acids. In this context it is important to realize that the functional state and the renewal of components of the immune system, as well as extracellular matrix, and rapid increase of competent cell populations (hematolymphopoiesis) and supporting epithelial and connective tissue cells are determined nutrition. For immunity, it is particularly important in the replacement of intestinal epithelium guaranteeing not only its physiological sorption-nutritional functions, but also translocation of antigens with food coming from the external environment, including potentially pathogenic vector in the direction GALT, which it is essentially the largest and most important immune organ of the body.

5 Beta Glucans

Polysaccharides in general and beta glucans in particular, have a long history as immunomodulators. As early as the beginning of the 18th century, it was known that certain infectious diseases showed a therapeutic effect on malignant processes. The dedicated use of such therapy dates from around the middle of the 19th century, at which time Busch (1850) performed experiments in search of curing sarcoma by infecting patients with an acute streptococcus bacterial infection of the dermis. Coley repeated these therapeutical procedures toward the end of the 19th century (Coley, 1894). However, the early researchers had no knowledge of the molecule responsible for the observed effects.

Eventually, other polysaccharide immunomodulators were sought. Their investigation began in the 1960s and 1970s. Two lines can be traced in the history of beta glucan, based on different starting points but gradually converging. The foremost origins were in Europe, United States, and Japan, respectively. Research on beta glucans in the Euro-American milieu was based on knowledge of the immunomodulatory effects of zymosan—a mixture of polysaccharides isolated from the cell walls of the well-known and widely used baker's yeast *Saccharomyces cerevisiae*.

During decades of research, numerous types of beta glucan have been isolated and described. In scientific literature, you can find hundreds of different components, all under the name beta glucan. Unfortunately, not all beta glucans were created equal and beta glucans widely differ not only in physicochemical properties, such as molecular weight, branching, or molecular weight, but also in biological properties. Some of the described beta glucans show little activity and some have no biological activities (Vetvicka and Vetvickova, 2014a).

The most commonly used beta glucans are isolated from mushrooms or yeast (*S. cerevisiae*). The main reasons behind this popularity are traditions—Euroasia has traditionally a surplus of yeasts due to the consumption of bread and beer, whereas in Japan and China, mushrooms are considered a folk remedy. These traditions together with easier access to resources led to the scientific evaluation of these types of beta glucan by groups from these regions.

The original studies on the effects beta glucan has on the immune system focused on mice. Subsequent studies demonstrated that beta glucan has a strong immunostimulating activity in a wide variety of other species, including earthworms, shrimp, fish, chicken, rats, rabbits, guinea pigs, sheep, pigs, cattle, and humans. Based on these results, it has been concluded that beta glucans represent a type of immunostimulant that is active over the broadest spectrum of biological species and that it is one of the first immunostimulants active across the evolutionary spectrum. Some experiments even show that beta glucan can help in the protection of plants, such as tobacco or turmeric (Anusuya and Sathiyabama, 2015). Beta glucan is, therefore not only a biologically active polysaccharide with strong

immunomodulating effects, but is also considered to be an evolutionary and extremely old stimulant of a variety of defense reactions (Vetvicka and Sima, 2004).

Beta glucans were found to be active in reduction of intestinal injury (Vetvicka et al., 2015a), protection against liver injury (Vetvicka et al., 2015b), regulation of hematopoiesis in cancer patients (Vetvicka et al., 2015c), and has positive effects on benign hyperplasia of prostate (Vetvicka et al., 2014a). In addition, several papers showed that beta glucan's activity could be improved by addition of some additional bioactive molecules, such as resveratrol and vitamin C (Vetvicka and Vetvickova, 2014b), humic acid (Vetvicka et al., 2014b), or selenium (Vetvicka and Vetvickova, 2016a).

6 Transport Through the Gastrointestinal Tract

Despite extensive studies, detailed information about the mechanisms of transport of beta glucans through the gastrointestinal tract is still inadequate. The main route for a particular antigen to the body is through M cells localized in Peyer's patches. Peyer's patches are traditionally considered the main site of entry, mainly due to their intimate localization with the intestinal lumen and due to the presence of M cells. Readers seeking detailed information on the role of gastrointestinal dendritic cells should see an excellent article written by Bilsborough and Viney (2004). The original suggestion that insoluble glucans are taken up by Peyer's patches M cells goes back almost 30 years (Hashimoto et al., 1991).

Availability of soluble glucans allowed better and more precise studies on gastrointestinal transport. A detailed study showed that the speed of transfer differs based on the type of beta glucans used, ranging from 0.5 to 12 h. Flow cytometric analysis of cells showed that oral administration of FITC-labeled glucans resulted in the presence of the labeled material inside the cells after 24 h. These beta glucans were internalized by intestinal epithelial cells. As only 10% of these cells were able to ingest beta glucans, it is possible that only a special subpopulation of these cells participate in beta glucans transfer (Rice et al., 2005).

It is important to note that beta glucans do not currently represent essential nutrients. On the other hand, it is clear that they may be successfully used during treatment of numerous diseases not only for strengthening of immune functions but also for maintenance of health, and improving the general quality of life (Liu, 2003). Beta glucans are resistant to digestion and might require bacterial fermentation (Lattimer and Haub, 2010). With more than 10,000 published studies, literature on the health effects of beta glucans is clearly extensive, but the cellular and molecular mechanisms behind the reported effects are still not fully established.

7 Beta Glucan and Immunity

Beta glucans represent evolutionary highly conserved structure often labeled as pathogen associated molecular patterns. Multicellular organisms developed the ability to recognize these molecules as nonself and, upon recognition, react via numerous defense mechanisms.

It means that the ability to recognize beta glucans as potential pathogens is phylogenetically coded in all living creatures, from invertebrates to humans (Iwanaga and Lee, 2005).

Beta glucans are generally considered to be potent stimulators of cellular immunity, with macrophages and neutrophils being the most important target. Beta glucan is recognized by receptors expressed on membranes of effector cells, such as macrophages, monocytes, dendritic cells, leukocytes, and NK cells. The most important receptors are Dectin-1 and CR3 (CD11b/CD18) receptors; additional receptors include Toll-2, lactosylceramide, and scavenger receptor family (Vetvicka, 2013).

Upon binding of beta glucan, these receptors transfer the signal resulting in cell activation. In macrophages, beta glucan binding causes not only an increase in functions, such as phagocytosis, but also production of cytokines, such as IL-1, IL-2, and IL-6. Phagocytosis is extremely important for elimination of pathogenic microbes and other materials from inside of cells. Increased level of cytokines subsequently stimulates effector cells of both specific and nonspecific immunity, resulting in increased antiinfectious and anticancer response (Vannucci et al., 2013). Besides direct effects on immunity, beta glucans also act as scavengers of free radicals. It is important to note that free radicals are the main risk factors in cancer development (Nakao et al., 1983; Sima et al., 2014).

8 Beta Glucan as Food Additive

For all living organisms, food is the source of chemical substances for growth and body building, the material for renewal and regeneration of cells and tissues. It is also the only single source, from which the energy is obtained that is needed for physiological reactions, metabolism, and as well for ensuring the course of the immune response against pathogens and for the control and destruction of tumor growth. Apart from the basic and essential components, proteins, lipids, saccharides, vitamins, minerals, the diet must contain a number of chemically diverse compounds that are nondigestible, but they are needed for ensuring the equilibrium within the large society of microbial species composing the intestinal microbiome and their digestive functions, which support the utilizing of a row of food components indigestible by macroorganism. These substances were in 1953 termed “dietary fiber” (Hipsley, 1953). However, much earlier, people were aware of the benefits of fiber in the diet. Already in 430 BC Hippocrates knew the beneficial (laxative) effects of meals prepared from whole wheat flour. In the modern era, around 1920, well-known American specialist J.H. Kellogg and health expert recommended “breakfast cereal” (corn flakes) (Slavin, 1987, 2013).

Dietary fiber was sometimes described as either “the skeletal remains of cell walls” or as “remnants of the plant cell wall” (Trowell, 1972, 1975). As it is difficult to distinguish between indigestible materials originating from the cell wall or other parts of the plant, the definition was later expanded to include all indigestible components of plant origin (Trowell, 1976; Trowell et al., 1976). Later, the Life Sciences Research Office of the Federation of American Societies for Experimental Biology adopted a new definition of

dietary fiber as follows: "the endogenous components of plant materials in the diet which are resistant to digestion by enzymes produced by humans." As referenced by Southgate (1991), this definition is in fact identical to that for "unavailable carbohydrates" as defined by Lawrence and McCance (1929). FDA nutrition facts panel on food products and nutrient content considers sources of dietary fiber as good (2.5 g fiber) and as excellent (5.0 g fiber).

Presently, dietary fiber, as numerous other indigestible nutritive substances, is generally ranked among prebiotics. The concept of prebiotics is relatively new. Prebiotics were first defined as indigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving their activity important for maintenance of the host health (Gibson and Roberfroid, 1995).

Chemically to the indigestible prebiotics, that is, dietary fiber, belong polysaccharides (cellulose, arabinoxylans) and various other plant components (resistant starch, dextrins), further inulin, lignans, chitin, pectin, and oligosaccharides, such as beta(alpha)-beta glucans. In addition, indigestible prebiotics also contain dietary important phenolics, carotenoids, vitamins, minerals, and antioxidants (Lattimer and Haub, 2010; Liu, 2003).

From these substances, the most important in terms of their beneficial impact on the health are beta glucans, which represent the noncellulosic polymers of beta-D-glucose containing glycosidic bonds in position beta-(1-3), eventually beta-(1-6). Beta glucans are indigestible for all mammals due to the lack of the beta-1,3-beta glucanase enzyme (Mueller et al., 2000; Ooi and Liu, 2000).

It was earlier supposed that the main part of the nutrition of preagricultural Paleolithic hominins was the meat of big game and their diets were primarily carnivorous in nature (Dart, 1957). In the later studies of authors studying dietary habits of contemporary ethnics, it was concluded that diets of ancestral man derived from diets of higher primates and were mainly plant based (Copeland, 2009). Fossil evidence shows that this pattern of nutrition was characteristic for early bipedal hominids living between 6 and 2 million years ago (White et al., 2009). Reliance on animal flesh increased substantially after 2 million years with the evolution of *Homo habilis* and especially *H. erectus*, who were already capable of hunting larger game. It was also shown that contemporary hunter-gatherer populations consumed more gathered plant food than hunted game meat (Lee, 1968). Ethnographic research in the South African !Kung tribes showed that plant foods formed 67% of their average daily nutritional dose and the rest was meat of small animals and insects of various species (Lee, 1979). Evidence from cave deposits in the Chihuahuan Desert suggests utilization of desert plants with a high content of prebiotics especially the inulin (Leach and Sobolik, 2010). It means that the Paleolithic diet had a much higher content of dietary fiber (prebiotics beneficial for health including beta glucans) and its total intake could reach up to 100 g per day (Eaton, 2006).

Foods high in prebiotics, including beta glucans as structural components of the cell walls of plants (grains), fungi, seaweed, yeasts (and also bacteria), have been consumed by early man since prehistoric times. From the viewpoint of the evolution of hominids, to which modern species of man *H. sapiens* belong (Mann and Weiss, 1996), these substances still formed a substantial part of the food of these hunter–gatherers during at least the whole Paleolithic Era. Their health and especially antiinfectious branch of their immunity have been supported principally by beta glucans, which represent important BRMs (Bohn and BeMiller, 1995; Novák and Větvička, 2009; Torrence 1985). This plant-based diet primarily formed the human genes controlling digestive metabolism and utilizing food components for growth, regeneration, and immunity long before the shift to meat-based diets. These genes were specific for direct predecessors of man (hominids), as well as hominins, and still determine nutritional behavior of modern man.

The character of the diet has changed considerably with the advent of the agrarian (Neolithic) revolution before about 10,000 years when the former man added to its diet new products from cereals and milk. Particularly, the composition of the nutrition has changed for a second time, since the beginning of the period of industrialization more than 200 years ago. The extent of the diversity of the diet significantly narrowed in industrialized countries, which have adopted quite uniform, so called "westernized lifestyle," which is obviously not always in accordance with genetically preadopted human metabolism during Paleolithic Era.

All aforementioned is forever the justification for the supplementation of the technologically produced "westernized" food by prebiotics and especially by beta glucans.

There is a large amount of studies on the beneficial health and nutritional benefits of beta glucans and reasons for the use of these substances as novel pharmaceuticals (Mälkki and Virtanen, 2001) and on their positive effect as support therapy of a row of communicable and noncommunicable diseases. Beta glucans have also suitable properties, which could be advantageously used in food processing and food supplementation of food commodities (preservation, stabilization, emulsification, gelation, etc.) (Burkus and Temelli, 2000). Beta glucans of various origin well suited for applications primarily in the brewing and bakery industry, meat production (Ahmad et al., 2012; Liu et al., 2015; Moroney et al., 2015; Panjagari et al., 2015), beverages and dairy based products (Kilic and Kankaya, 2016; Konuklar et al., 2004; Skouroliakou et al., 2016; Temelli et al., 2004), and further for the manufacture of low-fat ice creams and yogurts (Abdel-Haleem and Awad, 2015; Brennan et al., 2012), and can also be incorporated in combination with other soluble dietary fiber into low-fat dairy products and low-fat cheese curds to improve gelation and rheological characteristics (Tudorica et al., 2004). Insoluble (highly branched) yeast beta glucans (brewer's yeast beta glucans) are also convenient for food application for their emulsifying and water holding properties. Additional glucan may serve as fat replacers and as a source of dietary fiber (Thammakiti et al., 2004; Zhu et al., 2016).

Recently, some studies have brought about supporting reasons for the use of some sorts of beta glucans for supplementation of food and nutraceuticals. Beta glucans act in food as free radical scavengers (prevent lipid peroxidation) and induce postprandial production of interferon by blood leukocytes (Kozarski et al., 2011). Antioxidant capacity and free radical scavenging potential have beta glucans from mushrooms, for example, *Agaricus bisporus*, *Auricularia auricula*, *Flammulina velutipes*, *Ganoderma lucidum*, and *Lentinus edodes*, and have been recommended for food applications. The commercial importance of fungal polysaccharides has drawn a great deal of attention to the field of BRMs, as well as functional food. In this regard, the most commonly cultivated mushroom is of the genus *Pleurotus*. The annual production of these mushrooms is more than 900,000 tons.

Especially beta glucans from the mushroom *L. edodes* were successfully used as a partial replacement of wheat flour in baked foods and noodles, and in which they improve batter viscosity and elasticity (Kim et al., 2009, 2012). Also sclerobeta glucan produced by the fungus *Athelia rolfsii*, which is chemically analogous to schizophyllan (schizofiran) formed by the fungus *Schizophyllum commune* represents a versatile functional ingredient for stabilization and texturing of some foodstuffs (desserts, sauces). Its molecule is stable at low pH and in high temperatures (Survase et al., 2007).

The supplementation of beta glucans into food products for improving their nutritive value and fiber content may also have beneficial preventive and therapeutic effects (Parab et al., 2012; Slavin et al., 2009). As examples, it could be shortly mentioned the extracts from *Agaricus* sp., which exert antioxidative and free radical scavenging capacities (Richu et al., 2009), or extracts from *A. brasiliensis*, which acts as immunostimulants and alleviate obesity and diabetes type 2 (Yamanaka et al., 2013). As a novel food with high nutritional value and positive effects on health (cholesterol-lowering, antioxidant, anticolagulant, and immunomodulatory activities) the mushrooms of genus *Auricularia* are recommended (Sękara et al., 2015). Partial starch replacement in snack foods by extracts from *Agrocybe aegerita* brought about lower glycemic response (after consumption) (Brennan et al., 2012). A reduction of glycemic index was also reported in beta glucan enriched breakfast bars (Jenkins et al., 2002) and beta glucan containing bread (Cavallero et al., 2002). Beta glucans from *S. commune* (mushroom is used for production of cheese-like food as a live starter culture fermenting and coagulating milk through lactate dehydrogenase and proteolytic activities) have in final food substantial antithrombotic properties (Okamura-Matsui et al., 2001). Even if some studies provide inconsistent results, for instance, 5 weeks diet with a higher dose of oat beta glucan (5 g) significantly affected postprandial glucose levels and lowered insulin responses, while 5 weeks diet with barley beta glucan (5 or 10 g) did not (Biorklund et al., 2005), most of the other studies, in which food was supplemented with beta glucans, brought positive results. Significant reductions in glucose and insulin were found when fiber was added

to a standard breakfast (Nazare et al., 2011). Beta glucans have been shown to stimulate the immune system directly and thus can be potentially useful in alleviating symptoms of inflammatory conditions, such as irritable bowel syndrome (IBS) (Parisi et al., 2002). In addition, beta glucans can be used both as prebiotic and probiotic (Vetvicka and Vetvickova, 2016b).

The discrepancy in results obtained by various studies monitoring health benefits of beta glucan application (supplementation) of foods depends on at least two important factors: the variability of origin of beta glucan intake and type of diets or supplements and their culinary technologies (Kim et al., 2006). For instance, the amount of beta glucan is not substantial. It was shown that the intake of 3 g of beta glucan (20 g of oat bran concentrate) per day might not be sufficient to achieve a decrease in cholesterol level in patients with a mild hypercholesterolemia (Lovegrove et al., 2000), while feeding with either oat cereal or corn cereal (3 g of beta glucan daily) lowers significantly the total concentration of cholesterol (Karmally et al., 2005).

On the other hand, the positive effect of beta glucan could be dependent on the culinary technology of food preparation or the food composition. When mild hyperlipidemia patients were fed with the bread (5.9 g of beta glucan) no significant change in the cholesterol concentration was documented. In contrast, when the smaller amount of beta glucan was given to the same group of patients with a drink, a significant decrease of cholesterol was achieved (Kerckhoffs et al., 2003). It should be also noted that, in regard to the consequences of food production technologies and food processing, the interactions of beta glucans with food components might hinder or change their effectiveness (Giavasis, 2013). As an example, we can mention the ganoderan [beta glucan derived from the mushrooms *Ganoderma* sp. (reishi)], which is degradable by pectinases and dextranases. Therefore, in the food with this particular beta glucan its activity might be very low or none (Xie et al., 2012).

Conclusively, the growing interest in foods rich in dietary fiber prompted research on the application of beta glucans in nutrition. Despite their quite convincingly documented positive effect for maintenance of health, it was repeatedly shown that beta glucans could be utilized as excellent thickening agents, stabilizers, and fat replacement agents. They have the capability to form viscous solutions and emulsions supporting growth of beneficial colonic microflora and subsequently production of short-chain fatty acids. Therefore, they improve the peristalsis of the intestine and promote the faster removal of harmful, potentially carcinogenic substances from the colon, resulting in reduction of the risk of development of colorectal cancer. Moreover, beta glucans utilized as fat mimetics reduce intake of calories, so that nutritional products enriched with beta glucans could be recommended for the prevention and primarily the therapy of obesity (Siurek et al., 2011; Zhu et al., 2016).

9 Conclusions

There is no doubt about the importance of beta glucan for human health. Regardless of the use, that is, as a supplement or directly in food, beta glucan represents a unique modulator of numerous immune reactions. Another benefit includes no toxicity and the fact that beta glucan is active throughout the entire animal kingdom.

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CHAPTER 9

Nutritionally Enhanced Foods Incorporating Chía Seed

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1 Introduction

When looking to ensure and contribute to human health in general, a full and varied food based on fresh food and processed as little as possible is the best way to achieve this, in addition to preventing early deaths.

However, food availability is not always accessible to the entire population, especially for those living in rural areas, mainly in developing countries (Bhutta et al., 2013), where often they only have access to some foods but do not meet adequate consumption to obtain a balanced diet, and an adequate intake of minerals and vitamins is more complicated because almost always the diet is composed of a predominant food that tends to be nutritionally incomplete (Hefferon, 2015).

Taking into consideration and coupled with seeking improvement in quality of life, strategies have been sought to solve this deficiency of vitamins, minerals, and essential amino acids, mainly with so-called nutritionally enhanced foods.

These kinds of products are those that have been modified in their original composition by adding essential nutrients to meet the particular needs of food of certain groups of the population and that can almost always be classified as fortified foods. They may include or be called:

- Super foods (fortified with fiber), which are characterized by high levels of fiber present, as well as essential compounds as vitamins, minerals, fatty acids, phytochemicals, and that all of these compounds have been associated with a low incidence of deaths, as well as other health benefits.

Therapeutic Foods
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Nutritionists recommend that two or more servings of whole grain foods be eaten every day. Whole grain foods are rich in fiber, B vitamins, and minerals, such as iron. Whole grains include barley, oat, wheat, corn, millet, and wheat. These foods are a great choice among low-fat foods. Cereal energy comes mainly from complex carbohydrates. Nutrition experts recommend contributing more than half the calories in the form of carbohydrate-rich foods, among which are breads, rolls, muffins, biscuits, cereals, tortillas, potatoes and corn, peas, beans, and lentils.

A fiber-rich diet plan can help prevent cancer, maintain low cholesterol levels, and control diabetes. Most adults do not eat the recommended 25–30 g per day. Eating whole grain foods, fruits, and vegetables can increase fiber intake.

In advanced age it becomes necessary to eat food with higher density between each group. Therefore, breakfast cereals fortified with fiber can be selected as an excellent option. It is also wise to choose bread or toast made from flour fortified and enriched.

- Fortified foods, to which vitamins and minerals have been added in some crops like rice, corn, cassava, wheat through supplementation practices or fortification through the application of fertilizers biofortified with micronutrients.

However, if it is important to establish that companies use the fortification as differentiation strategy to develop food that may be perceived as higher value products. For this reason, these companies usually fortify foods, which can be more profitable with little additional cost, such as breads, breakfast cereals, dairy products, biscuits, and pasta. It is not customary to fortify meat and derived products, ice cream, sugary foods, fermented beverages, alcoholic beverages, waters, carbonated waters, and mineral waters.

For the company, the benefits of food fortification can be summarized as follows: (1) its high potential added value can increase profitability, and (2) it can develop a differentiation strategy for very low cost. The main disadvantages are summarized in the possible toxicity by excess micronutrients or by reactions between ingredients.

- Functional foods, which are designed to assist in the prevention or treatment of disease, but always take care to cover various criteria as the arising natural origin, to provide improved quality of life and especially to provide reproducibility, quality, safety, and efficacy. Through time, other terms have been created to characterize the “functional foods,” including therapeutic foods.

The International Food Information Council, defines functional foods as “foods that provide health benefits, beyond basic nutrition.” The Institute of Medicine of the Academy of Sciences, an academic institution in the USA, comes to an agreement that functional foods are “those in which has been manipulated or modified the concentrations of one or more ingredients to enhance their contributions a healthy diet.”

The consumption of functional foods is having a significant growth, especially in Europe and Asia. The increase of this market is reinforced with the commitment of international companies in product development under the slogan "rich in antioxidants and very healthy."

The offering provides a lot of combinations: multivitamins and minerals and natural plant extracts nutrients are part of the marketing of healthy foods. Many consumers are inclined by prebiotics with omega 3 and fiber.

These types of nutritionally enhanced foods are closely interrelated both in food and nutrition and health because its main goal is to help in the prevention or delay in chronic diseases and increase the quality of life.

Food fortification is one of the key strategies of WHO and UNICEF, which are seeking to eradicate malnutrition with ready-to-use therapeutic foods (RUTF), mainly based with nutrients, with iron, iodine, and vitamin A the most commonly used, followed by vitamin B complex, vitamin C, selenium, zinc, and calcium.

This should put attention on the major diseases of the 21st century that result in nutritional problems. The diets were not the most appropriate, because they were based on extremely white flours, processed, and subjected to a number of chemical mechanisms, and also they contained excessive quantities of dyes, preservatives, and sweeteners (Arroyo-Mera, 2011).

This type of eating in no way constitutes a true healthy source of carbohydrates, lipids, and proteins, so diseases, such as obesity, atherosclerosis, hypertension, and diabetes are increasingly frequent.

One thing that is interesting is that the global market for nutraceuticals is continuing to grow due to an aging population, interest from a broader spectrum of consumers in functional foods that include healthy ingredients, such as omega 3 fatty acids, and increasing interest and use in preventative medicine.

2 Therapeutic Foods

In the 1980s the term "functional food" was proposed in Japan and defines "food for specific health"(Pérez, 2006). It refers to those processed foods that contain ingredients playing a specific role in physiological functions of the human organism, beyond its nutritional content (Cagigas and Blanco, 2002).

The food–drug interaction recognized under the name of "functional foods" accepts the role of food components as essential nutrients for maintenance of life and health. Also, it highlights the role of nonnutritional items (Roberfroid, 2002), but helps prevent or delay chronic diseases of old age.

Commercial availability and the increase in this type of functional food, product of the improved micronutrient as supplements for malnourished populations, has proved successful (Das et al., 2013), so there is another term to characterize the "functional foods" as "therapeutic food."

The so-called therapeutic foods are prepared foods that contain calories, vitamins, and minerals (Santini et al., 2013) that can be used as a supplement in diets because they present certain properties for health maintenance. These have been directed toward populations that have special requirements in nutrition, such as the elderly.

Some of the main functions of these foods are related to optimal growth and development, the cardiovascular system function, antioxidants, xenobiotic metabolism, the gastrointestinal system, among others.

This range of natural foods (e.g., onions, garlic, gourd, tomato, olives, lemons) that generates benefits in the human body has been considered as medicated foods or foods that heal.

However, factors, such as poverty, insecurity, as well as unavailable necessary ingredients in order to have a healthy diet, are barriers to obtain or have access to a nutrient-rich diet that contains vitamins and minerals and similarly includes high-quality protein, content, and appropriate bioavailability of micronutrients, essential fatty acids, and so on (FAO, 2010; van der Kam et al., 2012).

This situation has led to nongovernmental international organizations generating means available to them to help this situation by programs for both general food distributions where the population can access at least the recommended daily intake of nutrients (Huybregts et al., 2012); even in emergency situations general distribution programs are implemented to provide basic food to affected populations.

Similarly, these institutions have worked in the development of food that contains certain properties for health maintenance or prevent and treat malnutrition problems; these have been classified as a ready-to-use foods (RUF) (Greiner, 2014; WHO, 2012).

Another procedure that has been sought with these foods, RUF is being used to restore both weight and health in undernourished people where they are deficient in micronutrients (e.g., vitamin A and B, iodine, iron, zinc), mainly pregnant women and malnourished children who reside in areas where nutrient-rich foods are limited or unavailable (Bhutta et al., 2013; Greiner, 2014).

A primary feature of RUF is that they are designed and manufactured to ensure they are ready to eat directly from the container, do not require cooking or water to be added for consumption (Huybregts et al., 2012), thereby they do not promote bacterial growth even without refrigeration and with the main objective to provide or help children and babies

with serious problems of malnutrition (Greiner, 2014). Moreover, the technology for the production of RUF is considered unsophisticated and production can be carried out in low-scale levels in developing countries (Wagh and Deore, 2015).

Because RUF does not contain water, it can create a problem with infants older than 6 months who need to take water, but not with infants less than 6 months old who are breast-feeding. Whereas RUF does not contain water, when it is consumed it can cause the need for supplementary water, which can cause contamination. This need for water could cause even one child fed with RUF to have a marked up risk of being infected by water-borne diseases.

As an example, a 12-month-old child who is being fed wholly with RUF would need to drink more or less 1500 mL of water daily, which might be contaminated water from a well or pond. In an opposite situation, when a 12-month-old child drink 850 mL of breast milk daily (composed mainly for bioactive fluids), and about 400 mL of water from some cereal sterilized by cooking, plus 250 mL of water from fruit and vegetables (such as oranges, papaya, banana, pineapples, and tomatoes), in reality there is no need for additional water or perhaps very little (Latham et al., 2011), compared to those who consume RUF.

According to its application, RUF has been grouped as those considered as food supplements, and which they have been called supplementary food ready for use (RUSF) (Huybregts et al., 2012), tested and used in situations considered as acute malnutrition to moderate whether a food is replaced used in food aid, it is these lipid-based nutritional supplements.

At present, RUSF manufactured with the assistance of diverse privately held companies and nonprofit organizations located in Europe, the USA, and in several developing countries, often close to where there is subsist acute malnutrition (Duclercq, 2014). The related expression “local producers” is adopted to refer to food-processing companies that make RUSF in developing or “programmatic” countries where malnutrition rates are increasing.

RUTF are a variety of therapeutic foods that create a distinction in the fight against problems of malnutrition. RUTF provides calories by means of carbohydrates, proteins, and fats. Also, this food source contains vitamins and minerals in sufficient quantities that allow treatment of malnutrition in young children (Linneman et al., 2007; Nasser, 2014), and it has been shown effective when used in children who have serious problems of nutrition (Linneman et al., 2007; van der Kam et al., 2012, 2016).

At the present time, RUTF be offered by way of RUTF paste and RUTF biscuits/bars. The energy concentrated in the paste is placed on a blend of peanuts, sugar, vegetable oils, and a pre-vitamin A mix and minerals. The industrial process to obtain RUTF paste is required to

ensure that all of the nutritional and chemical properties of the multiple ingredients are for a long time without any negative effects that affect the quality of the finished product (shelf life of up to 24 months) (Duclercq, 2014), although in locally produced ready-to-eat foods, this condition of shelf life is not necessary (Wagh and Deore, 2015).

RUTF has been extremely effective when used in the treatment of critical acute malnutrition, including kwashiorkor (inadequate intake of proteins), nutritional marasmus, and numerous forms of crucial wasting. On the other hand, in situations of critical acute malnutrition, RUTF is not a panacea and needs to be used carefully (Latham et al., 2011).

There are various studies carried out by Phuka et al. (2008, 2009), Defourney et al. (2009), and Isanaka et al. (2009), the potential intervention of corn/soy and cow milk-based treatment foods using RUTF are compared, and it may overstate the efficacy of RUTF. A grave deficiency of macronutrients (carbohydrate, protein, and fat), together with micronutrients, has been the predominant clinical sign of critical acute malnutrition, so-called extreme wasting, although the presence of infection and infestation is also likely.

Between the 1960s and 1970s in various limited-revenue countries, dietician treatment was based on a mixture of dried skimmed milk, casein, vegetable oil, and sugar, mixed with water. Also, vitamins and medicinal iron were supplied in those treatments. One attractive feature was that it was low-priced and available. Its use in hospitals had a good response with this regimen and this practice demonstrated that critical acute malnutrition could be attended with foods that are generally obtainable in the locality, at much lower cost than with RUTF or other commercially manufactured foods.

WHO has advised that the use of therapeutic foods, without supervision, when attending to serious problems of malnutrition, can result in some medical complications (Collins et al., 2006) in poor areas and especially for sick children (van der Kam et al., 2012).

In spite of this, and although since 2007 the UN has recommended the employ of therapeutic food ready for use (RUTF) in infants of low-income countries (Dibari et al., 2012), in the knowledge and the proven use of RTUF, only 15% of children with malnutrition could receive this type of food in 2014 (UNICEF, 2014).

One cause may be the foods that RTUF has identified as an imported and expensive food, based on ingredients outside the region where its use is proposed, has led to the establishment as a barrier for acceptance (Weber et al., 2016).

Additionally, until now, there is an inability to reduce the unit cost of RTUF in its production scale (Greiner, 2014); for example, peanuts are the foods most commonly contaminated with aflatoxin and therefore measures for quality control in both RUTF and RUSF must be of a high standard, and therefore it makes distribution more expensive (Greiner, 2014).

3 History and Ethnobotanical Aspects of Chia

Chia has a long history as food and its name derivative of náhuatl, means "seed from which edible oil is extracted" (Gerónimo et al., 2004). It is a species from the Mesoamerican region, where large tracts of land in Aztec provinces were dedicated exclusively for cultivation of this seed (Cahill, 2003, 2005).

The development of pre-Hispanic cultures in Mesoamerica was associated with the cultivation of various plant species, including chia (Vázquez-Alonso et al., 2014). Its cultivation was probably the third in economic importance, overcome only by corn (*Zea mays*) and poroto (*Phaseolus vulgaris*); in some areas chia was the most important crop species (Cahill, 2003; Rovati et al., 2012).

There is evidence to show that chia seed was used as a staple food by the year 3500 (BC) by pre-Columbian cultures of Mexico and Guatemala (Rovati et al., 2012). It was cultivated in the Valley of Mexico between 2600 and 900 (BC), mainly by Teotihuacan and Toltec civilizations (Guiotto, 2014; Ixtaina, 2010), as well as by the Tarahumaras (Giovanni, 2004).

Chia was an important element in religious ceremonies dedicated to the deities from the Aztec culture. The Aztec Empire received annually between 5,000 and 15,000 tons of chia seeds as a tribute from conquered nations (Ixtaina, 2010). But nevertheless, the cultivation of chia was persecuted from the Spanish conquest to near extinction for being considered a sacrilegious seed.

Through time, this crop declined leaving only very few domesticated varieties in addition to wild populations (Ayerza and Coates, 2005a; Cahill, 2003, 2005; Ixtaina, 2010; Mera et al., 2011).

The use of chia remains, thanks to the preservation of some traditions of descendants of Nahuas. Estrada (1989) and Guiotto (2014) refer to the information recorded in the Florentine Codex, where it is mentioned that plants that were integrated in the economics had both use value and exchange value. Also, in the Florentine Codex it became noticeable the magical and religious significance.

Faced with this situation, chia survived and remained only in a small region used for crops in the mountains of southern Mexico and Guatemala (Ayerza and Coates, 2005b), mainly because of the importance it had as a food and medicinal plant in the pre-Columbian period.

Cahill (2003) conducted an extensive review of literature in the 16th century codices, ethnographic texts, documents of naturalists, ethnobotanical information from herbarium specimens, and botanical floras on the uses of chia before and after the Spanish Conquest. However, at least in this work, little information on management techniques and morphological variation of this plant was reported.

Contrary to the previous report, morphological and phenological characteristics were documented (Cahill, 2005) to differentiate varieties of chia, between domesticated and wild species. Some characteristics in domesticated species may be mentioned as closed chalices, larger seeds, more compact inflorescences, and larger flowers.

The International Code of Nomenclature used for different organisms as plants, fungi, and algae (called Melbourne Code) (IAPT, 2012), and with some observations (Hernández, 2013), the systematic position of chia is:

Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Salvia*
Species: *hispanica*

The common name of chia is used to refer to a number of plants native to Mexico and the USA, belonging to other species of the genus *Salvia*, *Hyptis*, and *Amaranthus* (Ixtaina, 2010).

The red, white, and black chia so named for the color of its seed, are three local varieties in the region of Lake Patzcuaro, Michoacan, Mexico and are grouped into genus *Chenopodium* and *Amaranthus*. From elsewhere, the seed chia, called chia of water, corresponds to those species of *Salvia hispanica* L. (De la Cruz and García, 2010).

The family *Lamiaceae* has 170 genera and more than 3000 species widely distributed in tropical and temperate regions. *Salvia* genus includes about 900 species and it is distributed in various regions throughout the world, such as South Africa, Central America, North America, South America, and Southeast Asia (Guiotto, 2014).

S. hispanica is an annual herbaceous plant, which takes place from 1 to 1.5 m high, with branching stems quadrangular, with short and white pubescence. It has opposite leaves with serrated edges. The flowers are hermaphrodite, purple or white color, and they are in axillary pains or terminals. The fruit is of type schizocarp indehiscent, which has four very small seeds, oval, smooth, bright, and grayish brown with irregular spots (Giovanni, 2004; Guiotto, 2014).

The seed is, according to botany, part of the fruit that contains the embryo of a future plant. This part is protected by a seed coat (testa) and comes from seedpod of primordium. The seed is produced by the maturation of an ovum of a gymnosperm or angiosperm plant and, besides containing the embryo of a future plant, seed is a food source (Vázquez Yanes et al., 1997).

Seeds and their functions are of great importance for human consumption. Therefore, beyond the action of nature, man has developed a production process including planting,

harvesting, drying, sorting, selecting, washing, treatment, storage, and packaging of the seeds (Ixtaina, 2010).

For many years, chia seeds were marketed only in Mexican markets. In 1965 chia became available in health food stores southeastern California and Arizona (Hicks, 1966) and by the late 1980s it began marketing in the United States as a food supplemented with chia to feed pets.

Later in 1991 a Regional Project of Northwest Argentina was developed in order to identify and lead to the commercial production of new industrial crops that could help to diversify agricultural production and increase the earnings of farmers in that region, with chia identified as the most promising species (Guiotto, 2014).

Taking into account the importance of dietary-medicinal use of *S. hispanica*, assessments have been conducted on the possibility to perform cultivation in different regions, to find which is the most suitable date for planting this species (Busilacchi et al., 2013). Currently, the major countries producers of chia are Mexico, Guatemala, Bolivia, Colombia, and Argentina (Rovati et al., 2012), and it is estimated that its production reaches 3.000 kg/ha (Giovanni, 2004).

In countries where climatic conditions do not allow cultivation in the field, planting is done in greenhouses. Because it is a sensitive plant photoperiod, the choice of seasonality for cultivation depends on the latitude that is made (Guiotto, 2014). In accordance with Ayerza (2009), there is a strong relationship between the temperature of the station and the number of basic metabolic physiological processes.

Differences in the composition of seeds between ecosystems is due to the effects of one or more environmental factors. The effects of temperature, light, nutrition, soil type, even rising atmospheric CO_2 may affect proteins and the quality and quantity of seed oils (Pal et al., 2014).

3.1 Chia Seed as Valuable Food

Nowadays, the development of new products plays a strategic role in the food industry. Consumers demand food products with high nutritional value that provide additional health benefits, through the incorporation of new ingredients and natural products in such foods, that because of its particular composition they have protective effects against certain diseases.

This development has been thanks to scientific advances in both specialties, such as molecular biology, converging technologies in nutrition, and the importance that the consumer every day gives to their health, which provides the food industry starting points for the design and development of functional or therapeutic foods (Palou and Serra, 2000).

Thus, the procedures for obtaining functional foods are diverse, for example, techniques of supplementation and/or enrichment and modification or product formulation, have emerged to reach obtaining these foods.

But nevertheless, the development of these new foods is necessary to ensure that such previously incorporated ingredients in the food matrix are biologically active to gastrointestinal level to ensure proper absorption and consequently, its beneficial health effect (Palou and Serra, 2000).

There are already wide varieties of foods available to the consumer, but now the priority is to identify, which foods can improve health and well being, as well as to reduce the risk or delay the onset of major diseases, such as cardiovascular disease, cancer, and osteoporosis. If food is combined with a healthy lifestyle it can contribute positively to improving health and well being.

Usually, RUF are made from a mixture of proteins, carbohydrates, lipids, vitamins, and minerals (Bhutta et al., 2013; Santini et al., 2013).

To seek an increase in the nutritional value of a food, the employ of chia seed (*S. hispanica* L.) could be an alternative in the production of this type of food, mainly because of its high nutritional value (Rendón-Villalobos and Flores-Huicochea, 2016), then considering it as functional foods that have received massive attention in the latest years as constituent of healthy lifestyle changes (Silveira and Salas-Mellado, 2014).

This seed has several health properties that highlight its high quantity of fatty acids, such as omega-3 and 6 that provide health benefits, and it is also a source of other nutrients of great importance to health, such as antioxidants, proteins, amino acids, vitamins, minerals, and fiber (Chicco et al., 2009; Coates and Ayerza, 2009; Djousse et al., 2007).

The main uses of chia in antiquity were as food and medicine (Ixtaina et al., 2008), of which the main category was medical use (Reyes-Caudillo et al., 2008). Its use is reported in the care of diseases of the digestive, respiratory, urinary, and central nervous systems, besides being used in obstetrics, as a stimulant, with ophthalmic use, for skin problems and fever (Ayerza and Coates, 2005a).

According to Cahill (2003), parts of the plant of *S. hispanica* that are used for these various treatments are primarily seed, root, and leaves. Furthermore, there are reports on multiple uses of chia, such as fodder, medicine, religion, currency, food, and artistic character (Estrada, 1989; Guiotto, 2014).

The use of chia food is the most diverse, and frequently cited in the literature as a refreshing drink, called “fresh chia” or “chia water,” consumed both in Mexico and Central America and in California and Arizona (Ayerza and Coates, 2005a; Cahill, 2003; Mapes and Basurto, 2016).

Likewise, it has been ideally used for enriching a variety of foods, such as nutrition bars, yogurt, salads, bread, and "atole" (typical ancient drink of Mexico and Central America) (Ayerza and Coates, 2005a; Giovanni, 2004; Guiotto, 2014).

Chia is an oilseed, but it is different from other oilseeds in their fat content. Two-thirds of chia oil are compounds for essential polyunsaturated fatty acids (PUFA) (omega-3) and 10% are conformed by saturated fatty acids (SFA) (Ayerza and Coates, 2005a).

Consequently, and according to the presence of omega-3 in high amounts, chia seed also has other important components for human nutrition: antioxidants, fiber, protein, vitamins B1, B2, B3, and minerals, such as phosphorus, calcium, potassium, magnesium, iron, zinc, and copper (Ayerza and Coates, 2011; Capitani et al., 2012; Di Sapio et al., 2008; Ixtaina, 2010).

Moreover, dietary fiber has been an important component in the daily diet, because their consumption has beneficial health effects among which are cholesterol reduction, modification of the glycemic response, changes in bowel function, and antioxidant activity (Borderias et al., 2005; Esposito et al., 2005).

Similarly, it has been used in the production of eggs from hens fed with chia seed; these eggs have a ratio α-linolenic fatty acid (ALA) and docosahexaenoic acid (DHA), similar to that found in human breast milk (Giovanni, 2004); and it has also been used as food for cats, dogs, and horses in various cities of the European Union, the USA, and Argentina (Ayerza and Coates, 2005b).

Therefore, and according to these reports, it can be considered that chia seeds may be taken into account as potential sources of functional ingredients (Jiménez et al., 2013) for even produce food RUTF, such as bread (biscuit) with nutritional properties for the maintenance of life and health, and for the treatment of malnutrition problems.

Solid food RUTF, as an alternative food, aims to strengthen and promote transformation in the development of new therapeutic diets, to provide energy, antioxidants, proteins, amino acids, vitamins, minerals, and fiber (Chicco et al., 2009; Coates and Ayerza, 2009; Djousse et al., 2007).

Besides, during consumption does not require the child to drink extra water to eat, which in many developing countries this is a problem because of not having clean water available (Greiner, 2014), and could be a risk of diarrhea due to the proliferation of pathogenic bacteria.

However, even when the technology for the production of RUF it is not sophisticated and can be carried out for production in developing countries (Wagh and Deore, 2015), all these benefits presented in chia within a functional or therapeutic food must care mainly in the sensory aspects so it does not become a disadvantage in acceptance if this bread is incorporated or is suggested to grouped within RUTF.

This healthy food, which seeks to prevent or help malnutrition problems in the future, will also represent a new flavor in relation to the taste of local foods that are eaten, hence great challenges are created mainly in the sugar content of this proposed food RUTF, because being very sweet can mislead those who consume it, encouraging increased consumption of sweet foods with low nutrient density (Greiner, 2014).

Nevertheless, RUTF experiences acceptance in Africa and India, and suggests that the proposal of this food produced according to strict quality standards of products RUTF, as demanded by the UNICEF to be a quality product, bread (biscuit) added with chia seed, will quite likely be accepted in their consumption.

3.2 Health Benefits and Nutraceuticals of Chia

In recent years the consumption of high-fat diets has increased, notably saturated fat (Valenzuela et al., 2011), low in complex carbohydrates (whole wheat bread, oatmeal, brown rice) and very few micronutrients, combined with everyday lifestyles with little exercise. This has led to an increase in eating disorders (obesity, diabetes, depression), as well as in cardiovascular problems, hypertension, and cancer (Varela-Moreiras et al., 2010).

The recommendations of the WHO/FAO (Bull et al., 2004) are based on a total fat intake and must be at least 20% and up to 30% maximum according to the daily caloric intake to keep and care for an optimal state of health; despite this there has been an increase in fat intake worldwide, which has serious repercussions in the health of populations.

This situation on fat intake is mostly reflected by the appearance of the industrial revolution, where, for example, the Western diet has a caloric value that is above the recommended (30%–35%), where increased consumption of saturated fat (>10%) is observed, and thus to an imbalance in the omega-6/omega-3 ratio, as well as the increase in a transfat diet has also been significant (Gómez et al., 2011).

In general, the percentage of saturated fat in diets increases due to confinement and excessive energy content of the diet of livestock feed. Further, the fatty acid content (omega-6) increases considerably in response to the increase (to 70%) from grains rich in omega-6 and other oils of the same grains (Kang, 2005). This has caused the balance omega-6/omega-3 has been lost, also because foods that are rich in omega-3 are not consumed, despite being present in foods like chia seed.

Changes in the present way of life have modified the traditional eating and physical activity habits. In southern Europe these changes represent a pattern of Mediterranean diet and replace traditional foods for a more “modern” diet with a higher proportion of calories supply for substances of plant or animal origin (fats, oils, sugars) and processed starches and, in some sectors of the population, alcohol (OMS, 2010).

Faced with this problem, community dieticians have focused on chia seed (Rendón-Villalobos et al., 2011), basically in their fat content (Ayerza and Coates, 2005a) but without forgetting that it also has other important components for human nutrition as antioxidants, fiber, proteins (Chicco et al., 2009; Coates and Ayerza, 2009).

The richest food sources of omega-3 are fish oils, especially fish that live in cold waters. Omega-3 in form of EPA (*Eicosapentaenoic acid*) and DHA (*Docosahexaenoic acid*) due to consumption of phytoplankton by these fish (Travieso, 2010), while one of the best vegetable sources is chia oilseed (<60%), followed by flax (57%), soybeans, wheat germ, and nuts (between 7% and 13%) (Travieso, 2010).

Studies have shown that increased intake of ALA for a period of weeks to months has shown an increase in the proportion of EPA in plasma lipids, erythrocytes, leukocytes, plaquette, but is not observed an increment of DHA (Burdge and Wootton, 2002).

Moreover, EPA and DHA may have individual potential roles in the function of the human organism, since enriched supplements with EPA significantly improve psychological distress and depressive symptoms during transitions of menopause and are indicated as an antiinflammatory agent and effective against cachexic (Lucas et al., 2009). On the other hand, DHA is essential for growth and functional brain development in infants, besides being required in the maintenance of normal brain function in adults (Navarro-García et al., 2004).

In comparison with traditional grains (e.g., wheat, corn, rice, and barley), chia contains more protein, with values between 19% and 23 %, compared with the values of 13.7, 9.4, 6.5, and 12.5% in traditional grains. However, unlike other grains protein amino acids chia has no limiting factors in the diet of adults (Bushway et al., 1981). Chia is a source of good quality protein, which places it as important to be added to produce functional food.

Schep and Kellar-Munoz (2010) have suggested that 1 ounce (28.35 g) of chia seeds contains 4.4 g of vegetarian protein. Similar doses can be obtained by eating meat, and much higher than when consuming eggs. Another particular peculiarity in comparison with protein of chia is that this is free of gluten (celiac disease causing), then it is admissible for somebody with problems of this disease (Di Sapio et al., 2008).

Oxidation of food lipids is a serious problem, for both consumers and food manufacturers. If oxidation is not controlled, it can produce not only strange flavors (typically known as fishy taste), but also promote food aging and degenerative diseases of aging, such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction, which can be protected, just by eating omega-3 fatty acid (Okuyama et al., 1997).

The high proportion of antioxidant compounds in chia seed helps to keeps the seeds fresh for a long time, thus preventing rancidity of unsaturated fatty acids in foods that contain it (Reyes-Caudillo et al., 2008). Otherwise, the consume of these natural antioxidants content

in chia seed, such as flavonoids, tocopherol, phytosterols, carotenoids (Ixtaina, 2010) and phenolic compounds (chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol) have been related in the consumer protection toward many diseases and beneficial effects on human health (Capitani et al., 2012; Reyes-Caudillo et al., 2008).

The characteristic of the chia as a source of omega-3 excludes the need for artificial antioxidants like vitamins. It has been exhibited that antioxidant vitamins void the protective effects of cardiovascular drugs. Brown et al. (2001) established that the combination of antioxidant vitamins, such as vitamins E, C, and β-carotene obstruct the increase in HDL cholesterol (good cholesterol); this compared with simvastatin (a composite of cardiovascular protection).

Chia seed is considered as a good source of vitamin B. Some studies show that low levels of vitamin B in the blood are connected to an increased risk of fatal cardiovascular disease and apoplexy. Homocysteine (amino acid that is produced by chemically altering adenosine), a nonprotein substance that is not a constituent dietary normal, tends to increase if consumption of animal protein is high and low consumption of both fruits and leafy vegetables, hence folic acid and vitamin B levels are inadequate (Selhub et al., 1996).

Researchers believe that when the body cells send too much homocysteine in the blood, it has been linked to an increased risk of heart attacks and strokes. The inside of the artery walls become irritated, thus promoting plaque-fatty deposits damaging arterial walls (McBride, 1999). Nowadays, elevated serum homocysteine concentration is known as an important and independent factor of risk for cardiovascular disease and apoplexy (Malinazaw, 1996).

Nearly all basic vitamins cannot be synthesized (produced) by the body, so it cannot obtain more than through the balanced intake of vitamins contained in natural foods.

The minerals are at least as important as vitamins to achieve the maintenance of the body in perfect health. But as the body cannot manufacture them, the body must use external sources, such as food, nutritional supplements, breathing, and absorption through the skin, in order to ensure an adequate supply of them.

Chia seed is an excellent source of calcium, phosphorus, magnesium, potassium, iron, zinc, and copper (Ayerza and Coates, 2005a). It has been established that an edible portion of chia seed (100 g) be composed of 13–354 times more calcium, 2–12 times more phosphorus, and 1.6–9 times more potassium than foods such as wheat, rice, barley, oats, and corn. It also includes 6 times more calcium, 11 times more phosphorus, and 4.6 times more potassium than the same ratio of milk (Brown, 2003).

An important element for healthy nutrition has been fiber, because it plays remarkably important physiological functions, such as stimulating the intestinal transit (Reyes-Caudillo

et al., 2008); and it is located only in plant foods, such as minimally processed grains, fruits, vegetables, and legumes, such as chia seed. This fiber contains mucilage, which has the ability to absorb large amounts of water (14 times its weight in water), thus influencing proper bowel function ensuring slower digestion and a slower rise in blood sugar. Chia's fiber content, thus stabilizes blood sugar levels, along with creating a sense of fullness (Vuksan et al., 2010).

Also known, an intake of dietary fiber in small amounts has been associated with diseases, such as atherosclerosis, constipation, colon cancer, obesity, type-2 diabetes, and heart disease, the latter two a large percentage of cases, preceded by the metabolic syndrome and many other chronic disorders (Vuksan et al., 2010). In a portion of edible fiber (100 g of chia seed), there are big differences with traditional cereals. Chia has 1.6 times barley fiber, 2.3 more than wheat, oats more than 2.6, 8.3 times and 9.8 times more than corn and rice. When measuring nutrients, chia seed contains ~560% more (almost 6 times more) calcium than whole milk, ~285% more iron than spinach, ~113% more potassium (equivalent to that found in 1½ bananas), a quantity of vitamin C equivalent to the consumption of seven oranges, ~1690% more (almost 17 times more) magnesium than broccoli, and an antioxidant capacity considered 3 times more than blueberries (Vuksan et al., 2010).

While interest of weight loss and decrease in daily desire for food may be a bit far-fetched, increase in satiety due to the fiber and protein content may be valid. A study carried out by Nieman et al. (2009) showed that a daily intake of 50 g of chia seeds during 12 weeks did not influence body mass, composition, or disease risk factors in overweight/obese men and women.

Chia seed is considered a "dietary supplement" for federal agencies, such as the Food and Drug Administration (FDA). In addition, this completes the demanding standards of food nutrient content established by this organization to be a healthy food. The European Food Safety Authority delivered its position related to consumer safety of seed (whole and crushed) as an ingredient in foods (EFSA, 2009). Later they finished as improbable the use of chia seeds and ground whole chia seeds in bread products at a maximum of 5% would have an adverse effect on public health. The marketing was authorized on the Community European (2009/827/CE) to be used as an ingredient in bakery products with a maximum content of 5%.

The earlier characteristics have increased commercial interest in *S. hispanica*, which has been introduced to several countries as promising industrialized culture and have different food and medicinal products made from chia seeds. Mexico, Guatemala, Bolivia, Argentina, Paraguay, Peru, Ecuador, Colombia, Honduras, and Australia are the largest producers of chia seeds in the world.

These characteristics of the seed make it suitable for enriching a wide variety of products, thanks to its composition and nutritional value; also it gives great potential for use in the

production of a food RTUF, which serves to maintain the life and health and why not also contribute to the major problems of child malnutrition offering a food that has been accepted for consumption, mainly for not presenting unpleasant taste (González, 2016).

4 Some Reports on Use of Chia Seed as Food Ingredient

Given the fact that mainly food is used as a method to combat malnutrition and health problems, it has generated a range of new food products and the flood of information about its benefits is higher.

Called functional or therapeutic, foods have been quite interesting not by their mere nutritional value but because they try to add value health through changes in composition, adding new bioactive substances, combinations, and enrichment (Pacheco and Sgarbieri, 2001). Some universities have initiated programs for the study of this interesting area of food science and nutrition.

Many studies are available that work on ways to improve food, such as those discussed later, where chia seed is used, mainly because each and every one of the benefits that have been described earlier.

Pizarro et al. (2015) planned to study how the addition of whole chia flour (WCF) could present effects on the technical, nutritional, and sensory qualities of bread. Contents of 0% and 20% of WCF and vital gluten (VG) of 0% and 4% were added to bread. Results showed that in the bread loaves, WCF reduced the specific volume, lightness, and hue angle, in contrast to the chroma values that were not affected. Both WCF as VG provided the preservation of the moisture content of the loaves during the storage time. The expanded product firmness of the addition of levels of WCF from above 10% was countered by larger amounts of VG (more than 2%). This study showed that the optimum loaf contains 10% WCF and 2% VG, with 26% more lipids, 19% more protein, and 11% more ash than the control loaf (0% WCF and 0% VG). An excellent lipid profile was also obtained and displayed much larger content of omega-3 fatty acid as a better ratio between the fatty acids (omega-6/omega-3). Both breads were positively rated in the sensory profile analysis.

A similar study by Pizarro et al. (2013) used different contents of whole chia flour (WCF), between 0 to 30 g/100 g, and hydrogenated vegetable fat (HVF), 12–20 g/100 g to produce a cake that was evaluated for their nutritional and sensory qualities. A control cake (without WCF) was produced, too. The results exhibited that the addition of WCF decreased the specific volume and color parameters of the cakes. The variation in WCF and HVF contents contributed to maintenance of the moisture content during storage time. The best technological results were obtained when it was used amounts greater than 15 g WCF/100 g and from 16 to 20 g HVF/100 g in the cakes. The cake formulations containing 15 g WCF/100 g and 20 g HVF/100 g were choices for further evaluations, and values of

protein, lipid, and ash were higher than the control sample. This formulation also exhibited a considerable increment in the content of omega-3 fatty acid, also a good sensory acceptance and a greater purchasing intention.

Garda et al. (2012) evaluated the quality of gluten-free breads with a partial addition of ground flaxseed and chia. With this addition of seeds, the authors discovered that the bread offered improved rheological characteristics, longer shelf life, and better nutritional profile, compared with gluten-free breads. The authors found that the water activity and texture tests demonstrated that adding flax seeds and chia allowed the bread was kept fresh for longer. Bread dough supplemented with flaxseed and chia, achieved greater volume, raising and elasticity. Its chemical composition improved its profile by adding fiber and the relationship between lipids, because bread added with seeds showed an increase of 36% protein, 63% of polyunsaturated lipids, 40% of monounsaturated, and 185% in dietary fiber content. All sensory attributes evaluated had greater acceptance on bread with seeds, and the overall acceptability was the qualified attribute more positively.

Data published by Vuksan et al. (2010) relative to the chia as a functional food, showed that chia achieved two world records. It is also relevant that ω−3 fatty acids are determined to maintain health; regulate heart rate, both blood clotting and blood pressure, fertility, immune support, and reduction of inflammation. Both the National Institute of Health and the WHO coincide that most people are extremely low in fatty acids (omega-3) so that is necessary to increase daily consumption. To get a higher consumption at 3 g of this fatty acid (within an optimal ω−3 to ω−6 ratio), two tablespoons (15 g) provide a lot more than 100% of the recommended daily intake. Also, these researchers discovered that chia seeds possess the highest amount of fiber than any food, supplying 5 g exactly in 2 tablespoons (30% of daily requirement). Talking about the role of the fiber (soluble and insoluble) containing the chia seed, this allows increasing bulk and transit time, and also it allows it to alleviate constipation problems, therefore, it helps to keep healthy levels of glucose and blood cholesterol. Possessing a high fiber content, it allows that chia can incorporate 14 times its weight in water. If the fiber is added to meals, it to be converted to a bulking agent, ensuring slower digestion and a slower rise in blood sugar. Chia's fiber content, thus, stabilizes blood sugar levels, along with creating a sense of fullness.

Some studies have shown that the chia seed can be used to obtain enriched food products and consequent better nutritional value. Rendón-Villalobos et al. (2011) produced a nutritionally enhanced tortilla incorporating chia seed powder at different levels in a corn tortilla. With this the authors also attempted to produce a low-glycemic index (GI) and fibre-rich product taking care to preserve the sensory acceptability and physicochemical properties. Nutritionally, all chia tortillas had significantly higher levels of protein, lipids, and total dietary fiber than the corn tortilla. The in vitro digestibility of the tortilla showed differences between the products, and the relative digestibility values appeared to be

lower for chia powder added products. It is likely, then, that these chia tortillas may lead to lower glucose liberation in humans as well. They also point out that according to the test panel, chia powder did not alter the sensory properties of tortillas and have developed and characterized a highly acceptable tortilla with a high nutritional value, regarding the most recent observation on the effects of low GI food items and high intake of dietary antioxidants. The control sample showed a predicted glycemic index (pGI) of >101% and those tortillas with chia seed flour show pGI values between 72% and 98%. The pGI indicates that the chia seed-added tortilla may promote higher postprandial metabolic responses than standard corn tortillas. These results suggest that the intestinal glucose release after consumption of chia seed-added tortilla might be lower than when a common white tortilla is ingested. The uniqueness of this work is in having considered these two nutritional features at a time, thus creating a food product able to affect positively the health with two separate but strongly linked mechanisms.

Arroyo-Mera (2011) used chia seed in the preparation of new culinary recipes to improve the nutritional quality of consumers. The use of chia as an ingredient is referred to for the information of a well-documented nutritional functionality of this seed. In this work, chia has been used in different ways whose technicalities include, or not, cooking methods. The new products were appetizers, main dishes, desserts, and drinks. Appetizers include chia ravioli stuffed with cheese and spinach, chia and chicken tamales, chia bread, chia cream; main dishes like chia and quinoa risotto, breaded tenderloin with chia; Bavaresa cocada of rum with chia, blueberry pie with tocte and chia, chocolate cake with chia, cheesecake with passion fruit (maracuya) and chia, mouse chia with figs, to name a few desserts. Drinks include horchata chia, chia juice, and chicha chia. Arroyo-Mera (2011) showed that all dishes pleased the panel of 12 judges who knew how to appreciate the quality of products, as well as all the organoleptic characteristics of each.

Alvarado-Rupflin (2011) designed a nutritional bar using as ingredients: toasted amaranth, honey, cashew seed, and chia seed. The nutritional composition of the bar was 11% protein, 18% fat, 4% humidity, 2% ashes, and 2% raw fiber with energy value (463 calories). This author tried to obtain a content similar to those of other commercial bars, but with a notorious higher content in unsaturated fatty acids from chia seed. The sensory analysis results showed that it was defined that the consumer admits the aspect, aroma, flavor, and texture of the nutritional bar. Its nutrient content was similar to different bars designed in other investigations and most bars that already exist on sale for what the authors considered fit to compete in the market.

A study by Iglesias-Puig (2012) provides further information on how replacing wheat flour by chia seeds or their by-products at 5% level affects the bread performance, overall acceptation by consumers, and to assess their functionality as nutritious ingredients. The samples with chia significantly increased the levels of proteins, lipids, ash, and dietary fiber in the final

product compared to the control sample. In general, the samples with chia showed higher nutritional, technological, and sensory quality than the control bread. The inclusion of chia did not modify the dough mixing, with the exception of water absorption. However, the bread volume, crumb color, and the kinetic of amylopectin retrogradation showed significant differences in comparison to the sample used as control.

In an investigation released in the *Journal of Nutrition*, the effects of a specific dietary pattern on metabolic syndrome were examined (Guevara-Cruz et al., 2012). Sixty-seven participants with metabolic syndrome were implicated in the investigation and were given a mixture to drink twice a day. The mixture chosen was based on antihyperglycemic, antihyperinsulinemic, hypolipidemic, antiinflammatory, and antioxidants effects to protect the body from free radicals, aging, and cancer. Chia seeds were included in this mixture principally for the presence of fatty acids and antioxidants that promote a reduction in the inflammatory response. This research determines that a dietary pattern of nopal, chia seed, soy protein, and oat exhibited a reduction in serum triglyceride levels, serum CRP (C-reactive protein test, indicates acute inflammation or infection), and insulin area under the curve.

Chia seed for feeding animals have been used for Ayerza and Coates (1999), who tried to study the impact that can present the use of chia in feeding laying hens on egg production and composition. In this study, the amount of eggs produced was determinate parameters, as well as both content yolk fat and cholesterol, fatty acid composition, and egg flavor. The results showed significant findings when chia was used, because this is manifested in the egg yolks mainly when the content of the saturated palmitic and polyunsaturated ω-3 fatty acid was measured. This is very important because when there is a high consumption of PUFA, as it was in this study, this high dietary intake of PUFA could help to decrease synthesis of SFA. According to the results, chia seed enhances the relationship between egg yolk, PUFA, and SFA and it has become more consistent with the 1:1 ratio recommended by the American Heart Association, a nonprofit organization and the Canadian Federal Department of Health and Welfare Canada. The authors relate that the changes obtained in egg yolks in the composition of the fatty acids were due to the fact that the hens were fed with chia. They observed a decrease in the content of SFA content; the opposite situation was for the ω−3 PUFA content, this because of its increase presented; evenly enhanced the ratios of PUFA/SFA and ω−3: ω−6, and there were no off-flavors observed. All these factors involved in this study would create suitable products (e.g., eggs and egg products) to health-conscious consumers, situations that could transform the decrease in per capita consumption of eggs and egg products, as is happening in recent years.

Although these studies provide encouraging results, more research needs to be done with a combination of chia seeds that help to improve cardiovascular disease, lower cholesterol, triglycerides, blood pressure, and promote weight loss. To date there is little published

research on chia, and most of the information available is based on nonhuman laboratory animals and in vitro tests.

However, each day is expanding the knowledge and use of chia seed for the properties and benefits disclosed about their consumption, either alone or as an ingredient in food.

5 Conclusions

Despite the high nutritional values, for years the distribution of chia seed was only in Mexican markets and food stores from southeastern California and Arizona, because in Europe this plant was not well known. It wasn't until 2009 that the European Food Safety Authority delivered its confirmation about the safe use of chia seeds (whole and crushed seeds) as an ingredient in food, although, in the food industry its use is still rare.

Having said that, chia seeds from *S. hispanica* L. are commonly consumed in several countries, with the USA, Canada, and Australia now having a history of up to 8 years of use. Their use to produce several foods have been different and according the regional eating habits, but always taking into account the characteristics of the seed that make it suitable to enrich a wide variety of products, thanks to its composition and nutritional value, giving it great potential for use in food markets.

For example, in areas as Mexico, and South and Central America, where maize plays a fundamental role in the basic food of the population, it is more feasible that there are more maize-related food products, such as tortillas and arepas added with chia seed as an alternative to improve the nutritional value.

In contrast, in European countries bread consumption is very common and is considered to consume on average of 66 kg/person/year. In recent years, the European Parliament approved the seeds of chia as an innovative food that can be used as ground whole seeds in bread products at a maximum of 5%. This resulted in chia seeds that can be used to produce food products, such as biscuits, with an average intake of chia seeds of 9 g/person/day if all bread consumed contained 5% chia seeds.

Under these conditions of chia seed use as a whole can be considered as a functional food or therapeutic because it meets that fundamental premise of beneficially affecting one or more functions of the body, so as to provide better health and reduce the risk of disease.

This is how some countries have established basic standards for products clearly linked to human health. The scientific basis to consider as functional to various foods should be consistent, and is a function of nutrition experts disclosing the well-proven health claims each and every one of functional, natural, or processed foods. Countries like Japan and the USA have since the 1990s tried to regulate the marketing and labeling of functional foods

and nutraceuticals, and a few years ago the European Union has created the Functional Food Science in Europe.

Functional foods are not a panacea. Frequent consumption of a single food or supplement with healthy effects does not replace a healthy dietary pattern. Current public health recommendations place an emphasis on a diet rich in vegetables, cereals, fruits, and nuts, hence, rich in fiber and phytosterols. Further, it is very important restriction of foods rich in SFA (meat and meat products, whole milk) and those containing abundant refined sugar, often in combination with saturated vegetable fats (pastries, cakes, sweets, sweetened drinks, and commercial fried in general).

Nutraceuticals and especially functional foods must become a key element to make food a source of health in the coming decades. The nutrition in our society must incorporate value-added elements in the prevention and treatment of many diseases.

Consequently, promoting the use of chia seed could represent a valuable staple to enhance the nutritional value of the original food product, especially for its high content of fatty acids, proteins, antioxidants, and dietary fiber, all of them with health benefits known. However, all these benefits of a functional food are tied to physical activity, because regular physical practice reduces the risk of coronary heart disease and stroke, diabetes type II, hypertension, colon cancer, breast cancer, and depression.

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SECTION III

Medical Impact

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CHAPTER 10

Perspective Therapeutic Effects of Immunomodulating Acidic Herbal Heteropolysaccharides and Their Complexes in Functional and Dietary Nutrition

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Abbreviations

AG	Arabinogalactan
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BPH	Prostatic hyperplasia
BW	Body weight
DTH	Delayed-type hypersensitivity
EAE	Autoimmune encephalomyelitis
5-FU	5-Fluorouracil
GID	Gastrointestinal disorders
GIT	Gastrointestinal tract
GSH-Px	Glutathione peroxidase
HG	Homogalacturonan
IBD	Inflammatory bowel disease
LCC	Lignin-carbohydrate complex
LMWC	Lower molecular weight compounds
MCP	Modified citrus pectin
MDA	Malondialdehyde
PBMC	Peripheral blood mononuclear cells
PI	Prebiotic index
RG	Rhamnogalacturonan
SCFA	Short-chain fatty acids
SOD	Superoxide dismutase
SRBC	Antigen sheep red blood cells
TBARS	Thiobarbituric acid reactive substances

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1 Introduction

1.1 Trends in Herbal Medicine and Nutrition

Humans and animals have approached instinctively their health maintenance that relies on medicinal plants since time immemorial. Nowadays people can partly explain the physiological effects of a given herb and use its therapeutic potential by isolating its active constituents, proving their mode of action and developing a drug or a nutraceutical product. There are about 300,000 species of higher plants in the world, and only between 6% and 15% of them are investigated for bioactive compounds. Medicinal herbs that are exploited in phytotherapy are derived from more than 53,000 plant species (Pan et al., 2014). For example, Bulgaria has about 770 species of medicinal plants, and 200 are currently in use as more than 250 herbal drugs are produced from them and used in the prophylaxis, medicine, cosmetics, and the food industry (Evstatieva et al., 2007). According to the World Health Organization (WHO), 75% of the human population uses herbs for health care. Eleven percent of those evaluated by WHO as extremely important medicines are produced only by plants. For instance, the 2015 Nobel Prize in Physiology or Medicine was awarded half to the discovery of a plant drug artemisinin that saved millions of lives from malaria. About 60% of the antitumor drugs in clinical trial phase are of natural origin (Demain and Vaishnav, 2011). More than 70% of German physicians use in their practice plant-derived drugs and phytotherapeutics are even covered by the national health insurance in Japan (Azaizeh et al., 2010). Research and development studies on immunomodulating and antioxidant therapeutic effects of medicinal herbs have always been intriguing scientists, medical practitioners, producers, and entrepreneurs (Cravotto et al., 2010; Kratchanova et al., 2010a; Labadie et al., 1989; Liu et al., 2015b; Radoucheva et al., 1995; Ramberg et al., 2010; Wagner et al., 1999). Medicinal plants are the main and most perspective raw material for the growing nutraceutical industry (Kathirvel et al., 2015). Interestingly, green tea from *Camellia sinensis* is the most widely consumed liquid after water in the world (Nie and Xie, 2011). According to Transparency Market Research, the global nutraceutical market was valued at US $165.62 billion in 2014 and is expected to reach US $278.96 billion by 2021.

1.2 Importance of Bioactive Acidic Heteropolysaccharides

In a classical manner, most of the therapeutic effects of medicinal plants are related to their lower molecular weight compounds (LMWC), which are accepted as the most active constituents. For more than 30 years of scientific research in Chinese medicine, and especially in Japanese Kampo medicine, traditional European, African, and Indian Ayurvedic medicines has been proving that acidic herbal polysaccharides are also main contributors to biological activity of almost every herb (Liu et al., 2015c; Paulsen and Barsett, 2005; Popov and Ovodov, 2013; Srivastava and Kulshreshtha, 1989; Thakur et al., 2012; Wangensteen et al., 2015; Yamada, 1994, 1996; Yamada and Kiyohara, 2007). At the same

time, old medicinal books recommend for gastrointestinal disorders (GID) herbs like *Althea officinalis*, *Tanacetum vulgare*, and *Rheum palmatum*, which are currently well-known rich sources of acidic polysaccharides (Nedelcheva, 2012). On a cellular level in plants, acidic heteropolysaccharides and their conjugates are physiologically active dynamic structural blocks of the cell wall and intercellular matrix. They play a crucial role in cell growth, proliferation, and defense (Knox, 2002). All human cells express carbohydrates on their surfaces in the form of glycoproteins, glycolipids, and oligosaccharides that are important for immunological response and numerous primary biological functions (Nangia-Makker et al., 2002). From a physiological point of view, the human organism has created an adequate adaptation to acidic plant polysaccharides because they are present in the normal diet through fruits, vegetables, and herbs. Although herbal acidic polysaccharides do not have any nutritional value for humans, they are natural functional ingredients in our traditional foods and drinks, and remedies used in the folk medicine. These polysaccharides are famous for the lack of or low cytotoxicity and detrimental side effects, and are degraded in the cecum, ascending and transverse colon by residential microflora to bioactive short-chain fatty acids (SCFA) (Endress, 2011).

The family of herbal acidic heteropolysaccharides includes mainly two groups: pectins and acidic hemicelluloses. Both groups are referred to as mucilage and gums by pharmacists or to dietary fibers by nutriologists. Because of the cell wall complexity, negative charge, and extraction conditions these acidic polymers are isolated quite often in the form of covalently or physicochemically bound conjugates with glucans, proteins, lignins, polyphenols, and minerals (Kiyohara et al., 2000; Nie and Xie, 2011; Pawlaczyk et al., 2009). Special attention should be given to pectic polysaccharides, whose importance for maintenance of human health is determined by their beneficial effect on digestive, urinary, cardiovascular, endocrine, nervous, and respiratory systems (Chen et al., 2011; Endress, 2011; Šutovská et al., 2013; Wicker et al., 2014). Braconnot (1825) proposed the application of pectins in medicine because of its jellifying properties. The therapeutic effect of pectin in the famous apple diet of Heisler-Moro for the relief of GID was explained in 1931 by Malyoth (Kertesz, 1951). The first evidence-based study that showed clearly the cholesterol-lowering action of pectin was published in 1961 (Keys et al., 1961). Generally, acidic polymers express in vitro and in vivo anticomplementary, lymphocyte, and phagocyte modulating, antitumor, antiinflammatory, anticoagulant, prebiotic activities, and so on (Bijak et al. 2013; Ferreira et al., 2015; Paulsen, 2001; Yamada and Kiyohara, 2007). It is proved that after oral administration immunoactive herbal polysaccharides stimulate the intestinal immune system through interaction with lymphocytes in Peyer's patches, which further have a complex impact on the systemic immune system (Yamada and Kiyohara, 2007). Moreover, pectins form hydrocolloids in the gastrointestinal tract (GIT) that absorb steroids, lipids, hydrolytic enzymes, and other metabolites, which are further excreted from the body (Groudeva et al., 1997; Stantschev et al., 1979). Classical examples of immunomodulating

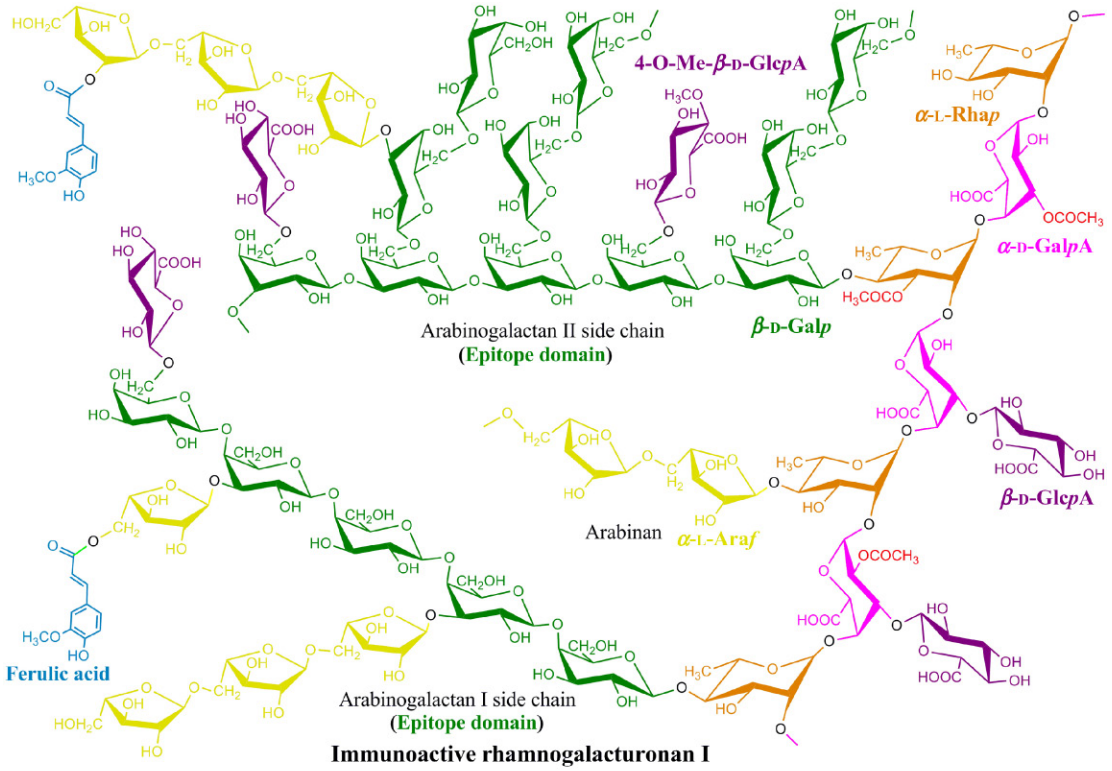
acidic polysaccharides are pectic polysaccharides isolated from *Angelica acutiloba*, *Urtica dioica*, *Bupleurum falcatum*, *Viscum album*, *Biophytum petersianum*, *Echinacea purpurea*, *Plantago major*, leek, citrus fruits, and glucuronoarabinoxylans from *E. purpurea* and *P. major* (Barsett et al., 2012; Georgiev et al., 2012; Inngjerdingen et al., 2008; Kratchanova et al., 2010b; Matsumoto et al., 2008; Mueller and Anderer, 1990; Ognyanov et al., 2014; Proksch and Wagner, 1987; Samuelsen et al., 1996, 1999; Wagner et al., 1989, 1994; Yamada et al., 1985, 1989). As a result of these continuous and comprehensive studies the immunomodulating therapeutic effects of crude herbal drugs were partly explained, leading to further development of numerous food supplements for treatment of different kinds of immunodeficiency from influenza to tumor diseases, as well as for regular detoxification and maintenance of human body. An extensive collaborative study on Malian medicinal plants focused on traditional wound healing water extracts revealed important role of immunomodulating pectic polysaccharides on the activity of traditional formulations (Wangensteen et al., 2015). Good commercial and clinically tested examples are crude mistletoe extracts, nettle root extracts, Kampo medicine formulations Juzen-taiho-to, Hochuekkito, *Astragalus* polysaccharide extract, and enzyme modified citrus pectin PectaSol-C (He et al., 2012; Kiyohara et al., 2004; Morris et al., 2013; Sekiya et al., 2013). Interestingly, it was found that the quality of life of cancer patients supplemented with apple pectin in parallel with chemo- and radiotherapy was improved (Botushanova et al., 1999; Tomova et al., 1999; Yaneva et al., 2002). In addition, functional drinks on the basis of chokeberry juice enriched with pectin and chokeberry juice with rosehip extract were developed in our laboratory and currently produced by Vitanea Ltd. These functional drinks express cardioprotective and antiatherogenic effects (Daskalova et al., 2015). Generally, acidic heteroglycans expand their role as technological food hydrocolloids to biofunctional food additives.

2 Chemical Diversity of Herbal Acidic Heteropolysaccharides and Relation to Immunogenicity

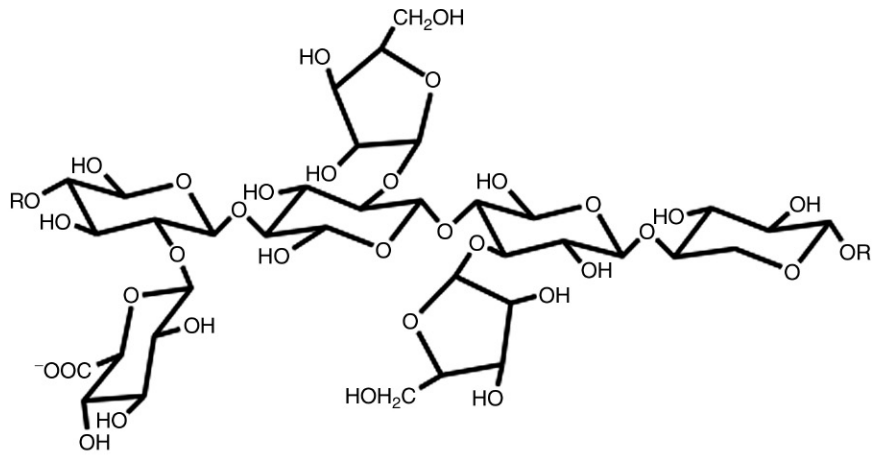
As already stated, medicinal plants contain different in structural complexity, charge and function homo- and heteropolysaccharides, such as cellulose, other glucans (starches, β-glucans), inulin, pectic polysaccharides, hemicelluloses (xylans, arabinoxylans, glucuronoarabinoxylans, xyloglucans, glucomannans), and their complexes with other compounds (Ebringerová et al., 2005; Liu et al., 2015c; Nie and Xie, 2011; Paulsen and Barsett, 2005; Thakur et al., 2012).

Pectic polysaccharides are the most complex heteroglycans on Earth. Although they were discovered 226 years ago (Vauquelin, 1790), polymer fine structure is still an open issue. Generally, pectic polysaccharides are composed of about nine different monosaccharides (Ara, Gal, Rha, Xyl, Fuc, Glc, 2-Me-Fuc, 2-Me-Xyl, and Api), three uronic acids (GalA,

GlcA, and 4-O-Me-GlcA), two ulosonic acids (Kdo and Dha) and one 3-carboxy-5-deoxyxylose (Ace) in certain anomeric, enantiomeric, conformational and substituted forms. On a structural point of view, pectins contain three main building blocks: unbranched homogalacturonan, ramified rhamnogalacturonan I, and II. Some galacturonans branched with Xyl, Api, etc. also occur (Albersheim et al., 1996; Schols and Voragen, 2002; Voragen et al., 1995). The most abundant is homogalacturonan (HG), followed by rhamnogalacturonan (RG) I. Linear HG is composed of α-(1→4)-linked poly-D-Gal*p*A, which carboxyl groups are methyl-esterified to different extent and some hydroxyl groups at C-2 and/or C-3 can be acetylated (Yapo, 2011a). Furthermore, it is covalently linked by an α-(1→2)-glycosidic bond with the linear repeating fragment [→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→]$_n$ that represents RG I backbone (Yapo, 2011a). The latter carries side chains of α-(1→5)-L-arabinans, β-(1→4)-D-galactans, arabinogalactan types I, and II at a specific branching point, -C-4 hydroxyl group of L-Rha residues (McNeil et al., 1980; Voragen et al., 2009). It is found for herbal pectins that GalA units are partly or highly substituted at O-3 with β-D-Glc*p*A units (Georgiev et al., 2017b). Similarly, Rha units are rarely acetylated at O-3 (Sengkhamparn et al., 2009; Georgiev et al., 2017b). The presence of methyl and acetyl ester groups in the neutral side chains of RG I backbone could not be excluded. Togola et al. (2008) reported a pectic polysaccharide with 40% mono-*O*-methylated monosaccharides in RG I side chains. Arabinogalactan (AG) I is an arabino-4-galactan, composed of a linear β-D-(1→4)-linked galactan (Gal*p*), which is branched at O-3 with mainly α-L-(1→5)-linked Ara*f* residues. AG II is an arabino-3,6-galactan composed of β-D-(1→3)-linked galactan backbone with side chains at O-6 of β-(1→6)-linked galactans. 1,3- and 1,6-galactans are substituted to different extent mainly with α-L-(1→3)-linked Ara*f* residues (Schols and Voragen, 2002). Glycosidic coupling of L-Ara in AG structures is highly diverse (1,2; 1,3; 1,5). Usually AG side chains are terminated with β-D-Glc*p*A, 4-O-Me-D-Glc*p*A, α-L-Fuc*p*, and other sugars (Matsumoto et al., 2008; Yapo, 2011a). Additionally, α-L-Ara*f* and β-D-Gal*p* residues could be esterified with ferulic acid at O-2, O-3, O-5, and O-6 respectively (Ralet et al., 2005). The complex chemical structures of RG I side chains or direct AG I and II structures are extremely important for the expression of immunomodulating activity (Yamada and Kiyohara, 2007). It is well known that RG I in herbal pectic polysaccharides is strongly ramified and it often contains AG II side chains. AG II structural motifs are of great importance for recognition from immunocompetent cells (Ferreira et al., 2015). This could be clearly seen from the works of Yamada and Kiyohara, and Paulsen (Paulsen, 2001; Yamada and Kiyohara, 2007). Fig. 10.1A presents a model structure of immunoactive RG I with AG I and II side chains. Furthermore, it is very common that the distribution ratio between HG and RG I in herbal pectic polysaccharides is lower than in fruit pectin (Ognyanov et al., 2016; Wangensteen et al., 2015). In fact, herbal pectins often have a lower molecular weight than those from fruits. Sometimes authors use the term acidic AG for native herbal pectic fractions (almost) lacking HG (Wang et al., 2005). Generally, these types of heteroglycans express very potent



(A)



(B)

Figure 10.1: (A) A model structure of immunoactive rhamnogalacturonan I with arabinogalactan I and II structural motifs. (B) The basic structure of glucuronoarabinoxylan (Caffall and Mohnen, 2009).

activity. The literature data on the importance of HG for expression of immunomodulating activity are still contradictory (Yamada and Kiyohara, 2007). Maybe the HG region could serve as a dynamic arm like in the plant cell wall for better interaction of AGs with biological receptors.

The third main structural pectic fragment is RG type II that is covalently linked for HG by α-(4→1)- and/or α-(1→1)-glycosidic bonds at the nonreducing end (Bar-Peled et al., 2012; Darvill et al., 1978). It has an extraordinary and conservative complex composition and structure. Generally, RG II is composed of a partly methyl-esterified galacturonan backbone, carrying four oligosaccharide side chains rich in rare sugars (2-Me-Fuc, 2-Me-Xyl, Api, Kdo, and Dha). RG II exists in nature as a dimer formed by borate-diester linkages (O'Neill et al., 2004). RG II has been found to be relatively abundant in red wine and other fermented beverages (Buffetto et al., 2014). This dimeric form is physiologically active for both plants and humans (Li et al., 2014b). At the same time dimeric RG II is absorbent for heavy metals and the number of publications for its immunomodulating properties is increasing (Yapo, 2011b). Shin et al. (1997) showed that RG II from the leaves of *Panax ginseng* could be macrophage Fc receptor expression-enhancing polysaccharide. Later, they reported for the first time that dissociation of the RG II dimer led to a decrease in the expressed immunomodulating activity against macrophages (Shin et al., 1998).

Elucidation of structure-immunomodulating activity relationship of pectic polysaccharides is realized by using pectinolytic enzymes (Georgiev et al., 2013; Sakurai et al., 1998; Voragen et al., 2009; Yamada and Kiyohara, 2007). Enzyme modification is combined with SEC and further GC-MS glycosidic linkage composition analysis and LC-EIS-IT-MS/MALDI-TOF-MS or 2D NMR for sugar sequencing (Li et al., 2014b; Ognyanov et al., 2016; Sakurai et al., 1998; Sengkhamparn et al., 2009; Zou et al., 2014a).

Another bioactive herbal acidic heteropolysaccharides are hemicellulosic glucuronoarabinoxylans. They are acidic heteroxylans composed of linear β-(1→4)-linked D-Xyl*p* units substituted with α-(1→3)-linked L-Ara*f* residues and α-(1→2)-linked D-(4-O-Me)Glc*p*A residues (Fig. 10.1B). Xyl*p* residues could be methylated and/or acetylated and Ara*f* residues could be feruloylated or coumarylated (Ebringerová et al., 2005).

During cell growth and extraction or decoction preparations polysaccharides can form immunoactive complexes with noncarbohydrate compounds that could influence immunomodulating properties of the former polymer. For example, acidic heteropolysaccharides can interact with phenolic acids and polyphenols, especially flavonoids: procyanidins, anthocyanins, and catechins. These interactions are based on the formation of weak noncovalent bonds (hydrogen bonds and hydrophobic interactions), covalent linkages, and adsorption. Hydrogen bonds are formed between –OH groups of polyphenols and –O- from the glycosidic linkages (Jakobek, 2015). Covalent ester bonds

are formed predominantly with phenolic acids (Ralet et al., 2005). Hydrophobic pocket or cavity formation in polysaccharide macromolecule is important for hydrophobic interactions with procyanidins (Watrelot et al., 2014). Interestingly, we separated from lavender pectins after an exhaustive ethanol treatment and SEC the ethanol-soluble catechin, epicatechin, quercetin, and other flavonoids (Georgiev et al., 2017c). Acidic polysaccharides can form glycoconjugates with lignins, as well (Sakagami et al., 2010). The chemistry of this interaction is still poorly understood but information on the composition of both separate parts is available. Generally, the carbohydrate part is a pectic polysaccharide or neutral heteroglycan. Lignin part is represented by vanillin, guaiacol, syringaldehyde, *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, cinnamic acid derivatives, and so on (Bijak et al., 2013; Kiyohara et al., 2000). Varying the ratio of polysaccharide to phenylpropenoids produces heterogeneity in the acidity, water-solubility, ethanol-insolubility, and molecular weight (Sakagami et al., 2010). Sakagami et al. (1987) reported that antitumor activity of lignin-carbohydrate complex (LCC) increased with the acidity. They also concluded that polymerized structure of phenylpropenoids in LCC is required for the induction of antiviral activity, and that the higher-order structure of phenylpropenoid polymers and polysaccharides is required for immunopotentiation. Kiyohara et al. (2000) demonstrated that ex vivo intestinal immune system modulating activity of LCC was significantly reduced by $NaIO_4$ or $NaClO_2$ treatments, concluding that the whole glycoconjugate was important for the activity. Additionally, herbal polysaccharides were found to be mostly conjugated with proteins where carbohydrate moiety is covalently attached to a polypeptide backbone usually via *N*- or O-linkages (Nie and Xie, 2011). For example, AG II is often linked to a protein moiety through Hyp or Ser residues (Tan et al., 2010). Zhang et al. (2011) reported that after digestion of protein moiety in polysaccharide-protein complex it lost activation ability on T-lymphocytes.

Fig. 10.2 presents our own recent results on the distribution of acidic (pectic) polysaccharides in 4 different organs of 34 widely used European medicinal plants. Acidic polysaccharides in herbs were estimated on the basis of anhydrouronic acid content (AUAC), corresponding to the total uronic acids and GalA content in water-extracted, insoluble in 70% ethanol and dialyzed macromolecular complexes. All investigated plants contain pectic polysaccharides, as their distribution is higher in fruits and flowers and lower in leaves and roots. The higher difference between AUAC and GalA amount in the linden sample was explained by the presence of a reasonable amount of GlcA in it. Later, it was proved by GC-MS methylation analysis and 2D NMR study that silver linden flowers contain a novel glucurono-rhamnogalacturonan I that is acetylated at O-3 of Rha units (Georgiev et al., 2017b). On the basis of sugar composition, determined by the corresponding alditol acetates, the crude herbal polysaccharides contain typical for pectins (Rha, Ara, Gal, Xyl, Fuc) and for other water-extractable herbal polysaccharides (Glc, Man) monosaccharides. Additionally, the predominant molecular weight is $\leq$100 kDa, as determined by HPSEC-RID analyses (Georgiev et al., unpublished results).

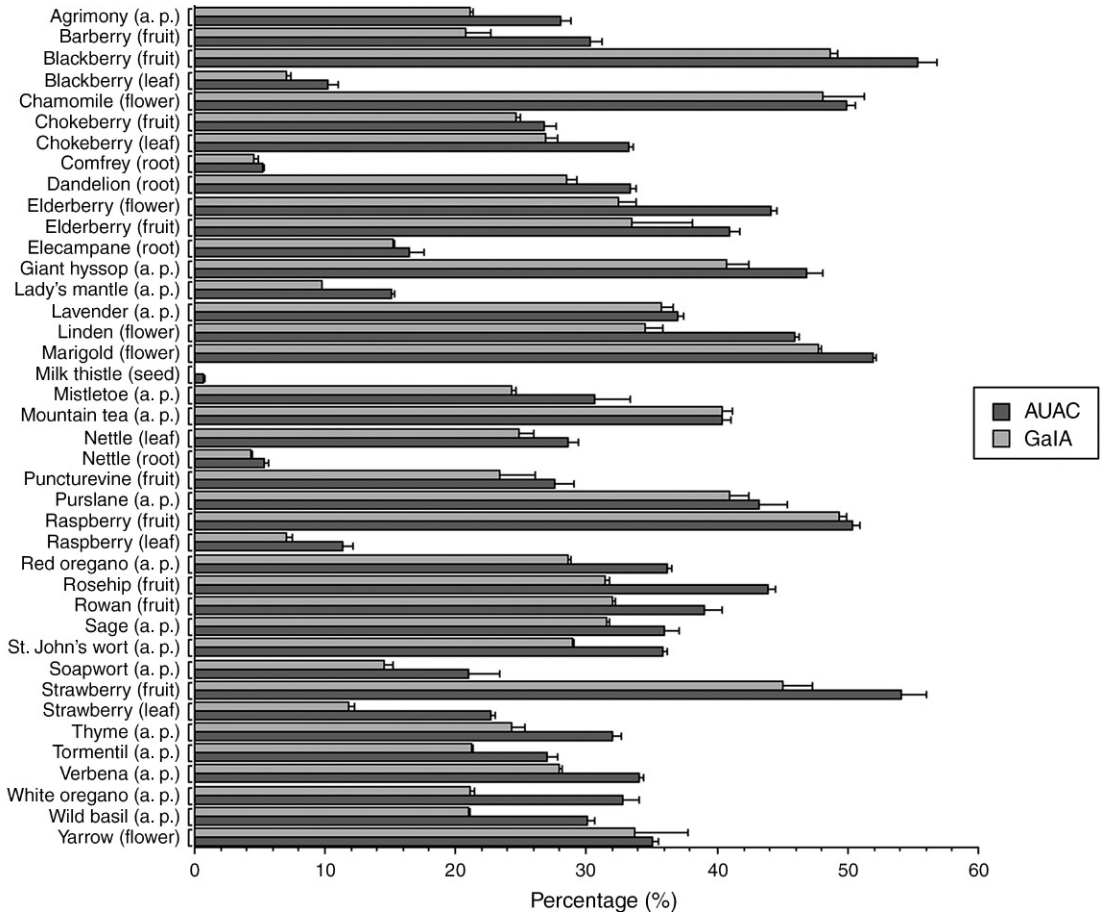


Figure 10.2: Anhydrouronic Acid Content (AUAC) and Galacturonic Acid (GalA) Content of Water-Extracted Crude Polysaccharides From Different European Medicinal Plants.

3 Perspective Therapeutic Effects of Acidic Heteropolysaccharides

3.1 Modulation of Innate and Adaptive Immunity

Originally, the health-promoting properties of medicinal plants and their extracts have been documented for centuries (Azaizeh et al., 2010; Nedelcheva, 2012; Pan et al., 2014). Later, some of these properties were specified as immunomodulating activities and now polysaccharide research revealed that carbohydrates are important for the observed effects (Wagner et al., 1999). More and more polysaccharides isolated from medicinal plants are reported for their potent immunomodulating activities (Ferreira et al., 2015). Immunomodulating activity of a given compound is explained by its pharmacological effects on humoral and cellular immunological factors. Regulatory interactions between these factors in the course of immune response from the immunomodulating compound

determine its immunostimulating or immunosuppressing effects (Labadie, 1993). Acidic heteropolysaccharides could mediate activity of certain immunological factors. Important factors from innate immunity are complement system, NK-cells, phagocytes (macrophages, neutrophils, dendritic cells), and their metabolites (monokines) and mediator cells (mast cells, basophils, eosinophils, and thrombocytes), producing proinflammatory molecules. Prominent immunomodulating cells from adaptive immunity are T- and B-lymphocytes, and their metabolites (immunoglobulins and interleukins) (Khan, 2008). The actual effects may be specific or nonspecific. For instance, acidic polysaccharides can stimulate phagocytes through interaction with some surface molecules (receptors) and initiate phagocytosis, production of NO, ROS, cytokines, etc. (Lojek et al., 2014). Matsumoto et al. (1993) and Matsumoto and Yamada (1995) demonstrated that pectic fraction bupleuran 2IIb from *B. falcatum* could enhance immune-complex binding to peritoneal macrophages through Fc receptor by a mechanism dependent on an increase in intracellular Ca^{2+}, followed by activation of the calmodulin, but not by a PKC or PKA pathway. Rhamnogalacturonyl arabinogalactans react with serum IgG and form a complex that initiates complement activation through the classical pathway (Kiyohara et al., 2006; Yamada et al., 1991). Furthermore, ramified pectic regions rich in AG II are recognized by certain receptors in lipid rafts of B-cells, which induce activation and proliferation of B-cells (IgM and IL-6 production) (Matsumoto et al., 2008; Sakurai et al., 1999). Therefore, polysaccharides could modulate innate and adaptive immune systems, which open their great potency for the development of new therapeutics and agents beneficial to human health. Fig. 10.3 shows a schematic overview of perspective therapeutic effects from immune system modulation by supplementation with herbal polysaccharides, which are further presented in the next sections.

3.1.1 In vitro complement system modulation

Some of the most studied immunomodulating effects of pectic polysaccharides are those on the complement system. The complement system is a vital instrument for the innate immunity and particularly general antimicrobial protection mechanism for humans (Austarheim et al., 2012a; Ehrnthaller et al., 2011). The complex pectic structure is recognized by the complement, similar to recognition of microbial cell wall polysaccharides, thus acting as an antigen-mimetic. Pectins activate complement system through the classical and alternative pathways (Michaelsen et al., 2000; Yamada et al., 1991). Complement activation is a complex immunological process in which different immunocompetent cells like macrophages, NK, T-, and B-cells are involved and activated. Therefore, pectins stimulate innate immune response and host defense through the complement. Generally, polysaccharides, expressing such effect, are termed anticomplementary or complement-fixing compounds (Wangensteen et al., 2015; Yamada and Kiyohara, 2007). Their effect is evaluated on inhibition of total complement hemolysis of targeted animal erythrocytes in vitro, as this evaluation system is not enough for differentiation between complement activation and inhibition. Interestingly, the classical view of complement as a major system of host defense that copes with infections

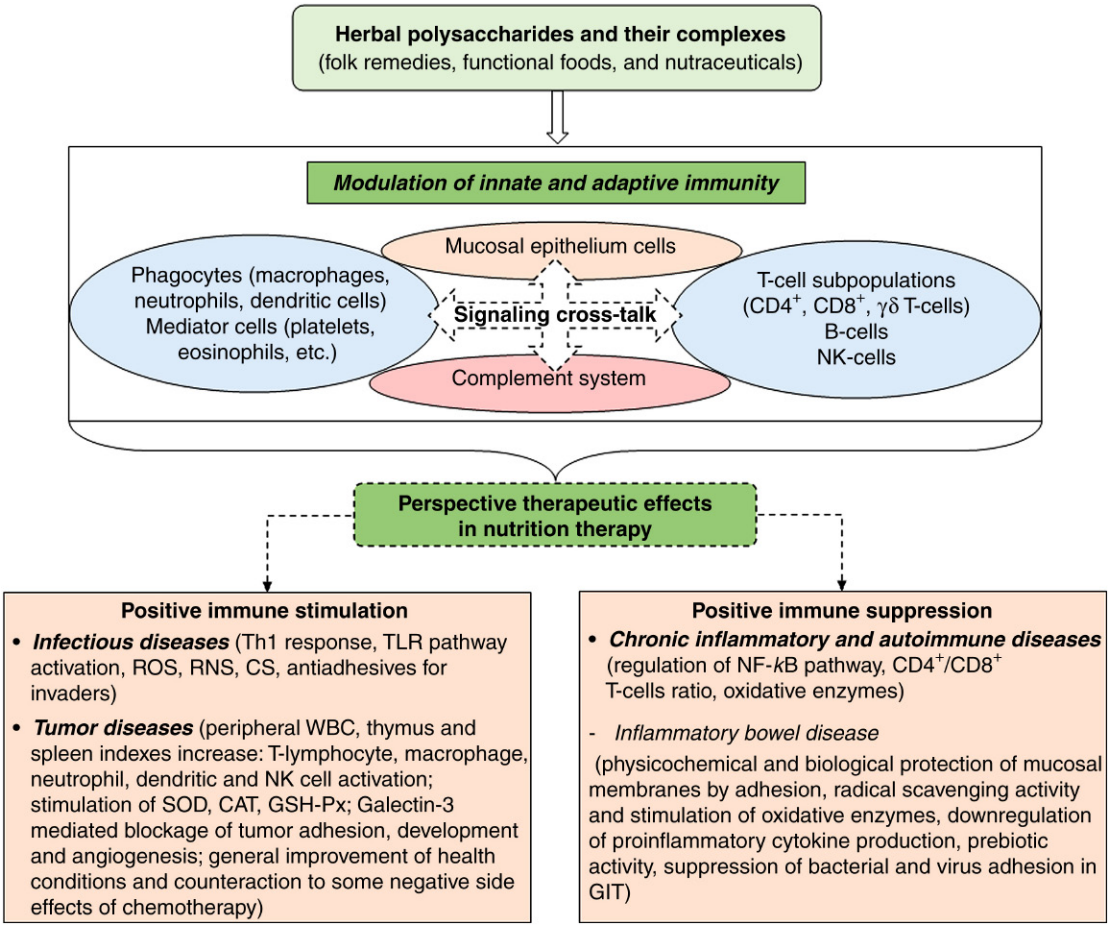


Figure 10.3: A Schematic Overview of Perspective Therapeutic Effects From Supplementation With Immunomodulating Herbal Acidic Heteropolysaccharides.

CS, Complement system; *GIT*, gastrointestinal tract; *GSH-Px*, glutathione peroxidase; *SOD*, superoxide dismutase; *WBC*, white blood cells.

is changing to that of a multifunctional player in tissue homeostasis, degeneration, and regeneration (Phieler et al., 2013). Complement modulation effect of acidic polysaccharides was extensively examined and reviewed by the groups of Yamada and Paulsen (Paulsen, 2001; Paulsen and Barsett, 2005; Yamada and Kiyohara, 2007) and some recently obtained literature results on this topic are reviewed here. For example, Zou et al. (2014a) obtained two pectic polysaccharide fractions 50WCP-II-I and 100WCP-II-I from the roots of *Codonopsis pilosula* with potent in vitro human complement fixation activity and $IC_{50} \approx 32.0$ μg/mL. 50WCP-II-I fraction presented long partly methyl-esterified HG region, interrupted by one short RG I with AG I and II side chains. Fraction 100WCP-II-I had two main ramified regions, one was unusual galacturonan region with AG-I side chain on position 2 of GalA, and the other

one was RG-I region with AG-II side chain. The authors concluded that responsibility for complement fixation activity in two fractions were mainly ramified regions on the basis of *endo*-polygalacturonase release of nonbranched galacturonans and reevaluation of biological activity of the corresponding subfractions. After enzyme treatment 50WCP-II-I-a subfraction expressed the highest activity with IC_{50} = 6.5 μg/mL, followed by 100WCP-II-I-a fraction with IC_{50} = 12.2 μg/mL. Zou et al. (2014b) also reported isolation of immunomodulating pectins from the bark of traditionally used in Malian medicine *Parkia biglobosa* that expressed potent complement fixation activity and stimulated macrophages to release NO. Zhang et al. (2014) isolated pectic polysaccharides from the leaves of the African plant *Lessertia frutescens* called "cancerbush" that possessed potent in vitro complement-fixating activity. These findings help for the elucidation of therapeutic effects of this traditionally used plant for treatment of cancer related illness. Košťálová et al. (2014) isolated two hemicellulosic neutral and acidic alkali-soluble fractions from the leaves of *Fallopia sachalinensis* that exhibited significant immunostimulating activity in the complement-fixation test. Similarly, Ho et al. (2015) showed that elderberries contain pectic polysaccharides with potent dose-dependent in vitro complement fixating activity and macrophage stimulating activity, as responsible for the immunomodulation were ramified regions. Interestingly, pectins from 100°C extract were more active. In another study on pectins from elderflowers they obtained higher immunomodulating activity and observed enhancement of bioactivity after removal of ester groups by NaOH. The authors concluded that their results could partly explain therapeutic effects of elderflowers in colds and influenza (Ho et al., 2016).

3.1.2 Intestinal immune system modulation

When orally administered, polysaccharides are transported through the stomach to the small intestine and colon. Immunostimulating polysaccharides modulate the intestinal immune system, which has been known to direct not only defensive but also regulatory functions of both mucosal and systemic immune systems through lymphocyte migration depending on homing receptor pattern (Jung et al., 2010). It was demonstrated by animal models that herbal polysaccharides stimulate the intestinal immune system through interaction with lymphocytes in Peyer's patches, which further have a complex impact on the systemic immune system (Yamada et al., 2009). For instance, Yu et al. (1998) isolated from rhizomes of *Atractylodes lancea* three pectic polysaccharide fractions with potent ex vivo intestinal immunomodulating activity against murine Peyer's patch cells. The most active ALR-5lla-1-1 fraction was rich in AG II and digestion with *exo*-α-L-arabinofuranosidase and *exo*-β-D-(1→3)-galactanase significantly decreased its activity. Kiyohara et al. (2002) demonstrated that oral administration of the isolated pectic polysaccharide fraction F-5 from the Japanese herbal prescription, Juzen-Taiho-To (150 mg/kg/day) to mice enhanced function of Peyer's patch cells. The increased activity after *endo*-polygalacturonase treatment of F-5 and further structural studies proved that the most potent intestinal immune system modulating activity was expressed by AG II-rich fractions. Kiyohara et al. (2010) obtained

14 pectic fractions from *A. mongholics* and 13 of them showed high dose-dependent ex vivo immunomodulating activity toward cells from Peyer's patches at 10, 50, and 100 μg/mL. The activity was sharply reduced by enzyme degradation of β-[1→3(6)]-D-galactans. Similarly, Grønhaug et al. (2011) showed by specific β-D-galactanases that β-(1→4)- and β-[1→3(6)]-D-galactans were important for the immunomodulating activity of pectin from *B. petersianum* against intestinal Peyer's patch cells and macrophages ex vivo. Suh et al. (2013) reported a pectic polysaccharide CUI-3IIb-3-2 from peels of *Citrus unshiu*, expressing a powerful immunomodulating activity on T- and B-cells from Peyer's patches. The polysaccharide was losing its activity after *endo*-polygalacturonase modification. It also increased proliferation of bone marrow cells in C3H/He mice at 1 g/body weight (BW) and GM-CSF/IL-6 production from T- and B-cells from Peyer's patches. Slavov et al. (2013) found that water-soluble pectin from waste petals of Bulgarian rose expressed ex vivo intestinal immune system modulating activity through Peyer's patch. Later, in our study on the search of immunoactive compounds from lavender, we found that lavender pectic polysaccharides contribute to the immune-enhancing properties of lavender. Pectic fraction chPS-L1 rich in AG II was free of LPS and expressed anticomplementary activity through the classical and alternative pathways with IC_{50}values of 94 and 38.9 μg/mL, respectively. At a concentration of 100 μg/mL, it also activated murine Peyer's patch immunocompetent cells, NO production from murine macrophages and spontaneous ROS production from whole blood and isolated human neutrophils. chPS-L1 showed ≥20% in vitro inhibition of A549 human lung adenocarcinoma, LS180 colon adenocarcinoma, and Jurkat T cell leukemia at 50 μg/mL after 48 h incubation (Georgiev et al., 2017c). Production of lavender oil and lavender-based products for maintenance of human health is increasing worldwide and lavender-derived nutraceuticals could be effective for improvement of human health. For illustration, Fig. 10.4A presents active carbohydrate chain units against Peyer's patch immunomodulation.

After immunomodulating polysaccharides are thought to incorporate into Peyer's patches through M-cells by pinocytic manner and/or as secretory IgA-polysaccharide complexes through polymeric immunoglobulin receptors, they are postulated to interact with immunocompetent cells, such as dendritic cells, macrophages, stromal cells and B cells. Polysaccharides are fairly well absorbed by intestinal epithelial cells, but their communication with the epithelium could also lead to immunomodulation of underlying dendritic cells because epithelial cells have been known to possess functions of immunocompetent cells. By these dual modes of action of active polysaccharides, functionally modulated T- and B-lymphocytes from Peyer's patches and dendritic cells from lamina propria interact in mesenteric lymph nodes. These lymphocytes are proposed to result in modulation of several mucosal and systemic immune systems with their by-stander effect in lymphoid and nonlymphoid organs through lymphocytes homing phenomenon (Jung et al., 2010; Tang et al., 2015; Yamada and Kiyohara, 2007). This mechanism is shown on Fig. 10.4B. The proposed mode of action of herbal polysaccharides on intestinal immune system is logically

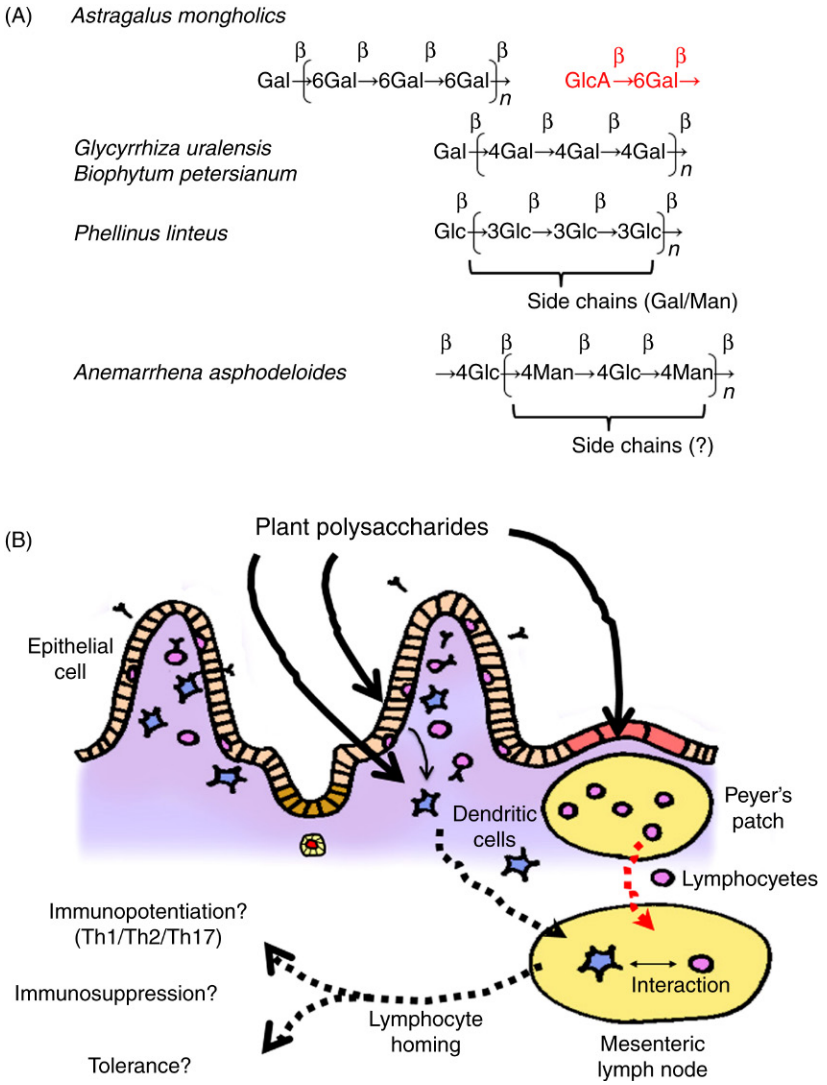


Figure 10.4: (A) Studied candidates of active carbohydrate chain units for Peyer's patch immunomodulation. (B) Hypothesized action mechanism of plant polysaccharides for immunomodulation through the intestinal immune system.

directed to immunopotentiation or immunosuppression through induction of effector and regulatory T-cells and B-cells. The potential therapeutic effect of this kind of polysaccharide immunomodulation is suggested in the next sections.

3.1.3 Combinatorial effects of immunomodulation with herbal polysaccharides

For further elucidation of immunomodulating potential of acidic herbal polysaccharides and their therapeutic perspectives were conducted numerous other in vitro and in vivo studies.

Kratchanova et al. (2010b) studied immunomodulating potential of water-extractable and acid-extractable pectic polysaccharides from the widely consumed leek (*Allium porrum*). The survival rate of mice pretreated with 2 mg/kg BW (i.p.) of two polysaccharides and subsequently infected with *Salmonella enteritidis* increased to 17.2 days in the case of water-extractable pectin, and to 10.5 days in the case of acid-extractable pectin from leeks, as compared with the survival rate of 5.5 days for control mice. Treating the mice with pectins led to an increase in the functional activity of their peritoneal macrophages and killing effect. Both polysaccharides activated complement system through classical and alternative pathways, as water-extractable was much more efficient. Additionally, Nikolova et al. (2013) showed that leek pectins could modulate ROS and RNS production from phagocytes in vitro and ex vivo. Yang et al. (2010) demonstrated that oral administration of herbal polysaccharides (LLPs) from *Lycopus lucidus* (50, 100, and 300 mg/kg) significantly enhanced the plaque-forming cells, serum hemolysin level, and delayed-type hypersensitivity (DTH) in response to antigen sheep red blood cells (SRBC) in a dose-dependent manner in BALB/c mice. In LLP-treated mice, phagocytosis capacity and concanavalin A-induced splenocyte proliferation were remarkably increased. Both higher doses of LLPs also caused a significant increase in the indices of thymus and spleen organs of mice. These findings show that *L. lucidus* polysaccharides could initiate nonspecific and specific immune-enhancing activities in vivo. Holderness et al. (2011) reported that acai fruit polysaccharides induced innate immune responses in human, mouse, and bovine peripheral blood mononuclear cells (PBMC). In mice, acai-PS incited neutrophil recruitment to the peritoneum and lung, as well as activated dendritic cells and macrophages in the lung after peritoneal injection and intratracheal administration. They induced dose-dependent IL-12 production within the lung, indicating proinflammatory Th1 response. These therapeutic effects show a potential use for acai polysaccharides in asthma and infectious disease. Kuang et al. (2011) studied the inhibitory effect of acidic polysaccharide from *Ephedra sinica* on DNFB-induced DTH in animal model in vivo. They showed that at a low dose of 50 mg/kg BW of ESP-B4 fraction ear tissue swelling and inflammatory cell infiltration were reduced, while in the high dose of 200 mg/kg BW tissue hypertrophy was reduced much obviously and a few inflammatory cell infiltration was presented. Furthermore, ESP-B4 in the higher dose decreased IL-2 and IL-4 serum levels and effectively suppressed $CD4^+/CD8^+$ ratio in serum of T-cell-mediated DTH mice. This shows immunosuppressive effects of studied polysaccharides and their potential use in the pathogenesis of T-cell-mediated autoimmune diseases and chronic inflammatory disorders. Liu et al. (2011) reported that *Astragalus* polysaccharides induced differentiation of splenic dendritic cells to IL-12-producing $CD11C^{high}$, $CD45RG^{low}$ dendritic cells, thus mediating activation of T-lymphocyte immune function and shifting of Th2 to Th1 response via IL-12. Therefore, *Astragalus* polysaccharides can regulate T-cell-mediated immunity by activation of dendritic cells, such as promoting cellular immunity and improving the immune functions in immunosuppression. Inngjerdingen et al. (2013) demonstrated that pectic fractions from *B. petersianum*, *Opilia celtidifolia*, and *Cola cordifolia* with complement fixation activity protected against *Streptococcus pneumoniae*

in a mouse pneumococcal infection model. This means that in vitro complement system activation led to an antibacterial protective effect in vivo. Kouakou et al. (2013) reported that polysaccharides from the leaves of *Clerodendrum splendens* exhibited beneficial effects in experimental autoimmune encephalomyelitis (EAE) in mice. One of the most efficient was pectic PS subfraction CSP-AU1 containing AG II. This subfraction-induced NO and cytokine production by human PBMC and monocyte/macrophages. CSP-AU1 treatment of animals with EAE (50 mg/kg BW, i.p., daily, 13 days) resulted in significantly reduced disease severity in EAE mice. Levels of IL-13, TNF-α, IFN-γ, IL-17, and GM-CSF were also significantly decreased, whereas TGF-β was increased in LN cells from CSP-AU1-treated EAE mice. The authors explained that CSP-AU1 stimulation of the innate immune system via TLR4 led to the induction of immunosuppression in EAE mice, which could be clinically beneficial effect of *Clerodendrum* polysaccharides on multiple sclerosis. Li et al. (2014b) purified RG II (KMPS-2E) from the roots of *Aconitum coreanum* that inhibited iNOS, TLR4, phospho-NF-*k*B–p65, phosphor-IKK, phosphor-IkB-α expressions, as well as the degradation of IkB-α and the gene expression of inflammatory cytokines, mediated by the NF-*k*B signal pathways in murine macrophages (50, 100, and 200 mg/mL). In rats, KMPS-2E (200 mg/kg BW) significantly inhibited carrageenan-induced paw edema as ibuprofen (200 mg/kg) within 3 h after a single oral dose. Polysaccharides are potent antiinflammatory constituents in *A. coreanum* roots and they have potential to aid inhibition of acute inflammation and also perspectives to be included in human diet for such purposes. Wang et al. (2015) obtained from the roots of *Panax quinquefolius* three acidic heteropolysaccharides with chemical characteristics of pectins and acidic hemicelluloses that upregulated macrophage production of NO, TNF-α, and IL-6 at 50 and 250 μg/mL in vitro.

Therapeutic potential of immunomodulating herbal polysaccharides could be also expanded to cardiovascular and liver diseases. Wang et al. (2014) showed that polysaccharides from selenium-enriched Ziyang green tea ZTPs displayed noteworthy scavenging effects against DPPH·, OH·, and O_2^-. The high in vitro antioxidant activity was further verified by suppressing CCl_4-induced oxidative liver damage in mice at 100, 200, and 400 mg/kg BW. Administration of ZTPs in mice prior to CCl_4 significantly prevented the CCl_4-induced increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactic dehydrogenase, as well as hepatic malondialdehyde (MDA) levels. Mice treated with ZTPs showed normal glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities, relative to CCl_4-treated group. Therapeutic potential of green tea polysaccharides could be related to hepatoprotective activity in preventing oxidative stress-related hepatotoxicity in liver. This study helps in the elucidation of therapeutic effects of the most consumed drink in the world, showing that polysaccharides are the essential functional ingredient of green tea, and its related nutraceutical products. Xie et al. (2015) extracted pectic polysaccharides (CASPs) from the roots of *Acanthioanax senticosus* that improved the symptoms of cerebral ischemia–reperfusion injury, and reduced the brain infarct volume and brain water content in Wistar male rats. In addition, CASPs elevated

SOD and GSH-Px activities and IL-10 levels, and reduced MDA, IL-1β, and TNF-α levels in the brain tissue of examined rats. The protective effect may be related to the improvement of CASPs on the antioxidant capacity of brain tissue and the inhibition of overproduction of inflammatory cytokines. Zhang et al. (2016) isolated a novel alkali-soluble acidic polysaccharide from Radix Polygoni Multiflori that exhibited significant immunomodulating potential by activating in vitro splenocytes and peritoneal macrophages. It also protected these immunocytes against immunosuppression induced by the anticancer drug 5-fluorouracil (5-FU). Rösch (2016) reported a novel approach, using mouse bone-marrow derived dendritic cells from TLR2/4 KO mice for evaluation of immunomodulatory properties of dietary fibers without interference from immunoactive LPS. They found that linear sugar beet pectic arabinan induced higher cytokine secretion than branched one. The authors concluded that pectic arabinans could modulate immune responses through C-type lectin receptors on immunocompetent cells, recognizing specific structural motifs.

3.2 Antitumor Activity Via Immune System Stimulation

Proposed therapeutic perspective of herbal polysaccharides, activating complement system and immune cells is expression of tumor-preventing, antitumor, and antimetastasis activities (Yamada and Kiyohara, 2007). For example, Mueller and Anderer (1990) showed that pectic RG, isolated from commercial *V. album* extract was able to enhance NK cytotoxicity of human PBMC by increasing formation of NK-cell/K562 tumor cell conjugation. The mechanism involves bridging of the NK and tumor cells by the active acetylated RG as follows: terminal acetylated Rha binds only to NK cells and on the other hand, terminal GalA binds only to tumor cells. Mistletoe was first proposed for treatment of cancer by Rudolph Steiner in 1920.

Similarly, nettle root extract is recommended for complaints associated with benign prostatic hyperplasia (BPH). The first clinical study was conducted in 1950 by Rückle, who showed that nettle root tea has a positive effect on patients with prostatic problems. More than 40,000 men suffering from BPH have been treated with various nettle root preparations. Usually, daily doses of 4–6 g as an infusion or 300–600 mg for 20% methanolic extract are applied in clinical studies. Twenty percent of methanolic extract has already proven its efficacy in BPH patients and is widely used in drug formulations (Chrubasik et al., 2007). Lichius et al. (1999) found that polysaccharide fraction POLY-M was one of the most effective constituents in 20% methanolic extract for inhibiting experimentally induced prostatic hyperplasia in mice. POLY-M was a mixture of glucans and pectic RG I (Wagner et al., 1994). Bazoton is a commercial drug on the basis of 20% methanolic extract from nettle root that is applied to treat prostate cancer and it contains about 1.7% polysaccharides. It is tested for acute toxicity, mutagenic and carcinogenic effects, as LD_{50} value for oral administration of Bazoton is greater than 30 g/kg in rats (Chrubasik et al., 2007). For comparison, in our two-stage screening study for immunomodulating acidic polysaccharides from 34 traditional European

medicinal plants, we found that crude nettle root polysaccharides expressed the most potent activity. Isolated rich in AG II pectic fraction was the most active constituent from the mixture against Peyer's patch cells (Georgiev et al., unpublished results). Neutral and pectic polysaccharides also stimulated ex vivo spontaneous ROS production in human neutrophils and in vitro NO production in murine macrophages (Georgiev et al., unpublished results).

Furthermore, it was found that commercial enzymatically or chemically produced modified citrus pectin (MCP) exhibits significant therapeutic effects on tumor metastasis and apoptosis in experimental animal models and in clinical trials (Eliaz et al., 2006). Monosaccharide and glycosidic linkage composition, defined molecular weight (10–20 kDa) and low degree of esterification (<10%) are key parameters for MCP to be able to enter blood circulation, and to bind cellular receptors on immunocompetent and tumor cells. It is considered that β-D-galactans in MCP are responsible for the activity (Morris et al., 2013). Beta-(1→4)-D-galactans interact with galectin-3 receptors on tumor cells through β-D-Gal*p* at O-3 and Arg144 from the receptor (Dumic et al., 2006). Generally, binding between pectic galactans and AGs to galectin-3 blocks tumor cell development, adhesion, and angiogenesis (Morris et al., 2013). In clinical trials with humans MCP PectaSol-C is administrated orally 3 times per day at a single dose of 5 g. The same dosage is recommended 1 week before surgery and between 2 and 4 weeks after the operation. Between 3 and 5 g/day are recommended for preventive therapy, but this is not clinically proved. MCP has a GRAS status and observed side effects in humans are loose stool and gastrointestinal discomfort (Eliaz et al., 2006). Interestingly, Courts (2013) found that unlike polyuronides, short-chain galactans and AGs derived from MCP were absorbed through an immobilized on porous membrane monolayer of tumor cells, inducing colon cancer from tumor cell line caco-2 (HTB-37). According to Niture and Refai (2013), 50 independent studies have demonstrated that MCP interacts with galectin-3 and inhibits tumor growth or cancer metastasis in vivo and in vitro in various types of cancers, including prostate, colon, breast, melanoma, and multiple myeloma. Currently, a clinical trial in Israel with duration 2013–17, entitled "Effect of MCP on PSA kinetics in biochemical relapsed PC with serial increases in PSA" is conducted with prostate cancer patients. The study is performed with commercial MCP PectaSol-C in a daily dose of 4.8 g in 6 capsules, 3 times per day.

Mistletoe, nettle root extracts, and MCP are classical examples for therapeutic efficacy of supplementation with polysaccharides. The next section presents some recent studies on perspective acidic herbal polysaccharides and their detailed therapeutic effects, examined predominantly in animal models.

Cao et al. (2010) demonstrated that treatment of S180 tumor-bearing mice with cyclophosphamid Cy (20 mg/kg/day), APS-3b, and APS-3c polysaccharide fractions from the roots of *A. sinensis* (100 mg/kg/day) increased their survival from 15.63 ± 0.68 days for the control to 19.38 ± 1.08, 22.75 ± 1.68, and 19.63 ± 1.12 days, respectively. Undoubtedly,

polysaccharides expressed lower activity than Cy, but appetite, activity, and coat luster of each animal in polysaccharide-treated groups were better. Additionally, polysaccharides increased the relative spleen and thymus weights. Polysaccharides stimulated NO production in peritoneal macrophages in vitro. The authors concluded that in vivo antitumor activity of APS-3b and APS-3c might be based on their T-lymphocyte activation via Th1 response and macrophage stimulation, rather than a direct cytocidal effect on the tumor cells. Lee et al. (2012) reported that an acidic *A. sinensis* polysaccharide possessed hematopoietic-enhancing and myeloprotective effects on human $CD34^+$ stem cells. They stated that it might be applied as a health-promoting agent for anemia patients and cancer patients under chemoradiation treatment. Xin et al. (2012) isolated two antitumor acidic polysaccharide fractions PTPa and PTPb (*Polygala tenuifolia*) with IC_{50} values of expressed cytotoxic activity against human lung adenocarcinoma cell line (A549) 47.8 and 41.2 µg/mL, respectively. For comparison, IC_{50} values for inhibition of human breast carcinoma (MCF7) and colon carcinoma (HT-29) were over 65 µg/mL for both polysaccharides. After daily i.p. administration of PTPa or PTPb to A549 tumor-bearing mice, the tumor volumes were statistically lower in polysaccharide-treated mice at low and high doses (50 and 100 mg/kg BW), compared with the control group. Treatment with two acidic polysaccharides caused an enhancement of SOD and catalase CAT activities in tumor-bearing mice and a reduction in thiobarbituric acid reactive substance (TBARS) level. The authors consider that it is reasonable to hypothesize that two polysaccharides prompt tumor regression at least partly through their antioxidative and radical scavenging properties. They found that initial PTP might express antitumor activity through mediation of GSH depletion and increase of intracellular ROS in cancer cells, thus resulting in apoptosis. In our in vitro study, we determined IC_{50} values for the cytotoxic activity of crude polysaccharide mixture from silver linden (flowers) linden PSC against A549 and HT-29 cell lines to 92.6 and 82.1 µg/mL (Georgiev et al., 2017a). At the same time linden polysaccharide fractions (free of LPS) obtained by ion-exchange chromatography stimulated in vitro NO production and corresponding iNOS expression in murine macrophages, and ex vivo ROS production in whole blood or isolated neutrophils at a concentration of 100 µg/mL (Georgiev et al., 2017b). Lin et al. (2013) isolated MDP-A1 and MDP-A2 acidic polysaccharide fractions with high GlcA content from the rhizoma of *Menispermum dauricum* that were able to inhibit cell proliferation of ovarian carcinoma SKOV3 cells. In addition, a significant reduction in tumor volume was observed in mice treated with doses of 25 and 50 mg/kg BW. Zhao et al. (2013) isolated antitumor acidic polysaccharide from *Portulaca oleracea* that inhibited in vitro HeLa cell proliferation and in vivo tumor growth (50–200 mg/kg BW) in U-14-bearing mice. The mechanism of antitumor activity was explained by sub-G1 phase cell cycle arrest, triggering DNA damage and inducing apoptosis. Similarly, in our in vitro study we obtained even higher cytotoxic effect of purslane polysaccharide mixture against HeLa cell line. Additionally, crude purslane polysaccharides purslane PSC stimulated human $CD4^+$ $CD25^+$ and $CD8^+$ $CD25^+$ T-cells and whole blood phagocytes ex vivo (Georgiev et al., 2017a).

Purslane is a widely distributed weed that is consumed in the Mediterranean and other countries and its polysaccharides are easily extracted with hot water in high yields and low content of LMWC. There is a perspective application of purslane polysaccharide-derived nutraceutical or food formulations for support of compromised immune status. Shu et al. (2015) found that supplementation (30 and 100 mg/kg BW) of Kunming mice bearing H22 ascitic hepatoma cells with a pectic polysaccharide (PSSC) from *Salvia chinensis* led to a decrease of tumor size and did not negatively influenced mouse body weight. Unlike cisplatin therapy (5 mg/kg BW) downregulated both parameters. Strong toxic impact of cisplatin on mouse immune system was shown by dramatic decrease of the amount of white blood cells in the blood, increased serum activities of AST and ALT, and serum levels of BUN and CRE. In parallel, polysaccharide-receiving mice did not show such changes. The polysaccharide elevated $CD4^+$ T-cell subpopulation in spleen and lymph nodes, increased cytotoxic activities of NK and $CD4^+$ T-cells, as all were compromised from the tumor implementation. Serum levels of IFN-$\gamma$and IL-2 increased and both IL-4 and 10 decreased by the polysaccharide administration. The authors concluded that suppressing tumor transplantation-induced PGE2 production was the key event mediating the antitumor immunostimulatory activity of the polysaccharide. The observed therapeutic effect initiates use of PSSC as a potential adjuvant reagent in the stimulation of an anticancer immune response in clinical HCC treatment.

3.3 Gastroprotective Effects Via Immune System Modulation

Still, oral administration of drugs remains the most convenient method for drug intake with the highest compliance rates among patients. Herbal polysaccharides from teas, folk remedies, functional drinks, and nutraceuticals pass easily through the GIT. That is why positive therapeutic effects of polysaccharides in the GIT are a direct result of their physicochemical nature and direct communication between their bioactive epitopes and immunocompetent cell receptors (Figs. 10.4A and B). Polysaccharides can be useful in all GID and corresponding diseases, such as inflammatory bowel disease (IBD). About 1.4 million people in the USA and 1 million in the EU suffer from IBD and the number of cases increases every year. Infiltration of inflammatory cells in the intestinal mucosa, disturbance of intestinal epithelium homeostasis, and abnormal production of proinflammatory and antiinflammatory cytokines lead to this inflammation. Crohn's disease and ulcerative colitis are examples of IBD that could lead to anemia, malnutrition, salt imbalance, and higher risk of colorectal cancer development (Hur et al., 2012). Polysaccharides are natural compounds that have already proven their efficacy in treatment and prevention of GID and could be used as part of the supplementation therapy and functional nutrition. Gastroprotective action of acidic polysaccharides is simply expressed by their antiulcer effect associated with protective adhesion to mucosal membranes, inhibition of hydrochloric acid, and pepsin secretion, and by their protective scavenging effect (Yamada, 1996). Sakurai et al. (1998) characterized with enzymes the antigenic epitopes in antiulcer pectic polysaccharides from *B. falcatum*.

They suggested that 6-linked galactosyl chains containing terminal GlcA or 4-O-Me-GlcA, attached to (1→3)-β-D-galactosyl chains, are important sugar residues in the antigenic epitopes of the ramified region of bupleuran 2IIc. Furthermore, acidic polysaccharides could exhibit a prebiotic effect, which influences intestinal microflora that has a vital role for IBD pathology (Xu et al., 2013). Thus, pectins and glucuronoarabinoxylans could be useful for prevention and treatment of IBD.

Cipriani et al. (2008) isolated two acidic heteroxylans with antiulcer activity from *Maytenus ilicifolia* and *Phyllanthus niruri* that are traditionally used for preparation of medicinal pills and teas for treating and preventing gastric ulcers. The polysaccharides reduced gastric lesions, induced by ethanol in Wistar rats by 65% and 78%, with ED_{50} = 40.0 and 20.4 mg/kg, each respectively. Later, Cipriani et al. (2009) demonstrated that polygalacturonic acid from the leaves of *M. ilicifolia* inhibited ethanol-induced gastric lesions in rats, with an ED_{50} of 103 mg/kg BW, suggesting that it had a protective antiulcer effect. Baek et al. (2010) showed that ginseng pectic polysaccharides expressed antirotavirus effect against human rotavirus HRV-Wa in an in vitro model with MA-104 cells. The authors stated that HG itself did not show an antirotavirus effect, so hairy regions might be the functional sites for expressed activity. These findings suggest that ginseng water extracts or polysaccharides are viable for prevention in rotavirus-induced diarrheal disease. Tanaka et al. (2010) demonstrated that RG with AG I side chains from the stems of cactus *Cereus peruvianus* significantly inhibited ethanol-induced gastric lesions in rats at an ED_{50} of 49 mg/kg BW. The cactus polysaccharide showed comparable activity with the positive control omeprazole (40 mg/kg BW, p.o.), but at a lower concentration of 30 mg/kg, revealing its potential as a phytotherapic drug. Austarheim et al. (2012b) reported antiulcer pectic polysaccharides from the leaves and bark of Malian plant *C. cordifolia*. The oral administration of 50 and 200 mg/kg BW of CCbark50 and CCleaf50 fractions 1 h before administration of 90% EtOH significantly inhibited ulcer formation in rats in a dose-dependent manner. Additionally, CCbark50 expressed a high complement fixation activity in vitro. Nascimento et al. (2013) showed that a pectic polysaccharide from the leaves of *Acmella oleracea* significantly inhibited ethanol-induced gastric ulcers in rats with an ED_{50} of 1.5 mg/kg BW. The authors mentioned that *A. oleracea*, locally known as jambu, is widely used in Legal Amazon in local dishes and in folk medicine. Simas-Tosin et al. (2014) reported that a glucuronoarabinoxylan (CNAL) from *Cocos nucifera* gum exudates reduced gastric hemorrhagic lesions, when orally administered (1 and 3 mg/kg BW) to rats prior to ethanol administration. The positive control treated with omeprazole (40 mg/kg BW, p.o.), also inhibited the gastric lesion area by 67%, which was similar to the inhibition observed for the dose of 1 mg/kg BW of CNAL. GAXs are around 80% of the gum exudate from coconut palm weight and are perspective candidates from technological and therapeutic point of view. Yang et al. (2014) found that polysaccharide fraction APS from *Astragalus membranaceus* (200 mg/kg BW) and dexamethasone (0.3 mg/kg BW) significantly downregulated TNF-α and IL-1β expressions,

and upregulated NFATc4 mRNA and protein expression in TNBS-induced experimental colitis in rats. Li et al. (2014a) demonstrated that purslane polysaccharides at doses 200, 400, and 800 mg/kg BW increased body weight, peripheral white blood cells, thymus, and spleen indexes, splenocyte proliferation and serum cytokines like IL-2, IL-4, and TNF-α in tumor-bearing rats. Furthermore, treatment with polysaccharides provided a dose-dependent protection against gastric cancer-induced oxidative injury by enhancing SOD, CAT, GSH-Px activities in rats. Thöle et al. (2015) revealed that acetylated RGs I from immature fruits of okra *Abelmoschus esculentus* exhibited antiadhesive activity against *Helicobacter pylori* to human gastric epithelial AGS cells in vitro. HBSS fraction inhibited more than 60% adhesion of three clinical strains (P12, 26695, TN-2) after preincubation at 1.0 mg/mL. They proved that methyl and acetyl esters were very important for the expressed antiadhesive activity. The inhibition mechanism was demonstrated by interaction of okra polysaccharides with several bacterial adhesins. Scientists concluded that okra water extract and isolated pectins might be interesting for development of antiadhesive products for establishment of preventive strategies against stomach infections caused by *H. pylori*. These products could be directly applied for prevention of early infections in children and during antibiotic eradication therapy. Harsha et al. (2016) demonstrated that pectic polysaccharide fraction MTrPP from *Curcuma longa* showed potent anti-*H. pylori* activity with IC_{50} value of 44 µg/mL, which was comparable to that of amoxicillin (26 µg/mL). The same fraction expressed anti-*H. pylori*-mucoadhesive property with IC_{50} of 36 µg/mL. MTrPP inhibited ulcer scores in a stress-induced gastric model in rats with rate up to 84% at 200 mg/kg BW, which was on par with the protection offered by lansoprazole (86%) at 50 mg/kg BW. The stomachs of rats pretreated orally with MTrPP significantly attenuated the process of ulceration. MTrPP could recover and prevent gastric mucin damage up to 87%, furthermore nearly 78% of increased H^+,K^+,-ATPase activity was normalized by its administration in ulcerative rats. Additionally, polysaccharide normalized TBARS, SOD, GSH-Px, and CAT levels. There were not any toxic effects observed. The preventive therapeutic effect of pectic polysaccharides on gastric ulcers was expressed by inhibition of excess acid production, maintenance of the gastric mucus layer, modulation of antioxidant mechanisms, and inhibitions of *H. pylori* growth and adherence.

3.3.1 Prebiotic effect

Immunomodulating acidic polysaccharides often possess prebiotic properties on the essential for gastrointestinal health and for the maintenance of human homeostasis lactobacilli and bifidobacteria (Aachary and Prapulla, 2011; Hartog et al., 2015). Intestinal microflora metabolizes polysaccharides to SCFA like acetic, propionic and butyric acids. SCFA play an important role in maintaining the function of epithelium barrier, regulation of epithelium proliferation, modulation of immune responses and prevention from colorectal cancer (Xu et al., 2013). For example, IBD patients have lower levels of SCFA, which is a result from the change in the comensal bacterial populations presented by increased number of proteobacteria, actinobacteria, and decreased populations of Firmicutes and Bacteroidetes.

IBD patients suffer from lack of bifidobacteria as well. Normalized dietary fiber-rich diet exhibits potential preventive effects against GID. Plant polysaccharides are assimilated to different extent from residential microflora, as increasing the complexity leads to more difficult degradation and lower SCFA production. Generally, amylopectin, β-glucans, and inulins are much better prebiotics than pectins. It is very important to reveal the relation between the structural features of different polysaccharides and the selective growth of microbial populations (Xu et al., 2013). On therapeutic point of view, this is important for development of polysaccharide formulations for manipulation of microbial flora and production of specific bioactive metabolites.

Yang et al. (2012) demonstrated that an aqueous extract (SAEAF) and pectic AG II (AFP) from *Anoectochilus formosanus* expressed prebiotic activity in vitro and in vivo. SAEAF exhibited prebiotic effects, including a decrease in cecum pH and increase of calcium absorption and fecal bifidobacteria after 4-week oral administration of 200 and 400 mg/kg BW in growing rats. Ethanol-precipitated AFCP showed effective promotion of *B. breve* growth. AFCP treated with enzymes to remove starch content provided AFP, which markedly enhanced *B. breve* proliferation. Mice receiving an AFP dosage of 20 mg/kg BW produced an increased number of *Bifidobacterium* colonies in the stool on day 3. In RT-PCR analysis of *Bifidobacterium*, AFP increased the expression of β-galactosidase, α-L-arabinofuranosidase and ABC transporter related to nutrient uptake. Compared to the same dose of inulin, AFP presented a higher prebiotic index (PI). Min et al. (2014) found that colon index in Kunming mice supplemented with 0.15, 0.3, and 0.6 mg/g BW crude acidic polysaccharide from *Cyclocarya paliurus* leaves significantly increased, as the administration time increased and the length of colon in the polysaccharide group was longer than the control. The pH values in caecum, colon, and feces of the polysaccharide group were significantly reduced. Furthermore, the levels of SCFA in caecum, colon and feces of mice fed with 0.6 mg/g BW significantly increased. Khodaei et al. (2016) found that enzyme-released galactooligosaccharides and oligomers from potato RG I were fermented by *Bifidobacterium* and *Lactobacillus* species with higher selectivity than the initial polymer. Potato RG I and its corresponding oligosaccharides/oligomers did not have a negative effect on Bacteroidetes and seem to reduce the population of the *C. leptum*. RG I-based oligomers and galactooligosaccharides exhibited similar PI to FOS. Total amounts of SCFAs, generated upon the fermentation of RG I oligomers and galactooligosaccharides were higher than those obtained with RG I and FOS. Therefore, complex acidic polysaccharides are difficult for microbial degradation but their enzyme-derived oligosaccharides are perspective prebiotics. Similarly to pectic oligosaccharides, hemicellulosic xylooligosaccharides, and arabinoxylan oligosaccharides show promising results (Aachary and Prapulla, 2011; Neyrinck et al., 2012).

Table 10.1 summarizes the results on in vivo experiments for evaluation of perspective therapeutic effects of herbal acidic polysaccharide presented earlier.

Table 10.1: In vivo evaluation of perspective therapeutic effects of herbal acidic heteropolysaccharides.

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
In vivo combinatorial effects of immunomodulation with herbal polysaccharides				
A. porrum, aerial part	Water- and acid-extractable pectic polysaccharides	2 mg/kg BW (i.p.)	Increased survival rate of *S. enteritidis* infected mice.	Kratchanova et al. (2010b)
L. lucidus, leaves	Water-extractable pectic polysaccharides	50, 100, and 300 mg/kg BW (p.o.)	Enhanced phagocytosis capacity, serum hemolysin level, and DTH in response to antigen SRBC; increase in the indices of thymus and spleen organs.	Yang et al. (2010)
Acai fruit	Water-extractable pectic polysaccharides	5 and 50 µg (i.p.); 1.56–500 µg (i.t.)	Neutrophil, macrophage, and dendritic cell activation; proinflammatory Th1 response in lungs.	Holderness et al. (2011)
E. sinica, stems	Water-extractable acidic polysaccharide	50 and 2000 mg/kg BW (p.o.)	Immunosuppressive effects on DNFB-induced DTH in mice.	Kuang et al. (2011)
P. ginseng, leaves	Water-extractable Rhamnogalacturonan II	20 mg/kg BW (i.p.)	Suppressed asthmatic reaction in an ovalbumin-induced murine model of asthma.	Jung et al. (2012)
B. petersianum; aerial parts; *O. celtidifolia*, leaves; and *C. cordifolia*, bark	Water-extractable pectic polysaccharides	125 and 25 µg (i.p.)	Protective effect in *S. pneumonia* infected mice.	Inngjerdingen et al. (2013)
C. splendens, leaves	Water-extractable pectic polysaccharides	50 mg/kg BW (i.p.)	Significant reduction of disease severity in EAE mice.	Kouakou et al. (2013)
A. coreanum, roots	Water-soluble rhamnogalacturonan II	200 mg/kg BW (p.o.)	Significantly inhibited carrageenan-induced paw edema in rats.	Li et al. (2014b)
Ziyang green tea	Water-extractable acidic polysaccharides	100, 200, and 400 mg/kg BW (p.o.)	Protective effect on the CCl_4-induced oxidative damage in mice.	Wang et al. (2014)
A. senticosus, roots	Water-extractable pectic polysaccharides	50, 100, and 200 mg/kg BW (i.g.)	Protective effects in cerebral ischemia-reperfusion injury in rats.	Xie et al. (2015)
Antitumor activity via immune system stimulation				
Nettle root	Water infusion or 20% methanolic extract, containing glucans and pectic rhamnogalacturonans	4–6 g infusion or 300–600 mg, 20% methanol extract	Effective in benign prostatic hyperplasia.	Chrubasik et al. (2007)

Citrus fruit	Modified citrus pectin (PectaSol-C)	4.8 g (p.o.)	Determination of effectiveness in improvement of PSA kinetics in men with biochemical relapsed prostate cancer and serial increases in PSA levels men with prostate cancer. Documentation of side effects.	Running clinical trial 2013–17, Institute of Oncology, Meir Medical Center, Kfar-Saba, Israel
A. sinensis, roots	Water-extractable acidic polysaccharides	100 mg/kg BW (i.p.)	Antitumor activity might be based on their T-lymphocyte activation via Th1 response and macrophage stimulation in S180 tumor-bearing mice.	Cao et al. (2010)
P. tenuifolia, roots	Acidic polysaccharides	50 and 100 mg/kg BW (i.p.)	Might express tumor regression at least partly through their antioxidative and radical scavenging properties in A549 tumor-bearing mice.	Xin et al. (2012)
M. dauricum, rhizoma	Water-extractable GlcA-rich polysaccharides	25 and 50 mg/kg BW (i.p.)	Significant reduction in tumor volume in SKOV3 carcinoma-bearing mice.	Lin et al. (2013)
P. oleracea, aerial part	Water-extractable acidic and neutral polysaccharides	50–200 mg/kg BW (p.o.)	Antitumor activity was explained by sub-G1 phase cell cycle arrest, triggering DNA damage, and inducing apoptosis in U-14-bearing mice.	Zhao et al. (2013)
S. chinensis, aerial part	Water-extractable pectic polysaccharide	30 and 100 mg/kg BW (i.p.)	Antitumor activity through augmentation the cytotoxic activity of NK and $CD8^+$ T cells and activity of $CD4^+$ T cells in mice bearing H22 ascitic hepatoma cells.	Shu et al. (2015)
Gastroprotective effects via immune system modulation				
M. ilicifolia, leaves and *P. niruri*, whole plant	Alkali-extractable acidic heteroxylans	ED_{50} = 40.0 and 20.4 mg/kg BW (p.o.)	Reduction of ethanol-induced gastric lesions in rats.	Cipriani et al. (2008)
M. ilicifolia, leaves	Water-extractable polygalacturonic acid	ED_{50} of 103 mg/kg BW (p.o.)	Protective antiulcer effect in ethanol-induced gastric lesions in rats.	Cipriani et al. (2009)
C. peruvianus, stems	Water-extractable rhamnogalacturonan I	ED_{50} of 49 mg/kg BW (p.o.)	Significantly inhibited ethanol-induced gastric lesions in rats.	Tanaka et al. (2010)
C. cordifolia, bark, leaves	Water-extractable pectic polysaccharides	50 and 200 mg/kg BW (p.o.)	Significantly inhibited ulcer formation in treated with 90% ethanol rats.	Austarheim et al. (2012a,b)

(*Continued*)

Table 10.1: In vivo evaluation of perspective therapeutic effects of herbal acidic heteropolysaccharides. (*cont.*)

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
A. oleracea, leaves	Water-extractable pectic polysaccharide	ED_{50} of 1.5 mg/kg (p.o.)	Significantly inhibited ethanol-induced gastric ulcers in rats.	Nascimento et al. (2013)
C. nucifera, gum exudates	Alkali-extractable glucuronoarabinoxylan	1 and 3 mg/kg BW (p.o.)	Reducing gastric hemorrhagic lesions in rats.	Simas-Tosin et al. (2014)
A. membranaceus	Polysaccharide mixture	200 mg/kg BW (p.o.)	Significantly downregulated TNF-α and IL-1β expressions, and upregulated NFATc4 mRNA and protein expression in TNBS-induced experimental colitis in rats.	Yang et al. (2014)
P. oleracea, aerial part	Water-extractable polysaccharides mixture	200, 400, and 800 mg/kg BW (p.o.)	Antioxidant and immune-enhancing activities against experimentally induced gastric cancer in rats.	Li et al. (2014a)
C. paliurus, leaves	Water-extractable acidic polysaccharide	0.15, 0.3, and 0.6 mg/g BW (p.o.)	Increased colon index, lowering pH, improving faecal moisture, and increasing SCFAs level in mice.	Min et al. (2014)
C. longa, roots	Ammonium oxalate-extractable pectic polysaccharide	200 mg/kg BW (p.o.)	Inhibition of excess acid production, maintenance of gastric mucus layer, modulation of antioxidant mechanisms in gastric ulcer rats, and in vitro inhibitions of *H. pylori* growth and adherence.	Harsha et al. (2016)
A. formosanus	Water-extractable pectic arabinogalactan II	20 mg/kg BW (p.o.)	Produced an increased number of *Bifidobacterium* colonies in the mice stool on day 3 and in vitro increased expression of their ABC transporter related to nutrient uptake.	Yang et al. (2012)

3.3.2 Unique target-specific drug delivery

Herbal polysaccharides expressing good jellifying properties could be used as target-specific carriers of different drugs in GIT, which is a promising application in drug formulations. For instance, Perera et al. (2010) tested in vitro novel pectin–4-aminothiophenol conjugate microparticles for colon-specific drug delivery of metronidazole that is used in various colonic diseases like Crohn's disease, amoebiasis, and diverticulitis. A 34.4-fold increased amount of metronidazole was retarded within Pec–ATP microparticles when they were exposed to simulated conditions of the small intestine. A rapid release of metronidazole from Pec–ATP microparticles was demonstrated by microbial pectinase degradation. Das et al. (2011) reported an optimized Zn-pectin-chitosan formulation for colonic-specific delivery of resveratrol in an in vivo rat model. They suggested that this optimized composite could be useful for further in vivo colitis or colorectal cancer disease studies. Elyagoby et al. (2013) developed zinc pectinate pellets for colon-specific delivery of the anticancer drug 5-FU. They showed in a rat model that less than 25% of the drug was released at the upper GIT but the majority of it was released upon prolonged dissolution and in response to colonic microbial pectinase. In vivo analysis indicated that a remarkably larger fraction of the drug was available in the colon when 5-FU was delivered by means of intracapsular coated pellets (322.18 ± 23.64 μg) instead of free drug (9.63 ± 4.10 μg). Furthermore, the colon-specific delivery of 5-FU could render a lower dose needed than that by injection route, which clearly shows feasibility of pectin in drug delivery systems. Villanova et al. (2015) reported chemically modified pectin with acrylic monomers for potential use as a mucoadhesive excipient for mucoadhesive drug delivery systems.

3.4 Perspective Therapeutic Effects of Polysaccharide Complexes

As mentioned earlier, acidic polysaccharides often present as bioactive complexes and glycoconjugates in herbal drinks, extracts and folk remedies. Interestingly, this kind of coupling with polyphenols, lignin, proteins, and minerals leads to formation of new immunoactive agents. In Table 10.2 results are summarized on in vivo experiments for evaluation of perspective therapeutic effects of polysaccharide complexes presented in the next section.

3.4.1 Polysaccharide-lignin conjugates

Kiyohara et al. (2000) demonstrated that water-soluble pectic LCC were involved in the in vitro and in vivo intestinal immune system modulation through murine Peyer's patch-mediated bone marrow cell-proliferation enhancing activity by the Kampo medicine formulation Juzentaihoto (Shi-Quan-Da-Bu-Tang in Chinese, TJ-48). Bone-marrow cell proliferation was used to estimate the relative production of hemopoietic growth factors from Peyer's patch cells. Later, the Japanese scientists found that similarly LCC were responsible for Peyer's patch-mediated bone marrow cell-proliferation enhancing activity of Hochuekkito (Bu-Zhong-Yi-Qi-Tang

Table 10.2: In vivo evaluation of perspective therapeutic effects of acidic herbal polysaccharide complexes.

Medicinal Plants	Polysaccharide	Treatment (Daily Dose)	Effects	References
Polysaccharide-lignin conjugates				
Kampo medicine formulation TJ-48, Tsumura & Co. (Tokyo, Japan)	Water-extractable pectic-polysaccharide-lignin	150 mg/kg BW (p.o.)	(1) Ex vivo murine Peyer's patch-mediated bone marrow cell-proliferation enhancing activity.	Kiyohara et al. (2000)
Kampo medicine formulation TJ-41, Tsumura & Co. (Tokyo, Japan)	Water-extractable pectic-polysaccharide-lignin	7.7 mg/kg BW (p.o.)	(1) +Enhanced influenza virus specific IgG antibody titer in sera of infected young mice.	Kiyohara et al. (2011)
Pinus densiflora, cones	Alkali-extractable LCC extract	1 mg tablets with vit. C or solution (p.o.)	Promoted faster healing, reduction of symptoms, and reducing the frequency of recurrence episodes in HSV-1 infection in patients.	Sakagami et al. (2010)
E. canadensis, flowering parts	Alkali-extractable pectic polyphenolic-polysaccharide conjugate	7–9 IU/mg of heparin standard (229.5 IU/mg)	Anticoagulant activity in rats.	Pawlaczyk et al. (2011)
M. chamomilla, flowering parts	Alkali-extractable polyphenolic-pectic polysaccharide conjugate	100 µg/mL	Ex vivo reduced platelet aggregation in platelet-rich plasma obtained from patients with cardiovascular disorders.	Bijak et al. (2013)
Polysaccharide-protein conjugates				
C. sinensis, low-grade green tea	Water- and alkali-extractable acidic polysaccharide-protein conjugates	150 mg/kg BW (p.o.)	Suppressed spontaneous diabetes mellitus in NOD mice.	Chen et al. (2010)
L. barbarum, fruit	Water-extractable polysaccharide-protein complex	100 µg/mL (i.p.)	Immunostimulatory activity predominantly on macrophages through CD86 and MHC-II expressions; induction of TNF-α and IL-1β production in macrophages.	Zhang et al. (2011)
S. canadensis, flowers	Alkali-extractable acidic polyphenolic–polysaccharide–protein	75 mg/kg BW (p.o.)	Antitussive activity in citric acid-induced coughs in guinea pigs.	Šutovská et al. (2013)
Polysaccharide-mineral complexes				
Se-enriched Ziyang green tea, Tea Industry Co., Ltd.	Water-extractable Se-polysaccharide complex	100, 200, and 400 mg/kg BW (p.o.)	Tumor regression in U-2 OS cancer xenograft model in BALB/c athymic mice.	Wang et al. (2013)
A. macrocephala	Selenylated polysaccharide	1 mg (i.m.)	Selenylation significantly increased immune-enhancing activity in chickens.	Liu et al. (2015a)

in Chinese, TJ-41). They obtained statistically significant positive correlation between enhancing activities of purified glycoconjugates against Peyer's patch immunocompetent cells and epithelial cells. TJ-41 formulation and its lignin-polysaccharide complex F-5 (7.7 mg/kg BW) succeeded to enhance influenza virus specific IgG antibody titer in sera of young mice (Kiyohara et al., 2011). In both studies, polysaccharides and lignin were important for the activity. The authors explained that TJ-48 and TJ-41 are clinically used in Japan to potentiate compromised immune status of patients with chronic diseases and after surgery. TJ-48 is useful formulation against tumor diseases and TJ-41 for respiratory diseases. Further, Jung et al. (2012) demonstrated that RG-II from *P. ginseng*, which is one of the constituents of TJ-41, suppressed asthmatic reaction in an ovalbumin-induced murine model of asthma. RG II reduced IL-4 production but increased INF-γproduction, and inhibited GATA-3 gene expression. RG-II also inhibited asthmatic reactions including an increase in the number of eosinophils in bronchoalveolar lavage fluid, an increase in inflammatory cell infiltration in lung tissues, airway luminal narrowing, and airway hyperresponsiveness. Sakagami et al. (2010) reviewed their studies on pharmacologically active LCC from pine and other sources, as summarized that they expressed antiviral, antimicrobial, antiparasite, antitumor, antimutagenic, and immunomodulating activities. They emphasized that phenylpropenoid polymer moiety of LCC has potent antiviral activity, whereas carbohydrate moiety has immunopotentiation activity, possibly via activation of TLR pathway and chemokine expression. They proved by radiolabeling that pine LCC was partly absorbed in the intestine and reached to blood and liver in experimental mice. A clinical trial with 48 patients, where evaluating anti-HSV-1 activity of a synergistic formulation between pine cone LCC and vitamin C was carried out, suggesting its possible applicability for the prevention and treatment of HSV-1 infection. Interestingly, Saluk-Juszczak et al. (2010) found that acidic polyphenolic-polysaccharide conjugates from six medicinal plants from Asteraceae family possessed antioxidative properties and protected ex vivo human platelet proteins against peroxynitrite toxicity. Pawlaczyk et al. (2011) reported a pectic polyphenolic-polysaccharide conjugate from *Erigeron canadensis* with in vivo anticoagulant and ex vivo antiplatelet activities. The most active anticoagulant fraction showed activity at 50 μg/mL, corresponding to 7–9 IU/mg of heparin standard (229.5 IU/mg), measured in a PTT test. The authors concluded that although this plant preparation had much lower anticoagulant activity than heparin, its antiplatelet activity might be an additional advantage in becoming an adjunctive agent in treatment like of deep vein thrombosis, especially of patients with some resistances on acetylsalicylic acid derivatives drugs. Bijak et al. (2013) showed that a pectic polyphenolic-polysaccharide conjugate from *Matricaria chamomilla* reduced platelet aggregation at a concentration of 100 μg/mL, induced by multiple agonists in platelet-rich plasma obtained from healthy donors and patients with cardiovascular disorders. Glycoconjugate did not show any in vitro cytotoxicity effects under the studied conditions. Blood platelets are the critical mediators of haemostasis and blood coagulation. They are implicated in the metastatic process of tumor cells, and in the pathogenesis of infection diseases. Anticoagulant activity of herbal polysaccharide conjugates expands their application potential.

Additionally, in our two-stage screening for immunomodulating polysaccharides from 34 traditional European medicinal plants through in vitro anticomplementary test and ex vivo Peyer's patch cell activation, we found that perspective complement immunomodulators were polysaccharides and polysaccharide-containing complexes from nettle (root and leaves), elecampane, thyme, purslane, soapwort, red oregano, bulhead (fruit), lady's mantle, lavender, verbena, linden, etc. (Georgiev et al., unpublished results). Polysaccharides are present in herbal teas and commercial water extracts, and must be partly responsible for their immunomodulating properties. Some polysaccharide complexes were rich in polyphenols and showed high antioxidant activity through ORAC and HORAC methods. The polysaccharide complexes from thyme, nettle leaves, red oregano, lavender, and tormentil were examples with high immunomodulating and antioxidant activities. The polysaccharide complexes contained not only polysaccharides and polyphenols. In our recent study on crude polysaccharides from linden, lavender, and purslane, we found that aside polysaccharides, polyphenols (phenolic acids, phenylpropanoids, etc.), saturated and unsaturated fatty acids, and higher paraffins presented in different amounts in the three samples on the basis of GC-MS and HPLC-UV analyses (Georgiev et al., unpublished results). The polysaccharides were responsible for expression of high anticomplementary activity through the classical and alternative pathways and polyphenols through the classical pathway. Further, rich in saturated fatty acids and hydrocarbons petroleum ether extracts showed weak activity. Linden butanolic extract expressed much lower anticomplementary activity through the classical pathway in comparison to lavender butanolic extract. It even showed antagonistic effect in combining with active extracts that was further explained by its high fatty acid content. After combination of SEC on Sephacryl S-200 and solid-phase extraction on Amberlite XAD7HP, we obtained potent chPS-L1 (free of LPS) pectic and chPP-L polyphenolic fractions that expressed IC_{50} values of 38.9 and 539.4 μg/mL or 94.0 and 86.9 μg/mL through the alternative or classical pathway, respectively. Thus, polysaccharides and polyphenols showed high anticomplementary activity. Their combined action deserves more scientific attention and possesses potential for further development of multifunctional nutraceuticals. Polysaccharides could serve as transporters and at the same time as protectors of polyphenols through the GIT to the colon, where they may express their antioxidant and different physiological effects (Jakobek, 2015). Polysaccharides and polyphenols could express protective activity against GIT inflammation and related disorders like IBD and colorectal cancer (Denev et al., 2014), and both positively influence lipid metabolism and neutralization of exogenous and endogenous toxicants in the human body (Groudeva et al., 1997; Stantschev et al., 1979; Yaneva et al., 2002). We have demonstrated that the addition of rosehip extract to chokeberry, blackberry, blackcurrants, or elderflower extracts led to a synergistic effect in ORAC antioxidant activity of the mixed extracts that reached up to 64%. The removing of rosehip polysaccharides inhibited the synergistic effect, indicating that rosehip pectic polysaccharides were at least partially involved in the observed synergism (Kratchanova et al., 2014). Herbal polysaccharides with considerable antioxidant and antiinflammation capacities have been shown to be beneficial in aging and age-related

neurodegenerative diseases (Li et al., 2014c). Polysaccharide-polyphenol complexes influence organoleptic qualities of foods and drinks as polysaccharides could prevent negative interactions between polyphenols and digestive enzymes (Jakobek, 2015). Practically, therapeutic combinatorial effect of polyphenols and polysaccharides has a great perspective in treatment of socially important diseases of our century, including cardiovascular, tumor diseases, and diabetes (Daskalova et al., 2015; Tomova et al., 1999).

3.4.2 Polysaccharide-protein conjugates

There are numerous studies on immunomodulating polysaccharide-protein conjugates and their perspective therapeutic effects (Nie and Xie, 2011). Particularly, antidiabetic potential of such glycoconjugates is very promising. For example, Chen et al. (2010) showed that acidic polysaccharide-protein conjugates from low-grade green tea could suppress spontaneous diabetes mellitus in NOD mice. Oral administration of 150 mg/kg BW TPC-W fraction significantly decreased the level of blood glucose and antiglutamic acid decarboxylase (GAD) antibody level in NOD mice. Lee et al. (2011) isolated three lignin-carbohydrate-protein complexes from seeds of *Pimpinella anisum* with in vitro antiviral activity against HSV-1 and 2, human cytomelagovirus and measles virus and in vitro immunostimulating activity against murine macrophages. Complexes were found to interfere with virus adsorption to the host cell surface and directly inactivate viruses. Zhang et al. (2011) reported that after digestion of protein moiety from the polysaccharide-protein complex LBPF4 from *Lycium barbarum* was obtained LBPF4-OL that lost activation ability on T-lymphocytes. LBPF4-OL expressed immunostimulatory activity predominantly on macrophages through CD86 and MHC-II expressions in vivo. It induced TNF-α and IL-1β production in a dose-dependent manner in macrophages. Additionally, B-cell proliferation was induced in the presence of macrophages. Šutovská et al. (2013) found that acidic polyphenolic–polysaccharide–protein complex from the flowers of *Solidago canadensis* expressed in vivo antitussive activity in citric acid-induced coughs in guinea pigs. Antitussive effect of the highest dose 75 mg/kg BW was 10% lower in comparison with that of codeine and it was without any side toxicity. Wang et al. (2015) isolated two potential prebiotic acidic polysaccharide-protein complexes (RP1 and RP2) from rapeseed that contained uronic acids and sulfates. Proliferative potency of crude polysaccharide, RP1 and RP2, against bifidopbacteria was 41.93, 68.15, and 60.08% of that of FOS. Clearly, the bacteria supplemented with RP1 and RP2 produced large amounts of organic acids, showing that both fractions were metabolized to some extent. Rapeseed is currently oilseed crop in Europe and this study opens some utilization possibilities of its waste.

3.4.3 Polysaccharide-mineral complexes

Herbal polysaccharides form complexes with minerals that also could potentiate and expand their immunomodulating activities. Wang et al. (2013) studied tumoricidal effect of a selenium-polysaccharide complex from Ziyang green tea on human osteosarcoma U-2 OS cells in vitro and in vivo in an animal model. Se-polysaccharide complex contained 94.5%

total sugars, 2.1% uronic acids, and 2.14 μg/g Se. In U-2 OS cancer xenograft model in BALB/c athymic mice, Se-ZYTP oral administration at three doses (100, 200, and 400 mg/kg daily for 28 days) resulted in obvious tumor regression, as compared to model control. The authors postulated that Se-polysaccharide complex might have cancer-preventive and cancer-therapeutic benefit for human osteosarcoma. Liu et al. (2015a) reported that selenylation of *A. macrocephala* polysaccharide AMP led to higher immune-enhancing activity in vitro and in vivo. In the in vivo test 14-day old chickens after vaccination with ND vaccine were intramuscularly injected with 1 mg $sAMP_9$ and AMP in solution, as lymphocyte proliferation rate in $sAMP_9$ group (59.63%) was significantly higher than those in AMP group (29.79%). Additionally, IFN-γ, IL-2, and IL-6 serum levels in experimental chickens treated with sAMP were higher than these with AMP or control groups. That is why selenylation significantly increased immune-enhancing activity of studied polysaccharides.

4 Preparation of Immunomodulating Acidic Heteropolysaccharides and Their Complexes

Some herbal polysaccharides are perspective immunomodulating agents for modern phytotherapy, functional and dietetic nutrition, but their profitable industrial purification and preservation of the natural formulation are still an open issue. The main problems are the low yields, expensive and exhaustive isolation, and purification procedures from herbal matrices. However, heparin is a good example for polysaccharide with unique biological properties that initiated its production from complex animal tissues, even on a pharmaceutical grade (Bhaskar et al., 2012). On the other hand, there are plenty of cheap industrial herbal residues that are waiting for a proper utilization, as especially attractive are by-products from cosmetic industry (Slavov et al., 2013, 2016). Therefore, it is clear that the most critical point is discovery of effective know-how purification technologies to attract industrial interest. That is why different immunoactive tea polysaccharide-containing complexes called polysaccharide extracts from traditionally used plants in the popular herbal medicine are currently produced. Interestingly, Corrêa-Ferreira et al. (2014) reported that traditional infusions from *Artemisia absinthium* and *A. vulgaris* contained 5.8 and 7.0% crude polysaccharide mixtures or 58 and 98 mg in each 200 mL infusion prepared from 1.0 and 1.4 g herbal material, respectively. Up to now clinically applied in Japan and China are mushroom β-glucan extracts (lentinan and schizophillan, registered as drugs in Japan), and pectic polysaccharide-containing extracts from *A. membranaceus* and *Achyranthes bidentata* (He et al., 2012). They could be referred to tea-like products. Nevertheless, this preparation technique may put in suspicion the therapeutic effect of the complex mixture and opens toxicological and adverse effect concerns. At the same time there is a Chinese *Astragalus* polysaccharide product (Jirek Animal Pharmacy Co., Ltd.) for intravenous use in animal health care. Undoubtedly, hot water is one of the best solvents for extraction of bioactive pectic polysaccharides and sometimes acids, alkali, or chelating agents are added to hot water for increasing

polysaccharide extractability or for sequential extraction of pectic and hemicellulosic polysaccharides. It is obvious that the addition of chemicals in water leads to essential changes in the structure and biofunctionality of polysaccharide macromolecule in comparison to hot water itself. Polysaccharides are separated from water extracts by alcohol precipitation, further fractionated and purified by ion exchange, size exclusion, and sometimes by affinity chromatography on a laboratory scale. Continuous boiling with water is used in the Japanese Kampo medicine, where decoctions are prepared from mixture of carefully selected herbs for specific health disorders and multiorgan targets. During preparation complicated ingredient interactions appeared and vital combinatorial pharmacological effects are reached. Therefore, synergistic or antagonistic effects occur, which are believed to be expressed in the potentiation of pharmacological effect of a single herb or a compound (changed extractability), reduction of particular adverse effects, and modulation of drug transport and metabolism (Yamada, 2005). Generally, hot-water extraction leads to extraction or formation of complex mixtures of different bioactive macromolecular and LMWC-acidic and neutral (hetero)polysaccharides (pectic polysaccharides, glucuronoarabinoxylans) and their conjugates with lignin, proteins and minerals (B, Se), as well as interacted physicochemically phenolic acids, flavonoids (catechins, tannins), saponins (triterpenic acids), fatty acids, hydrocarbons, phytosterols, and so on. Another preparation method, more applicable for glucuronoarabinoxylans and LCC, includes extraction with acidic solutions and/or diluted alkali. Sonication and microwave heating are also used to aid extraction of bioactive polysaccharides (Chen et al., 2016; Ebringerová and Hromádková, 2010; Liu et al., 2016; Zhu et al., 2016). Particularly, in our previous study microwave pretreatment of fresh orange peels led to considerable increase in pectin yield and quality (Kratchanova et al., 2004).

5 Concluding Remarks

Traditional therapeutic effects of herbs, such as immune potentiation, antiinflammatory, gastroprotective, and detoxifying actions could be partly explained by the presence of acidic heteropolysaccharides. Bioactive herbal pectic polysaccharides are easily coextracted with hot water, as is demonstrated by the industrially applied Kampo medicine technique. Arabinogalactan side chains contribute to the high carbohydrate-specific bioactivity of the whole molecule of herbal polysaccharides (Figs. 10.1A and 10.4A–B). This chapter shows that herbal pectins adequately contribute to both immune activation and immune suppression, when it is needed for helping in recovery and maintenance of homeostasis in living cells and whole organism. They could be useful in the treatment of immune disturbances like infectious and tumor diseases as immune system activators and for chronic inflammatory disorders like IBD (i.e., ulcerative colitis) and autoimmune diseases as immune system suppressors and regulators of oxidative enzymes. On the other side, bioactive glucuronoarabinoxylans that are generally extracted with diluted alkali have the potential to be used for prevention and treatment of gastric lesions. Additionally, it was

demonstrated that acidic polysaccharides could express prebiotic properties and also to be used for unique target-specific drug delivery in the GIT.

Acidic polysaccharides are often conjugated with noncarbohydrate polymers, (physico) chemically bounded with LMWC, and form complexes with inorganic elements. Interestingly, these kinds of coupling with polyphenols, lignin, proteins, and minerals lead to formation of new immunoactive agents. It was demonstrated that LCC express intestinal immunomodulating properties through Peyer's patch cells, possess antiinfectious and anticoagulant activities. The antioxidant activity of polyphenols and combinatorial antiinflammatory, anticomplementary, immunostimulating activities of polyphenols and polysaccharides have great potential in the treatment of chronic inflammatory diseases. Polysaccharide-protein complexes show antidiabetic, antiinfectious, and immunostimulating potential. Selenylated polysaccharide preparations have immune-enhancing activity and could be applied in preventive and nutritional therapy of tumor diseases.

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CHAPTER 11

Soybeans, Flaxseeds, and Fish Oil in the Treatment of Renal Disease

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1 Introduction

Therapeutic agents, including nutraceuticals, may possess therapeutic or protective effect toward kidney dysfunction. Current guidelines highlight that diet rich in fruits, vegetables, and some sorts of oil, seeds, and nuts could be used for prevention and/or treatment of a disease. This is due to the presence of different active constituents called nutraceuticals. A number of nutraceuticals, such as $n-3$ polyunsaturated fatty acid (PUFA) from dietary marine sources, soy protein, isoflavones, soy fibers, and flaxseed derivate α-linolenic acid (ALA), lignans, and fiber have potential beneficial effects on the progression of renal disease (Fig. 11.1) (Friedman, 2010; Huang et al., 2013; Lee et al., 2013; Ristić-Medić et al., 2011). These nutraceuticals with lipid-lowering, antioxidant, antiinflammatory properties and hypotensive effect are derivates between nutrients and drugs that may possess a renal protection effect (Al-Okbi et al., 2014).

Chronic kidney disease (CKD) is an important health problem that could progress to end-stage renal disease (ESRD), need for dialysis treatment, or transplantation. Overall, approximately 30%–40% of patients develop significant renal failure 10–15 years after the diagnosis of nephropathy. Different etiopathogenesis forms, such as glomerulonephritis, hydronephrosis, diabetic nephropathy, hypertensive renal disease, kidney malignancy, and renovascular disease are defined as causes for CKD. Abnormal fatty acid metabolism may contribute to clinical problems in patients on hemodialysis treatment including, dry and scaly skin, itching, pruritus, abnormal perspiration, susceptibility to infection, delayed wound healing, anemia, platelets dysfunction, increased hemolysis incidence for ischemic heart disease (Christensen et al., 1998; Koorts et al., 2002; Peck et al., 1996). Dialysis patients consume insufficient amounts of $n-3$ PUFAs and consequently have low blood fatty acid levels (Huang et al., 2013; Lee and An, 2013; Ristić-Medić et al., 2011). In addition, a study with participants free of renal insufficiency with lower baseline plasma PUFA levels showed that they were

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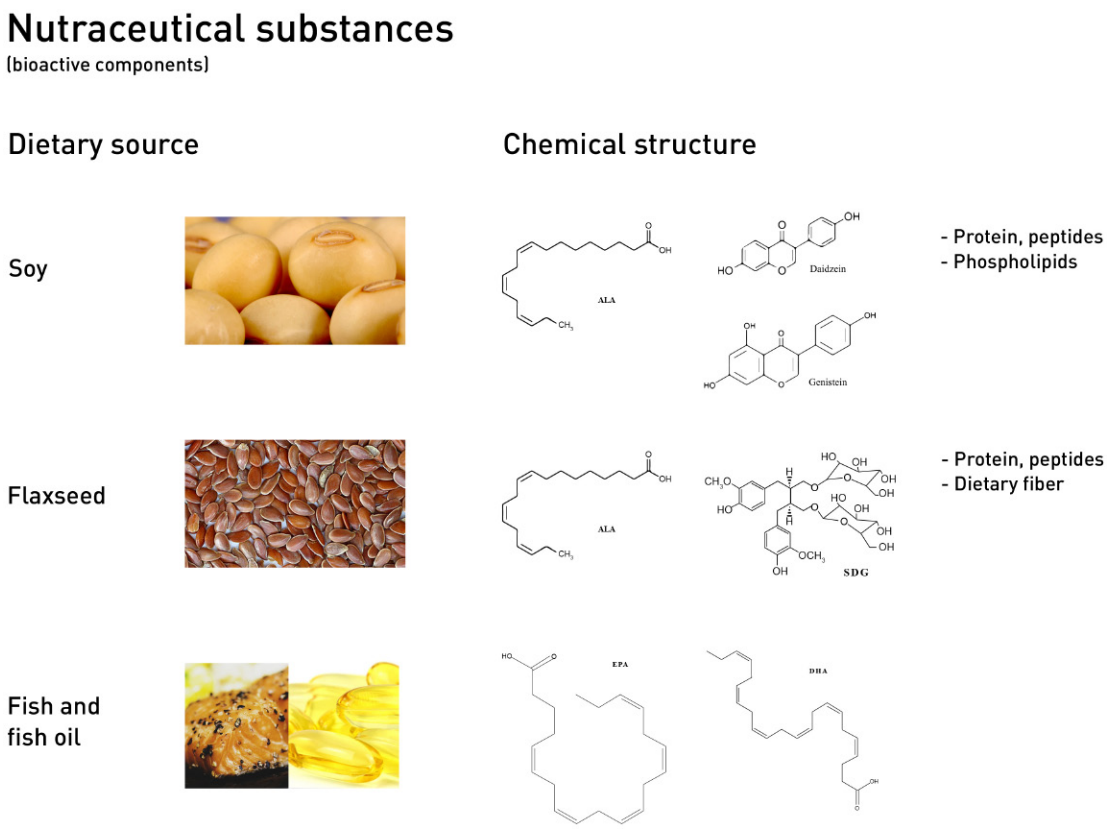


Figure 11.1: Bioactive Components of Soybean, Flax, and Fish Oil.
AA, Arachidonic acid; *ALA,* α-linolenic acid; *DHA*, docosahexaenoic acid; *EPA,* eicosapentaenoic acid; *SGA*, secoisolariciresionol diglucoside.

significantly more likely to develop renal insufficiency at the 3-year follow-ups compared to those with higher plasma PUFAs levels (Al-Okbi et al., 2014).

Human organisms cannot produce $n-6$ and $n-3$ PUFAs. Therefore, PUFAs must be contained in the diet. Linoleic acid (LA, 18:2 $n-6$) and ALA (18:3 $n-3$) are representatives of essential fatty acids (EFA) (Fig. 11.2). Dietary LA and ALA serve as the precursors for the $n-6$ and $n-3$ series of PUFA, respectively, and play an important role in modulation of cardiovascular and inflammatory diseases, immune system and blood pressure regulation (Benatti et al., 2004; Ristić-Medić et al., 2003a, 2013). Their products modulate biosynthesis of potent cellular mediators: eicosanoids, resolvins, protectins, and maresins. $N-6$ PUFAs are converted to arachidonic (AA)-derived eicosanoids. In contrast, $n-3$ PUFAs compete with the AA cascade. EPA is a competitive inhibitor of cyclooxygenase. EPA reduces the production of the 2-series prostacyclins, tromboxanes and prostaglandins and the 4-series leukotrienes, but produces the 3- and 5-series prostanoids, which are less biologically active. DHA can inhibit AA metabolism and platelet aggregation by reducing the affinity of the platelet TXA2/PGH2 receptor (Arterburn

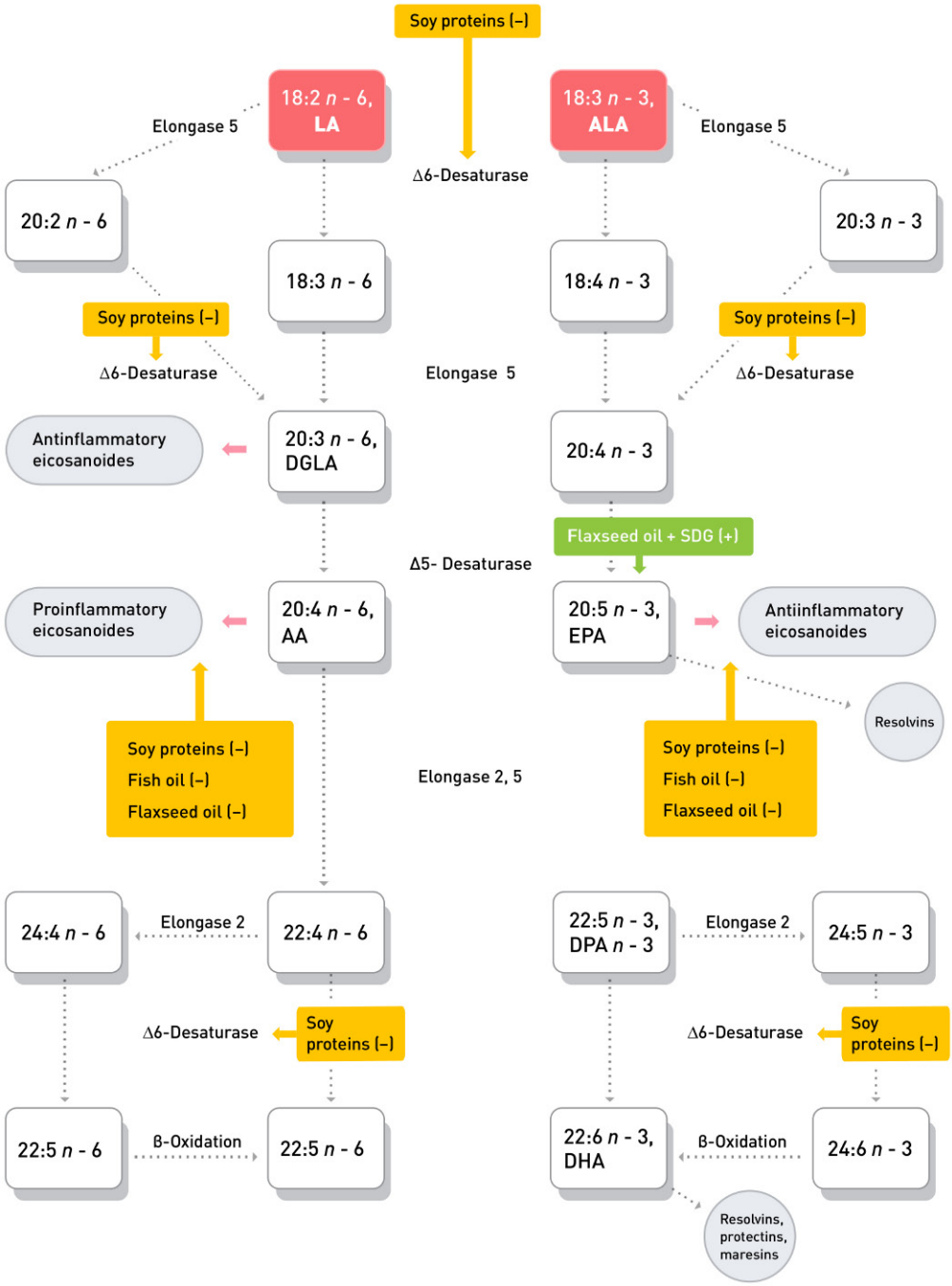


Figure 11.2: Elongation/Desaturation of Essential Fatty Acids and the Role of Soybean, Flax, and Fish Oil on Metabolism of *N* − 6 and *N* − 3 Pufas and Eicosanoids Synthesis.

+, Stimulation; –, inhibition; AA, arachidonic acid, ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihommo-gamma-linolenic acid; DPA, docosapentaenoic acid.

et al., 2006). Long-chain (LC) $n-3$ PUFAs, represent a unique group of nutraceuticals because of their multiple therapeutic effects on: triglyceride levels, thrombosis, and inflammation. $N-3$ PUFAs (omega-3 fatty acid) are tested as nutraceuticals that have more than one mechanism of action.

Animal models of CKD are important to study nutraceutical that may possess the therapeutic or protective effects toward kidney function. In animal models dietary flaxseeds and flax oil attenuated to decline the renal function and reduced glomerular injure (Caligiuri et al., 2013). The greatest market of soybean products for human consumption is in the form of soybean oil and lecithin (flour, textures, and beverage powder). There are a number of soy components (soy protein, genistein, daizdein) that have been studied with regard to their possible effect on lipid metabolism (Setchell et al., 2002a). Nutraceutical efficiency is usually measured by the change in a parameter from baseline physiological value before treatment to a new physiological value after treatment.

2 Sources, Dietary, and Supplement Intake

2.1 Soybean-Dietary Sources of Soy Protein, Isoflavones, Fatty Acids, and Fiber

Soy, as well as soybean products, are rich in proteins, isoflavones, phospholipids, PUFA, and fibers. Various factors have been proven to affect and change significantly the quantity and quality of soybean components and products, such as genetic and environmental factors, plant maturity, and processing conditions. The average amount of protein in soybeans is 36%–40% of their total weight (wt.) (McGraw et al., 2016; Medic et al., 2014). Soy protein is similar in quality to animal protein sources, as found in casein and eggs. Soybeans are rich in fat (18–24 wt.%) and contain saturated (SFA), monounsaturated (MUFA), and PUFA (about 15, 23, and 58% of total fats, respectively). The PUFA in soybean are LA and ALA (Medic et al., 2014). Soy foods rich in fiber are soybeans, soynuts, green soybeans, soy flour, textured soy protein, and tempeh. In isolated soy protein, dietary fiber is not present. Dietary soluble and insoluble fibers of soybeans offer many health benefits (Medic et al., 2014). Soybeans are processed into defatted meal of flakes, and the oil and lecithin that are removed are important commercial products, too (Ristić-Medić et al., 2003b).

Isoflavones are found in small amounts in many legumes, seeds, grains, and vegetables, but soy is their most concentrated dietary source (soy milk and soy flour). The amount of isoflavones varies between different soy products and soy-based dietary supplements. The isoflavones in soybeans consist mainly of genistein, daidzein, and smaller amounts of glycitein. In plants, they are usually found in the glycoside form. After ingestion, intestinal beta-glucosidases hydrolyze the glycosides in the intestinal wall, releasing the aglycones (genistein, daidzein) (Setchell et al., 2002a). Gut microflora metabolize isoflavones, daidzein is converted to equal, which has greater estrogenic activity than

daidzein (Rowland et al., 2003). There are particular differences in the metabolism of isoflavones as a consequence of individual variations in gut flora. About 20%–50% of the Western population converted daidzein to equal (Setchell et al., 2002b). Equal is the key of isoflavones in clinical effectiveness (Kreijkamp-Kaspers et al., 2005). The failure to distinguish subjects who are "equal-producers" from "nonequal producers" could plausibly explain the variance in reported data on the health benefits of soy in clinical studies.

Bioavailability of isoflavones is associated with gut transit time, fecal digestion rates, lactase, some dietary factors, and on keeping conditions of the soy-milk (Nielsen and Williamson, 2007). In some cases, lactase may be needed for hydrolization of isoflavones glucosides in the small intestine (Rau De Almeida Callou et al., 2010). Isoflavones of liquid soy food may be absorbed faster than other types of soy foods. However, it is very difficult to predict whether the consumption of whole soybean product has the same effect on health as well as isoflavones supplementation.

Many factors affect the amount of isoflavones in soy products: type of soybean, geographic area of cultivation, and method of processing soybeans. Soy products rich in protein, such as mature soybeans, roasted soybeans, soy flour, and textured soy protein, are excellent sources of isoflavones (5.1–5.5 mg isoflavones/g soy protein). Intermediate sources of isoflavones are green soybeans providing 3.3 mg/g and tempeh 3.1 mg/g, isoflavones/g soy protein, while tofu, an isolated soy protein, and some types of soy milk provide around 2 mg isoflavones/g soy protein. Alcohol-extracted products, such as soy-protein concentrate, have the lowest amounts of isoflavones ($\leq$0.3 mg/g soy protein). Plasma and urinary concentrations of isoflavones are frequently utilized as biomarkers for subject compliance in dietary intervention studies (Ranich et al., 2001). Individual variability of isoflavones biomarker depends on renal function.

2.2 Flaxseed-Dietary Sources of ALA, Lignans, Fiber, and Flaxseed Protein

Flaxseed (linseed, *Linum usitatissimum*) is a potential functional food source because of its unique nutrient components: high ALA 50%–62% of flaxseed oil, liganans level of 0.2–13.3 mg/g flaxseed and essential source of dietary fiber 28 wt.% (Bernacchia et al., 2014; Imran et al., 2015; Kajla et al., 2015). The lipid content of flaxseed varies from 37 to 45 g/100 g of the seed and it is rich in ALA. ALA is found in green plant tissues, in some common vegetable oils, including soybean and rapeseed oils, in some nuts, and in particular in flaxseeds and flaxseed oil (Rodriguez-Leyva et al., 2010; Vučić et al., 2012) (Table 11.1, Fig. 11.1). ALA is essential fatty acid that can be metabolized to LC-PUFA. It is reported to exhibit antiinflammatory, antithrombic, and antiarrhytmic properties (Simopoulos, 1999). Dietary guidelines recommend higher intake of $n-3$ PUFA and in nonfish eaters the flaxseed may be important dietary

Table 11.1: Dietary sources of *n* − 3 PUFAs and therapeutic option in dyslipidemia in CKD.

Common Name	Chemical Structure	Dietary Sources
n − 3 Subfamily		
Alpha-linolenic acid	18:3 *n* − 3	Rapeseed, soybeans, walnuts, flaxseed, perilla, chia, hemp, and their oils
Eicosapentaenoic acid	20:5 *n* − 3	Oily fish, seafood, seaweed, krill, and seal oil
Docosapentaenoic acid	22:5 *n* − 3	Seal meat and oils
Docosahexaenoic acid	22:6 *n* − 3	Oily fish, seafood, seaweed, krill, and seal oil
Proposed treatment for lipid management in patients with chronic kidney disease (CKD) (Harper and Jacobson, 2008)		
CKD stages	Moderate to severe CKD stages 3–4 (GFR 15–59 mL/min/1.73 m^2)—*very high triglycerides >500 mg/dL*	CKD stage 5 (hemodialysis or GFR < 15 mL/min/1.73 m^2)— *very high triglycerides >500 mg/dL*
Therapeutic option	1. Gemfibrozil 600 mg/day 2. Omega-3 fatty acids 3–4 g/day 3. Fenofibrate 48 mg/day	Omega-3 fatty acids 3–4 g/day or gemfibrozil 600 mg/day

CKD, Chronic kidney disease; GFR, glomerular filtration rate.

source of *n* − 3 PUFA. Flaxseed contains about 21% of proteins with amino acid composition comparable to that of soy protein (rich in arginine, aspartic and glutamic acid, cystein, and methionine) (Kajla et al., 2015). Phenolic compounds are present in good amounts in flaxseed (secoisolariciresionol diglucoside (SDG) 0.5–20 mg/g, ferulic acid 10.9 mg/g, chlorogenic acid 7.5 mg/g, gallic acid 2.8 mg/g (Setchell et al., 2014). It serves as a good dietary source of minerals phosphrous (650 mg/100 g), magnesium (350–431 mg/100 g), calcium (236–250 mg/100g), and potassium (5600–9200 mg/100 g) (Bernacchia et al., 2014; Kajla et al., 2015). Flaxseed edible products include the whole seed, ground meal, and extracted oil or mucilage and are used in preparation of numerous dietary items, such as baked cereal products, ready-to-eat cereals, fiber bars, bread, and muffins.

2.3 Dietary Sources and Rrecommendation of N *– 3 PUFAs (ALA, EPA, and DHA)*

It is known that fish oil's health-promoting effect is mainly due to its high content of *n* − 3 PUFAs. The main *n* − 3 PUFAs in fish oil are EPA and DHA. Fish do not synthesize them; they obtain them from the algae or plankton. Carnivore cold-water fish, such as salmon, herring, mackerel, anchovies, and sardines that synthesize LC-*n* − 3 PUFA are the richest available source of EPA and DHA. Oils from these fish have about 7 times higher than content of *n* − 3 compared to *n* − 6 PUFAs (Sinclair et al., 2007). Flaxseed and nuts are also common sources of *n* − 3 PUFAs. Fish oil supplements are often consumed as a source of marine, fish oils (Ciccone et al., 2013). Commercially, omega-3

supplements are usually in the form of soft gel capsules or microencapsulated power. Fish oil products in market pass safety standards for potential contaminants. Heavy metals selectively bind with protein in the fish flesh rather than accumulate in the oil. The $n-3$ LC-PUFAs are present in foods and food supplements mainly as triacylglycerol and as free fatty acids and bound to phospholipids in smaller amounts. EPA and DHA may be found in the form of ethyl-esters in synthetically produced concentrated supplements. Omega-3 fatty acids bound to phospholipids (with the highest content in krill oil) are considered as their best dietary sources due to better bioavailability and stability compared to nonesterified omega-3 fatty acids (Bjørndal et al., 2014). Even in high doses, omega-3 fatty acids are well tolerated with gastrointestinal discomfort reported. Daily EPA and DHA intake for European adults, based on consideration on cardiovascular disease (CVD) risk is recommended to be from 250 to 500 mg (EFSA, 2012). Even though there is much evidence to support the usage of $n-3$ PUFAs for CVD prevention, it is not readily recommended for those with CKD at this point in time.

3 Clinical Association of Soybeans, Flaxseed, and Fish Oil Treatments in CKD

The beneficial effects of soy on renal function may result from its impact on certain physiological risk factors for CKD, such as dyslipidemia and hyperglycemia (Yokoyama et al., 2014). Soy intake is also associated with improvements in antioxidant status and systemic inflammation in early and late-stage CKD patients (McGraw et al., 2016). Lower erythrocyte ALA is found in HD patients probably due to low intake of foods rich in ALA, such as flaxseed, that which not generally recommended in uremic patients because they are rich in potassium (Dessì et al., 2014). High phosphorous and cadmium may also be a problem for people with changes in renal function (Bernacchia et al., 2014; Steiber, 2014). Dietary ALA also serves as the precursors for EPA and DHA that are abundant in fish oil and play an important role in modulation of CVD, inflammatory diseases, immune system, and blood pressure regulation (Benatti et al., 2004; Ristić-Medić et al., 2003a,b, 2013). EPA and DHA modulate biosynthesis of potent cellular mediators, eicosanoids, protectins, resolvins, and maresins. ALA and other nutritional components of flaxseed (lignans, fiber, proteins) contribute to flaxseed consumption on renal function, hypolipidemic, blood pressure–lowering, antiinflammatory, antioxidant effects. In addition to reducing lipid levels, fish oil can affect the cardiovascular risk in patients with CKD through other mechanisms that involve antiarrhythmic, antiinflammatory effects, stabilizing arterial plaque with the improvement of endothelial function (Fig. 11.3) (Adkins and Kelley, 2010; De Caterina 2011). Therefore, consumption of food with high $n-6/n-3$ PUFA ratio increases production of inflammatory and thrombosis reaction mediators, while high $n-3/n-6$ ratio show cardioprotective, antithrombotic, and antiinflammatory action (Friedman, 2010; Ristić-Medić et al., 2013).

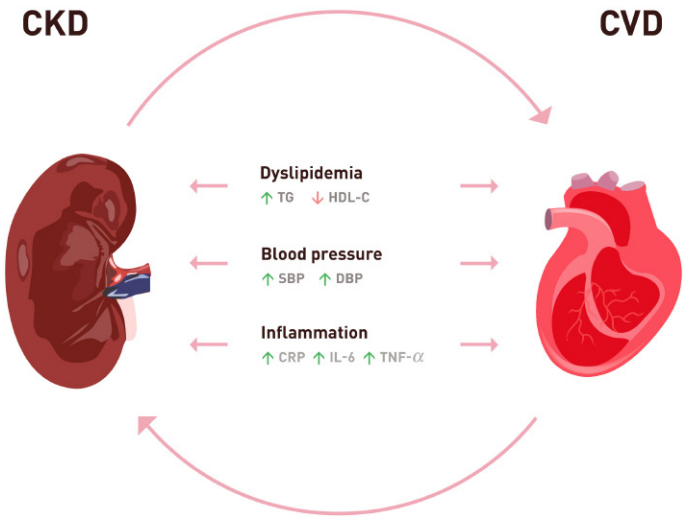


Figure 11.3: Risk Factors for High Incidence of CVD in CKD Patients.
CKV, chronic kidney disease; *CRP,* C-reactive protein; *CVD*, cardiovascular disease; *DBP,* diastolic blood presure; *HDL-C*, high-density lipoprotein cholesterol; *IL-6,* interleukin 6; *SBP*, systolic blood pressure; *TG,*triglycerides; *TNF-α*, tumor necrosis factor α.

3.1 Effect on Renal Function

According to the National Kidney Foundation (2002), one of the following two characteristics is required before a patient is diagnosed with CKD: (1) kidney damage for ≥3 months, as confirmed by biopsy or markers of damage, such as microalbuminuria with or without a decrease in glomerular filtration rate (GFR); or (2) GFR < 60 mL/min/1.73 m^2 for ≥3 months (Harper and Jacobson, 2008) (Table 11.1). Long-term intakes of soy-protein diets were associated with lower renal blood flow and GFR (Jing and Wei-Jie, 2016). Recent studies suggest that there may be an association between $n-3$ PUFAs and flaxseeds and the development of CKD.

3.1.1 Soybean supplementation and renal function

Dietary intervention has long been considered effective in the prevention, control, and treatment of CKD. The recommended protein intake for nondialysis clinically stable patients with stage 3–5 CKD, is 0.6–0.8 g/kg of ideal body weight (BW) per day in order to preserve their protein stores throughout the progression of kidney disease (Ikizler et al., 2013). In ESRD patients on maintenance dialysis, there are additional needs for protein because of catabolic stimulus and unavoidable loss of amino acids and albumin in the dialysate and due to the inflammatory stimuli associated with the dialysis procedure. The recommendations for protein intake are least 1.2 g/kgBW/day for patients on dialysis treatment to maintain muscle mass and the nutritional status (Ikizler et al., 2013).

Low protein and low phosphorus diets demonstrate effectiveness in slowing the progression of kidney disease. However, these diets demand careful consideration. Nutritional challenge is to maintain adequate total energy intake and not overdo the intake of protein and phosphorus in CKD patients (Steiber, 2014). Thus, not only the quantity but also the type of dietary protein has important implications in renal disease. Vegetarian diets are the focus in the field of renal nutrition, due to a favorable impact on renal function (Goraya and Wesson, 2015; Piccoli et al., 2011; Steiber, 2014). Energy/protein and energy/phosphorus ratio is higher in foods of plant origin, so that is suitable for low protein and low phosphorus diets. Furthermore, they are low in saturated fats (Filipowicz and Beddhu, 2013; Steiber, 2014), tend to improve glycemic control (Yokoyama et al., 2014) and may reduce oxidative stress and inflammation (Kuhlmann and Levin, 2005). Health benefits of vegetarian diets in CKD patients have been confirmed in human studies (Kitazato et al., 2002; Moe et al., 2011).

Soybean protein, as a plant protein, has been extensively studied in CKD. Research shows that soybeans provide high-quality plant protein and unique isoflavones (genistein and daidzein) associated with a potentially favorable effect on renal function (Anderson, 2008). Evidence from animal studies suggests that consumption of soy protein demonstrated protective effects by improving renal flow reducing proteinuria and renal histological damage (Teixeira et al., 2004; Trujillo et al., 2005) and consequently slowing the progression of renal disease when compared to animal protein diet. However, human studies have inconsistent results on the effects of soy intake on renal function in patients with CKD. These studies have different treatment duration (4–12 weeks).

Soy-based diets could be renoprotective and able to reduce deterioration of glomerular function and proteinuria in healthy individuals and patients with renal disease (Azadbakht et al., 2003, 2008). However, these effects have not been confirmed by all conducted studies (Ahmed et al., 2011; Miraghajani et al., 2013). Two metaanalyses in predialysis patients that examined soy protein (14–30.7 g/day) containing isoflavones (ranged from 35.5 to 65 mg/day) intake showed significant decrease of serum creatinine, serum phosphorus, and proteinuria while nonrelevant change was found in creatinine clearance, GFR, and nutrition status (Jing and Wei-Jie, 2016; Zhang et al., 2014). Moreover, soy protein intake in dialysis patients has proven able to maintain the nutritional status, causing no significant change in blood urea nitrogen and serum phosphorus (Jing and Wei-Jie, 2016).

Even though there are extensive recommendations on protein intake for patients with CKD (Ikizler et al., 2013), there are no specific dietary preventive strategies for persons at high risk for renal dysfunction or in individuals with mild renal function decline. Different subjects show different results, for example, subjects with initial renal dysfunction or individuals with CVD risk may be susceptible for a detectable improvement by soy treatment (Liu et al., 2014a). In accordance to that in prehypertensive postmenopausal women, who are

equal producers, a 6-month consummation of 40 g soy flour/day (12.8 g soy protein/day) does not indicate a significant effect on renal function. On the contrary, in subgrouping analyses women with lowered basal renal function tended to improve markers of renal function (Liu et al., 2014a). Future trials in subjects with middeclined renal function are necessary.

Whole soy (less processed soy products, such as soy milk, soy nuts, soy flour, and tofu) dietary intake may be more effective on renal function than isolated soy component. Purified single isoflavone are more effective than complex isoflavones (Hooper et al., 2008; Liu et al., 2014a). Consumption of whole soy tends to improve renal function relative to daidzein and milk control in individuals with lowered renal function who are even equal producers (Liu et al., 2014b). However, future trials are necessary to investigate the effects of daidzein on renal function.

3.1.2 Flaxseed and flax oil and renal function

Several studies indicate that dietary flaxseed can show renal protective effects in some animal models of chronic renal disease (Hall et al., 1993; Ingram et al., 1995), diabetic nephrophaty (Velasquez et al., 2003), and in humans with lupus nephritis (Clark et al., 2001). As fully defatted flaxseed meal with very high amounts of SDG (17 mg/g) showed favorable effect on renal function (Velasquez et al., 2003), it is possible that actions of lignans may significantly contribute to the obtained flaxseed effects. Treatment with flaxseed or flaxseed oil showed beneficial effects on kidney function (reduced increase of renal function parameters urea and/or creatinine and/or uric acid) in the conditions of renal toxicity (Abdel Moneim et al., 2011a,b; Khan et al., 2012) and in hypertensive rats (Al-Bishri, 2013). Based on the results of these studies it can be concluded that flaxseed shows potential renal protective effect in various models of renal injuries.

3.1.3 N − 3 PUFAs and renal function

Findings from studies suggest that there may be an association between PUFAs intake and blood status and the progression of renal failure (Baggio et al., 2005; Friedman, 2010; Goraya and Wesson, 2015). Older adults with higher total plasma PUFAs concentration had a lower risk of developing renal insufficiency, defined by creatinine clearance levels <60 mL/min, over a 3-year follow-up (Lauretani et al., 2008). Observational studies suggest that increased PUFAs intake may be protective against the age-related decline in GFR in the general population. In Italian and Australian cohorts, dietary intake and status of $n-3$ PUFAs showed negative association with mean GFR change, but this relationship was not observed in Spanish population (Díaz-López et al., 2012; Gopinath et al., 2011; Lauretani et al., 2008). A number of studies in population-based cohorts have reported no direct association between $n-3$ PUFAs and albuminuria (Lin et al., 2010; Nettleton et al., 2008). Only one study in diabetic patients demonstrated an inverse association between dietary $n-3$ LC-PUFAs and the degree of albuminuria (Lee et al., 2010).

Randomized controlled studies (RCT) testing the benefits of $n-3$ PUFA supplementation on proteinuria/albuminuria and renal function in humans are mostly in patients with diabetic nephropathy, IgA nephropathy, or lupus nephritis (Huang et al., 2013). Only a few studies observed significant effects of reducing proteinuria/albuminuria or retarding the reduction of GFR (Alexopoulos et al., 2004; Ferraro et al., 2009; Svensson et al., 2004). Also, nonrandomized intervention trials suggested that $n-3$ PUFA supplementation might be renoprotective (Parinyasiri et al., 2004; Zeman et al., 2006). A pooled metaanalysis of these interventional studies showed that $n-3$ PUFAs supplementation reduce urine protein excretion and lead to slower decline in GFR in CKD patients (Miller et al., 2009). Results of these trials may be inconsistent due to differences in chronic renal failure etiology, persistent different risk factors for disease progression, sample sizes, doses, and durations of $n-3$ PUFA administered, compliance of participants, use of medications and outcome evaluations (Huang et al., 2013).

$N-3$ PUFAs treatment can prevent or slow down the decline in renal function in a variety of animal models of renal disease (Baggio et al., 2005). The potential mechanisms by which dietary intake of $n-3$ PUFA putatively protects the kidneys from damage include suppression of mesangial cell activation and proliferation, antiinflammatory influence antiproteinuric effects, reduction in blood pressure, improvement of endothelial function, and alterations of cell membrane fluidity and functionality, as well as general hypolipidemic effects (Huang et al., 2013).

3.2 Fish Oil and IgA Nephropathy

The efficacy of $n-3$ PUFAs in IgA nephropathy remains a controversial issue. IgA nephropathy is a chronic form of glomerulonephritis characterized by deposits of predominantly immunoglobulin A in the glomeruli (Song et al., 2013). Earliest studies reported that dietary fish oil treatment reduce progression of renal disease among patients with IgA nephropathy (Huang et al., 2013). Animal models of CKD showed that fish oil suppress activation and proliferation of mesangial cell. Renal failure develops in 20%–40% of patients 5–25 years after IgA nephropathy diagnosis (Song et al., 2013). Fish oil group supplementation (1.8 g/day for 12 months) showed significant improvement in estimated creatinine clearance in small Japanese clinical trial among patients with IgA nephropathy. This trial indicated that fish oil therapy was a safe and worthwhile supplement to the drugs used to treat IgA nephropathy (Uchiyama-Tanaka and Mori, 2010). In contrast, a metaanalysis, which included five RCTs, suggested that there were insufficient data to confirm the efficacy of fish oil therapy for proteinuria and renal function in IgA nephropathy (Liu and Wang, 2012). Another metaanalysis of 5 RCT reported that currently available evidence suggests that $n-3$ PUFAs has no benefit in preserving renal function, but can ameliorate proteinuria in IgA nephropathy but this association was not dose dependent ($n-3$ PUFA >3 g/day or ≤ 3 g/day) (Chou et al., 2012). Further large-scale trials are needed to shed more light on this issue.

3.3 Fatty Acid Status in Patients With CKD

Cold-water fish consumption (main dietary source of EPA and DHA) is often related to income status, national and social eating habits. Patients with uremia often have compromised taste and dislike foods rich in $n-3$ PUFAs, which in part, explains their low PUFA consumption (Filipowicz and Beddhu, 2013; Ristić-Medić et al., 2011). However, it is a favorable dietary habit to control daily intake of phosphorus in CKD. Fish and flaxseed are rich in phosphorus. Potassium restricted diet in hemodialysis patients exerts only a minor influence on the reduction of $n-3$-LC-PUFAs intake, but flaxseed rich in ALA (its intake is generally low) is food with high potassium level (Moe et al., 2011). A plasma, erythrocytes and tissue fatty acid profiles in hemodialysis patients are inadequate and possibly reflects the presence of an EFA deficiency (Christensen et al., 1998; Dessì et al., 2014; Elshamaa et al., 2010; Koorts et al., 2002; Madsen et al., 2011; Rasic-Milutinovic et al., 2007; Ristić et al., 2006). Earlier studies investigating whether CKD patients eat a diet with a healthy fat profile indicate that the blood contents of $n-6$ PUFA and EPA are often decreased in hemodialysis patients (Dessì et al., 2014; Elshamaa et al., 2010; Madsen et al., 2011). Moreover, uremic patients may present defects in the conversion of ALA to LC-PUFAs. Then, given the frequent limitation of the whole caloric intake of uremic subjects, higher ALA intake could be recommended to overcome malnutrition, but it may fail to converse into EPA and/or DHA. This reinforces the need for fish and/or fish-derived products. Moreover, these patients exhibit chronic inflammation, increased oxidative stress, in connection to PUFAs oxidation and high intracellular calcium concentration, which also contributes to their low EFA profile (Lee et al., 2013; Peruničić-Peković et al., 2012; Ristić-Medić et al., 2011).

Long-term therapy and inadequate dietary intake in patients on hemodialysis lead to weight loss. Dialysis procedure may accentuate malnutrition by removing important nutrients. In addition, malnutrition is characterized not only by impaired lipid metabolism but also results in modification of plasma and erythrocyte fatty acid profile. Hemodialysis patients with adequate nutrition status, also show a deficit of essential PUFAs, particularly $n-3$ PUFAs (Friedman and Mo, 2006; Friedman et al., 2012; Ristić et al., 2006; Rasic-Milutinovic et al., 2007). In fact, composition of fatty acids of serum and erythrocyte phospholipids clearly reflects $n-3$ PUFAs intake (Arsić and Ristić-Medić, 2015), more attention should be given to the impact of dietary fat content and quality to prevent distribution in fatty acid metabolism in all patients with ESRD, and not only in malnourished cases. Reliable method for dietary assessment intake in dialyses patients is very important to make clear concept of the relationship between fatty acid intake and clinical response (Bross et al., 2010).

To date, few studies noted lower plasma EPA and DHA levels and low erythrocyte membrane EPA content in patients with CKD (Hooper et al., 2008; Koorts, 2002; Ristić-Medić et al., 2006). Also, it was concluded that the high relative abundance of SFA and MUFA in plasma of hemodialysis patients is associated with concomitant lipid disorders and

cardiomyopathy (Varga et al., 1997; Ristić-Medić et al., 2011). A single standard hemodialysis session effects patients' plasma fatty acid profile, increase $n-3$ PUFAs (EPA and DHA) levels, and decreases plasma palmitic acid e.i. SFA (Varga et al., 1997). It is well known that the hemodialysis treatments upregulate lipoprotein lipase (LPL) and phospholipase A2 (PLA_2) activity, and affect release of the free fatty acids from triglycerides and phospholipids (Vishwanath et al., 1996). $N-3$ PUFAs may be preferentially released compared with SFA because they have greater affinity for the sn-2 phospholipid position on which PLA_2 acts. In addition, the acute effect of hemodialysis on fatty acid levels may have important clinical implications during hemodialysis. Further studies are needed to confirm that theory.

Altered fatty acid composition with decreased $n-3$ PUFAs and increased SFA and SFA/PUFA ratio in erythrocyte membrane may be associated with lower membrane fluidity in chronic renal failure patients (Mills et al., 1995). In CKD patients, the advice to consume dietary higher amounts of $n-3$ PUFAs has the potential to increase the fluidity of their plasma membranes, which in turn can elevated glucose uptake (dietary glucose plus glucose absorbed from dialysate to avoid the loss of nutrients during hemodialyses procedures) and reduce hyperinsulinemia (Varga et al., 1997) often present in hemodialyses patients. Hyperinsulinemia stimulate delta-9 desaturase activity, which consequently increase endogenous synthesis of MUFA. CDK patients treated with hemodialysis have lower levels of $n-3$ PUFAs (ALA, EPA, and DHA) and EPA/AA and $n-3/n-6$ ratios when compared to those not exposed to hemodialysis (Nakamura et al., 2008). Moreover, exposure to hemodialysis resulted in a significantly higher concentration of LA and AA. Thus, hemodialysis may affect the enzymes involved in fatty acid synthesis, including desaturases and elongases and may contribute to AA-related inflammatory status. There are many limitations in the published studies, which deal with changes in fatty acid metabolism and PUFAs content in CKD. In general, published studies, which investigated fatty acid content and changes in their metabolism in CKD showed that patients on hemodialysis need adequate nutritional care programs with appropriate $n-6/n-3$ ratio in their diet regime (Friedman, 2010; Lee and An, 2013; Ristić-Medić et al., 2011).

Uremic pruritus is one of the most common symptoms in patients with ESRD, approximately 60%–90% of patients on hemodialysis suffering from this problem (Ghanei et al., 2012; Lopes et al., 2012). The pathogenesis of dry, scaly skin, and pruritus is not known but could be related to the abnormal fatty acid profile reported in these patients (Ristić-Medić et al., 2011). It is shown that intervention with fish oil and mixture seed may improve symptoms of pruritus in hemodialysis patients (Peck et al., 1996; Ristić-Medić et al., 2014). It was shown that $n-3$ PUFAs levels are easily modifiable by oral supplementation, which quickly and effectively raises the blood $n-3$ PUFAs levels (Fig. 11.4) (Saifullah et al., 2007). In that fact, further work is need to confirm the association between plasma PUFAs levels with clinical outcomes and renal function in ESRD patients.

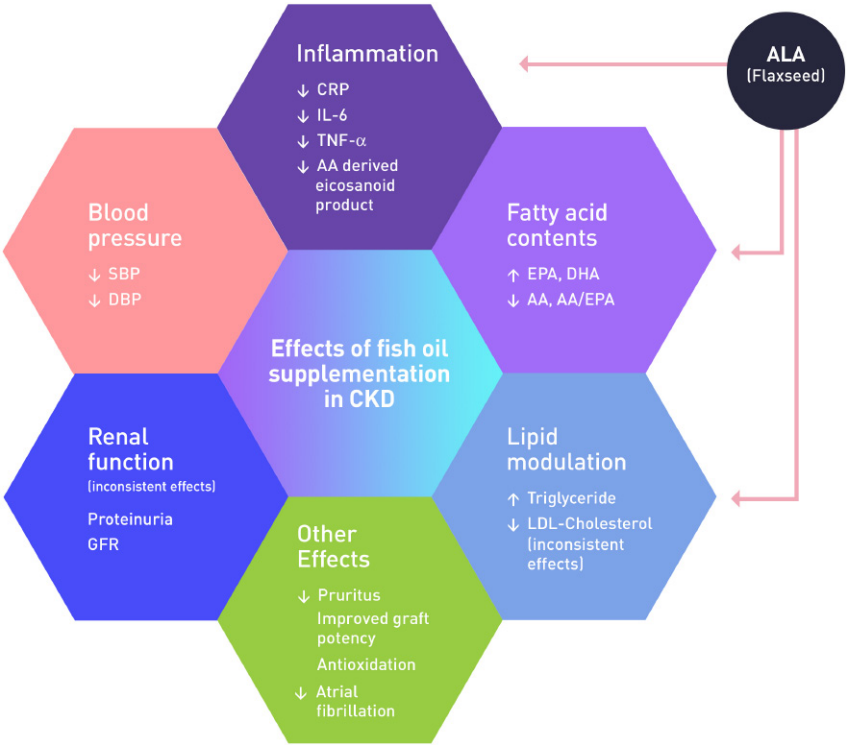


Figure 11.4: Flax and Fish Oil: Cardioprotective Effects and Influence on Renal Function in CKD.
AA, α-Linolenic acid; *CKD*, chronic kidney disease; *CRP*, C-reactive protein; *DBP*, diastolic blood presure; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *GFR*, glomerular filtration rate; *IL-6*, interleukin 6; *LDL-C*, low-density lipoprotein cholesterol; *SBP*, systolic blood pressure; *TG*, triglycerides; *LDL-C*, low-density lipoprotein cholesterol; *TNF-α*, tumor necrosis factor α.

3.4 Soybean, Flaxseed, and Fish Oil Effects on Dyslipidemia

Dyslipidemia is one of the possible risk factors for atherosclerotic vascular disease in patients with CKD. The most common lipid abnormalities in CKD patients include increased plasma concentration of triglycerides and low high-density (HDL) cholesterol levels, while concentrations of plasma total and low-density lipoproteins cholesterol (LDL) are usually not elevated (Lee et al., 2013; Zhu et al., 2014). More than 40% of patients with CKD have triglyceride levels greater than 200 mg/dL. In these patients dyslipidemia usually develops during the early phase of chronic renal failure and is connected with development of cardiovascular complications due to inhibition of the enzyme LPL, which is responsible for degradation of triglyceride-rich particles. (Fig. 11.3). However, the concentrations of total and LDL cholesterol as well as triglyceride level are sometimes normal or low in CKD patients with malnutrition CKD patients have reduced levels of enzymes involved in lipoprotein metabolism (LPL, hepatic lipase, lecithin-cholesterol acyltransferase) that result in increased

triglycerides, which is accompanied with reduced cholesterol-ester content of their lipoprotein particles. The aforementioned abnormalities in lipoprotein metabolism enzymes together with defective receptors for very-low-density lipoprotein (VLDL) and LDL lead to accumulation of chylomicron remnants, VLDL and intermediate-density lipoprotein, which are susceptible to oxidation. These oxidized remnants lipoprotein particles are usually atherogenic and play a role in CVD pathogenesis in CKD patients (Varga et al., 1997). Changes in antioxidant and antiinflammatory properties of HDL are noted partly due to reduced activities of paraoxonase and glutathione peroxidase in renal failure patients (Khandelwal et al., 2013; Varga et al., 1997).

3.4.1 Soybeans and dyslipidemia

Soybean ingredients, as soy proteins, sterols, fatty acids, fibers, isoflavones, and lecithin can modulate blood lipid levels, some of them able to act synergistically. Furthermore, some constituents may act via more than one lipid-lowering mechanism (Table 11.2). Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms (Jenkins et al., 2010; Potter, 1995; Sacks et al., 2006). Lipid profiles can be improved by substituting soy protein for animal protein (Cupisti et al., 2004; Tokede et al., 2015; Wofford et al., 2012).

The average daily intake of 30 g/day soybeans for 8 weeks leads to a substantial 3% decrease in serum LDL-cholesterol, 2% total cholesterol, and 4% serum triglyceride levels (Tokede et al., 2015). However, an increase of 3% in serum concentrations of HDL-cholesterol is also present. The effect would be stronger for patients with high levels of cholesterol. Whole soy foods are more effective in lowering serum cholesterol than the intake of processed soy.

Table 11.2: Proposed mechanisms for the hypocholesterolemic effect of soy.

	Metabolic Changes
Soyprotein, 25–50 g/day	• ↑ Cholesterol synthesis • ↑ Bile acid synthesis (or fecal bile acid excretion) • ↑ Apolipoprotein B or E receptor activity • ↓ Hepatic lipoprotein secretion • Upregulates LDL receptors • ↑Clearance of cholesterol from the blood
↑ Arginine/lysine ratio	• ↓Insulin and glucagon secretion • ↓Inhibit lipogenesis
Isoflavones: genistein	• Inhibit tyrosine kinase • Antioxidants • Inhibit LDL oxidation • Improved systemic arterial elasticity

Adapted from Potter, S.M., 1995. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J. Nutr. 125, S606–S611; Sacks, F.M., Lichtenstein, A., Van Horn, L., Harris, W., Kris-Etherton, P., Winston, M., 2006. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. Circulation 113, 1034–1044.

This metaanalysis showed that serum lipid profile was not notably influenced by isolated isoflavone supplementation (Tokede et al., 2015). Soy-milk consumption significantly reduces plasma levels of total, LDL-cholesterol, and triglycerides. Dietary intake of soy plus prebiotics was effective in reduction of LDL/HDL cholesterol ratio-cardiovascular risk parameter (Wofford et al., 2012; Wong et al., 2010).

In renal transplant patients, daily intake of 25 g soy protein for 5 weeks lowered the total and LDL-cholesterol level, but not HDL-cholesterol level (Cupisti et al., 2004). Also, in nephrotic patients vegeterian soy diet with protein intake of 0.7 g/kg body weight per day for 8 weeks, lowered serum cholesterol concentrations (total, LDL and HDL), as well as Apo AI and Apo B levels with no effect on triglycerides concentrations (D'Amico et al., 1992). Long-lasting treatment with protein intake of 0.8 g/kg body weight (35% textured soy protein) in patients with diabetes nephropathy lead to decreased levels of total and LDL-cholesterol, and also expressed effect on triglycerides levels in follow up period of 4 years (Azadbakht et al., 2008). However, soy protein intake of 0.8 g/kg for 8 weeks had no influence on serum lipids in patients with proteinuric glomerulopathies (Ahmed et al., 2011). The lowering effect of soy protein cholesterol is more efficient in hemodyalysis patients with elevated concentrations of cholesterol (Chen et al., 2005). Intake of 28 g/day textured soy flour that contains 14 g soy protein for 8 weeks reduced serum concentration of lipoprotein (a) in peritoneal dialysis patients. However, total, LDL and HDL-cholesterol, triglycerides, apo B100 and apo AI were not changed (Tabibi et al., 2010). It is therefore appropriate to view cholesterol-lowering foods, such as soy, as contributing to a diet that can achieve a clinically significant LDL-C reduction. (Jenkins, 2010; Ristić-Medić et al., 2003b, 2006).

3.4.2 Flaxseed and dyslipidemia

Edel et al. (2015) showed that flaxseed supplementation works independently of the cholesterol-lowering medication and that it is most probable that the high fiber content of flaxseed is responsible for the LDL-cholesterol-lowering actions of flaxseed, consistent with other studies (Almario and Karakas, 2013; Harper et al., 2006). A metaanalysis of studies that investigated the relationship between flaxseed supplementation (20–50 g/day) and lipid status parameters, showed that flaxseed consumption leads to significant decrease in plasma concentrations of total and LDL- cholesterol, but no significant effects on levels of triglyceride and HDL-cholesterol were found (Pan et al., 2009). Dietary consumption of whole flaxseed was associated with significant decrease in total as well as LDL-cholesterol levels, whereas flaxseed oil has the similar effect on blood triglycerides and HDL-cholesterol as MUFAs or $n - 6$ PUFAs (used in control diet). Flaxseed contains a large amount of dietary soluble fiber and it has been proven to have cholesterol-lowering effects (Almario and Karakas, 2013; Edel et al., 2015; Harper et al., 2006).

Additionally, flaxseed lignans have been shown to reduce levels of total and LDL-cholesterol in animal studies and human clinical trials (Adolphe et al., 2010; Zanwar et al., 2014).

According to the results of the aforementioned metaanalysis, it appears that initial cholesterol concentrations influence obtained effect on changes in lipid levels and that cholesterol-lowering effect of flaxseed was more pronounced in women (especially in postmenopausal women) than in men (Pan et al., 2009). It was concluded that the intervention using whole flaxseed, in females or in individuals with high initial lipid concentrations, reduce total or LDL cholesterol by 0.2 mmol/L, which is estimated to result in 6% reduction of coronary heart disease (Pan et al., 2009).

In the short-term studies flaxseed treatments reduced circulating cholesterol in patient populations with hyperlipidemia (Bloedon et al., 2008; Mandasescu et al., 2005) and in postmenopausal women (Patade et al., 2008). The effects of flaxseed are less consistent in a healthy population with a modest cholesterol-lowering effect at higher flaxseed concentrations (40–50 g/day) (Dodin et al., 2008) and no effect at lower flaxseed consumption (30 g/day) (Austria et al., 2008; Edel et al., 2015). Based on results of flaxseed effects on lipid parameters, Health Canada has recently approved a cholesterol-lowering health claim for 40 g of flaxseed (Bureau of Nutritional Sciences Food Directorate, 2014).

The findings from most of the conducted studies in nonuremic individuals with normal triglyceride levels showed that flaxseed consumption had no significant effect on serum triglyceride and HDL-cholesterol levels (Lucas et al., 2002; Patade et al., 2008). In patients with hyperlipidemia on hemodialysis treatments receiving 40 g/day ground flaxseed (containing 13.5 g of fat and 6.7 g of fiber) serum concentrations of triglyceride, total and LDL-cholesterol were reduced after 8-week treatments (Khalatbari Soltani et al., 2013). In the present study, at baseline, about 83% of patients on hemodialysis had serum triglyceride concentration higher than 200 mg/dL and after treatment with flaxseed, mean serum triglyceride levels decreased by 31%. Synthesis of fatty acids in the liver is inhibited by LC $n-3$ PUFA (mainly EPA and DHA) and consequently triglyceride levels are reduced due to lower gene expression of fatty acid synthesis enzymes (acetyl-coenzyme A carboxylase and fatty acid synthetase complex) (Jump et al., 2005). Flaxseed is one of the richest dietary sources of ALA (Rodriguez-Leyva et al., 2010), which can be metabolized to LC-$n-3$ PUFA (especially EPA) in the body. The obtained results suggest that the effective metabolism of ALA can mediate flaxseed effect on serum triglyceride concentration (Khalatbari Soltani et al., 2013). Over 8 weeks, flaxseed consumption also changed mean serum HDL-cholesterol concentration increasing its level by 16% in hemodialysis patients, although most studies have indicated that flaxseed consumption has no effect on serum HDL-cholesterol (Lucas et al., 2002). In the present study, flaxseed consumption decreased mean serum levels of total and LDL-cholesterol by 14.5% and 17%, respectively, and these findings are similar with results of the majority of previous studies in nonuremic individuals.

In another study, in hemodialysis patients supplementation with 1.0 g of flaxseed oil and 3.5 mg α-tocopherol twice a day during 120 days resulted in significant reduction of total,

LDL-cholesterol and increase in HDL-cholesterol (Lemos et al., 2012). Dietary treatment with 30 g of milled flax/sesame/pumpkin seed mixture (3:1:1, weight ratio) in hemodialysis patients for 12 weeks, reduced triglyceride, total and LDL-cholesterol plasma concentrations by 30%, 7%, and 5%, respectively (Ristić-Medić et al., 2014). This dietary intervention study clearly demonstrates the lipid lowering capacity of milled flax/sesame/pumpkin seed mixture rich in $n-6$ and $n-3$ PUFA in hyperlipidemic patients on hemodialysis treatment. At the same time, EPA and DHA levels of serum phospholipids were increased after 12 weeks of dietary treatment suggesting that ALA from seed mixture (3 g/day) can be efficiently metabolized to LC-$n-3$ PUFA. However, there are no other reports on the effects of dietary supplementation with seed mixture flax/pumpkin/sesame (rich in LA and ALA) in patients with renal disease compared to many studies with marine $n-3$ PUFA. Beside flaxseed lipid-lowering effects sesame seed (50 g/day) (Wu et al., 2006) have been shown to reduce blood lipids levels and the high content of dietary fiber and LA in the sesame seed are components that show capacity to lower serum cholesterol levels (Namiki, 2007). It is assumed that the possible potential mechanism of lipid lowering by pumpkin is probably due to fibers effects, too. Makni et al. (2008) demonstrated on an animal model that flax and pumpkin seed mixtures added in the diet of diabetic rats had hypolipidemic effect. In general, the available literature data suggest that 1% increase in concentration of LDL cholesterol results in 2%–3% increase in risk for coronary artery disease and 1 mmol/L increase in level of serum triglyceride lead to 32% increase of CVD risk. The obtained decreases of serum LDL-cholesterol and triglyceride levels by 5% and 30% in hemodialysis patients after consumption of milled flax/sesame/pumpkin seed mixture added to patients' regular diet suggest that its dietary intake could be effective in prevention of CVD in CKD patients.

3.4.3 N − 3 PUFAs and dyslipidemia

Fish oil can safely be employed in treatment of dislipidemia (Khandelwal et al., 2013). $N-3$ PUFAs affect lipid metabolism by reducing VLDL synthesis and increase postprandial clearance through suppression of VLDL secretion from liver. Fish oil supplementation promotes β-oxidation in the mitochondria and/or peroxisomes, and reduces remnant lipoprotein levels, such as apo B (Lee et al., 2013). By inhibiting the activities of diacylglycerol acyltransferase and phosphatidic acid phosphohydrolase and stimulating LPL activity-mechanisms $n-3$ PUFAs reduce postprandial hypertriglyceridemia (Park and Harris, 2003). Therefore, physicians usually prescribe $n-3$ PUFAs to control triglyceride levels in circulation. The American Heart Association, addressing hypertriglyceridemia and CVD, recommends daily intake from 2 to 4 g of EPA plus DHA for treatment of patients that need to lower triglycerides (Miller et al., 2011). More recently, a combination therapy of statins and fish oil supplementation has been proposed as its efficiency to reduce both concentrations of triglycerides and cholesterol (total LDL, both small and large LDL, IDL particle) (Bays et al., 2012; Lee et al., 2013; Oelrich et al., 2013).

Specifically, $n-3$ PUFAs are involved in gene expression regulation (Lapillonne et al., 2004) as they can modulate function of sterol regulatory binding proteins (SREBP)-1c and peroxisome proliferator-activated receptors (PPARs) (Kliewer et al., 1997; Omrani et al., 2015; Schmidt et al., 2012). Fish oil and its $n-3$ PUFAs content lower VLDL particle level due to enhanced of its catabolism mediated by LPL and production by repressing expression of apo CIII and apo B. Thus, triglycerides clearance was ameliorative and activity of three fundamental genes involved in triglycerides synthesis (MOGAT3, MOGAT2, and DGAT1) was reduced with lower mRNA expression in treated patients. Numerous intervention clinical trials that investigate the effects of fish oil supplements on serum lipids in CKD have been published (Peruničić-Peković et al., 2007; Rasic-Milutinovic et al., 2007; Sørensen et al., 2015; Vernaglione et al., 2008). However, the results are still inconclusive, because the trials included a small number of patients, short study duration, and different doses of EPA (900–2010 mg) and DHA (250–960 mg). Recently metaanalyses of RCT showed that fish oil supplements reduced serum triglyceride (0.23 mmol/L) and total cholesterol (0.12 mol/L) levels, and increased HDL-cholesterol levels (0.12 mol/L), without affecting LDL-cholesterol levels among dialysis patients (Zhu et al., 2014). There were no obvious differences in effects of different doses and components of fish oil on lipid levels (compared <2 g/day EPA + DHA supplement with ≥ 2 g/day). It was concluded that a high dose ≥ 2 g of fish oil supplement for lowering lipid levels might not be needed. Previously, metaanalyses obtained that fish oil reduce serum triglycerides levels significantly by 0.78 mmol/L with no significant effect on cholesterol levels (total, HDL and LDL-cholesterol) (Pei et al., 2012).

A metaanalysis concerning the differential effects of monotherapy with EPA or DHA showed that DHA significantly increased mean LDL-cholesterol levels by 7.23 mg/dL, whereas EPA nonsignificantly reduced mean LDL-cholesterol compared with placebo (Zhu et al., 2014). LDL-cholesterol concentration reduction remains the primary treatment goal concerning management of dyslipidemia. Prescription formulations and dietary supplements with a high content of EPA and DHA may reduce triglycerides levels, but increase LDL-cholesterol levels (Bradberry and Hilleman, 2013). The active components of fish oil that account for the lipid-altering effects is still unclear. After a period of DHA supplementation, when reported no significant changes in LDL or total cholesterol and a concomitant increase in HDL-cholesterol it was speculated that only EPA had lipid-modulating effects (Harrison et al., 2004). Recently, metaanalyses confirmed that EPA had a significant influence on lipid profile and EPA + DHA showed the same lipid-modulating effects as EPA alone in dialysis patients (Zhu et al., 2014).

Daily intake of 4 g of LC-$n-3$ PUFAs, consuming fish oil in the form of capsules, reduced triglycerides levels by 35%–45% (Kris-Etherton et al., 2002). Fish oil supplements rich in omega-3 fatty acids show minimal drug interactions and are safe for use in CVD patients. Until recently, an important limitation was that four capsules per day of over-the-counter

preparation with about 900 mg omega-3 fatty acids per capsule are required for daily treatment doses (Harper and Jacobson, 2008). Future large-scale randomized trials for long-term use treatment with adequate doses to measure site effects and tolerability are needed to evaluate the effect of omega-3 supplements on lipid status in CKD patients with focus on those with CVD on dialysis.

3.5 Soybean, Flaxseed, and Fish Oil Effects on Inflammation

As the kidney is the major site for elimination of many of cytokines, the delicate equilibrium of proinflammatory cytokines and their inhibitors is clearly dysregulated in CKD patients (Carrero, 2011). The C-reactive protein (CRP) level, proinflammatory cytokines, such as interleukins (IL)-1 and 6 and tumor necrosis factor-α (TNF-α), are significantly elevated in patients with ESRD.

3.5.1 Soybeans and inflammation

Inflammation is commonly associated with malnutrition and CVD in ESRD patients, and it has been proposed that soy products may have antiinflammatory properties (Beavers et al., 2009; Fanti et al., 2006). A significant decrease was present in serum CRP in animal model and in patients with diabetic nephropathy after 4 years of a soy consumption (protein intake 0.8 g/kg body weight per day with 35% soy protein) (Azadbakht et al., 2008). However, other studies have not confirmed any significant decrease in CRP, TNF-α, or IL-6 in patients on hemodialysis or with diabetic nephropathy (Fanti et al., 2006; Miraghajani et al., 2012). The explanation for this discrepancy in data may be the difference in duration of soy protein administration from 4 to 8 weeks and a follow-up period up to 4 years and low-dose of soy protein (less than 5 g/day) used in one study (Miraghajani et al., 2012). There is some evidence that consumption of 27 g/day of soy protein for 6 months resulted in a significant decrease in neutrophil–lymphocyte ratio, which is a marker of systemic inflammation (Tomayko et al., 2015).

Furthermore, substitution soy protein instead of animal protein for 8 weeks resulted in inverse correlation between serum isoflavones levels and CRP concentrations in patients with diabetic nephropathy (Fanti et al., 2006). Recently metaanalysis, which involved 12 studies, concluded that CRP level is reduced in soy-treated patients with CKD. However, no significant changes were detected in the dialysis subgroup compared to nondialysis patients (Jing and Wei-Jie, 2016).

3.5.2 Flaxseed and inflammation

Functional food-flaxseed and its components (oil or lignans) could have beneficial effects on human health due to their antiinflammatory effects. According to metaanalysis of long-term prospective studies CRP level is a good biomarker of inflammation and it is strongly positively associated with CVD risk (Kaptoge et al., 2010, 2014). Results of RCT suggest that

several dietary factors (fiber, lignans, isoflavones, ALA, EPA, and DHA) show cardiovascular protective properties (Alissa and Ferns, 2012).

Flaxseed (rich in ALA) could be an alternative source of EPA and DHA (synthesized from ALA in humans) that also show antiinflammatory effects. However, recent metaanalysis that evaluated the effectiveness of flaxseed and its derivatives on CRP showed no CRP-lowering effect (Ren et al., 2016). One possible explanation for this result could be low efficiency of ALA conversation to EPA and/or DHA (Plourde and Cunnane, 2007). Also, the effects of flaxseed oil on inflammation marker CRP may have been masked by the use of MUFA or $n-6$ PUFA-enriched oils as the control regimen (as they also show antiinflammatory effects compared to SFA). People with chronically elevated levels of inflammation (obese or elderly people, patients with CKD) tend to have higher levels of CRP in circulation and may be most likely to have benefit from flaxseed consumption (Dewell et al., 2011; Khalatbari Soltani et al., 2013; Lemos et al., 2012). This has been confirmed in the subgroup analysis in metaanalysis (Ren et al., 2016) that showed that CPR level was significantly reduced by 0.83 mg/L in studies among people with mean BMI over 30 kg/m^2. In addition, a trend toward a greater reduction of CPR in ages over 50 years is found.

In patients on dialysis dietary treatment with unsaturated fatty acid (Ewers et al., 2009) and fish oil (Bowden et al., 2009) led to decreased levels of CRP. Two studies indicate that flaxseed consumption reduces systemic inflammation in hemodialysis patients (Khalatbari Soltani et al., 2013; Lemos et al., 2012). Flaxseed oil contains ALA as EPA and DHA precursor and supplemented to hemodialysis patients in doses as 1.0 g/day in form of flax oil plus α-tocopherol (3.5 mg) twice a day for 120 days resulted in significant decrease of CRP levels (Lemos et al., 2012). Interestingly, in patients with CRP levels higher than 5.1 mg/L (inflamed patients), flax oil supplementation showed a greater CRP-lowering effect. The results of this study support the hypothesis that flaxseed oil consumption in CKD may have beneficial effects on CRP levels. A second study in hemodialysis patients showed that supplementation with 40 g/day ground flaxseed during 8 weeks significantly reduced CRP levels by 37% during 8 weeks (Khalatbari Soltani et al., 2013). The CRP-lowering effect of flaxseed consumption appears to be due to its high content of ALA, but it is unclear whether ALA itself exerts these effects or its product (EPA and/or DHA) after bioconversion.

3.5.3 N − 3 PUFAs and inflammation

The $n-3$ and $n-6$ series play a significant role in health and disease by generating potent modulatory molecules for inflammatory responses, including eicosanoids (prostaglandins and leukotrienes), and cytokines (interleukins) and affecting the gene expression of various bioactive molecules. Evidence from animal studies suggests a close relationship between PUFAs and renal inflammation and fibrosis, which are crucial stages in CKD (Baggio et al., 2005). This observation is important because it suggests that physiological levels of

fatty acids modulate inflammation. Although some epidemiological studies have shown an inverse correlation between $n-3$ PUFAs consumption and biomarker of inflammation, intervention trials have not yet confirmed this effect.

Data in dialyses patients of $n-3$ PUFAs effect on inflammation markers are sparse, with primary uncontrolled small cohorts, short study duration (8–12 weeks) and different doses of supplementation (capsules, 1.3–4 g/day) (Noori et al., 2012). In general, clinical trials show that supplementation with $n-3$ PUFA has the potential to reduce inflammatory markers in CKD patients (CRP, IL-6, TNF-α) (Huang et al., 2013) and soluble intercellular adhesion molecule type 1 (Gharekhani et al., 2014; Kooshki et al., 2011). There is some evidence that reduced circulating $n-3$ PUFAs in dialysis patients has an effect on few antiinflammatory properties. In older adults, high levels of plasma PUFAs were associated with lower concentrations of CRP as well as proinflammatory factors, such as IL-6 and TNF-α, but higher concentration of antiinflammatory parameters, such as IL-10 and transforming growth factor (TGF) (Ferrucci et al., 2006). Thus, more research focus should be given to the potential antiinflammatory effect of $n-3$ PUFA in patients with CKD. However, studies on dietary intake should be complemented by studies that investigate the relationship between fatty acid plasma levels and serum levels of multiple inflammatory markers.

Possible mechanisms of $n-3$ PUFAs protection of kidney function is based on preclinical studies by modulating the inflammatory response through transcriptional downregulation of the proinflammatory cytokines productions, activation of leukocytes and resident macrophages, vascular surface expression of endothelial leukocyte adhesion molecules, leukocyte transmigration, and the release of substances that lead to tissue injury (Calder, 2006; De Caterina, 2011). In a clinical study, supplementation with $n-3$ PUFAs with follow-up for 8 weeks significantly decreases leukotriene B4 (LTB4) and 5-hydroxyeicosatetraenoic acid, and increases 5-hydroxyeicosapentaenoic acid in patients with CKD (stages 2–5) (Maaløe et al., 2011). There was a theory that proinflammatory LTB4 may be of importance in the progression of CKD.

Given the evidence relating CKD to persistent low-grade inflammation, and that literature does not show a consistent association between $n-3$ PUFAs and inflammation in CKD, however, it was concluded that dialysis patients consume insufficient $n-3$ PUFA and consequently have low blood levels of fatty acid (Endres et al., 1993; Jump, 2002; Saifullah et al., 2007). Further clinical studies are necessary to verify the antiinflammatory properties of $n-3$ PUFAs in patients with CKD, especially those with chronic inflammation.

3.6 Soybean, Flaxseed, and Fish Oil Effects on Oxidation

An increased level of lipid peroxidation occurs in ESRD patients who lead to atherosclerosis (Caligiuri et al., 2013; Friedman, 2010; Wander and Du, 2000). Oxidative stress causes the

most pathological changes in the kidney, initially followed by an inflammatory reaction (Lauretani et al., 2008).

3.6.1 Soybeans and antioxidative status

Reactive oxygen species (ROS) and free radicals have significant role in the pathogenesis of nephrotic syndrome. Soy protein diet causes impressive changes in oxidative status markers and histopathological aspects of nephrotic syndrome as adding genistein to soy protein caused a greater improvement in this disease (Javanbakht et al., 2014). The imbalance between oxidant and antioxidant factors causes the glomerular damage in renal diseases (Ghodake et al., 2011). Intervention studies showed that soy protein consumption was associated with reduction in plasma lipid peroxides concentrations in renal transplant patients (25g/day for 5 weeks) and oxidized LDL level in hemodialysis patients (25 g protein, 4 times a week for 4 weeks) (Cupisti et al., 2007; Siefker and DiSilvestro, 2006). But, a soy protein diet in peritoneal dialysis patients has no effect on oxidized LDL concentrations after 8-week intervention (Imani et al., 2009).

3.6.2 Flaxseed and antioxidative status

Plant polyphenols are dietary antioxidants that show beneficial effects on human health (Kardum et al., 2014). As already mentioned in previous sections, SDG is the most abundant polyphenol found in flaxseed. SDG and its gut metabolites enterolactone and enterodiol exert high antioxidant potency in vitro (Kitts et al., 1999; Takić et al., 2016), but of its antioxidant activity in vivo are in not entirely clear. In a recent study (Moree and Rajesha, 2013) animals intoxicated with CCl_4 were treated with SDG (12.5 and 25 mg/kg/bw). The SDG treatment increased activities of catalase, superoxide dismutase, peroxidase, and decreased malonyl dialdehyde levels in kidney. Results of this study suggest that flaxseed consumption could improve kidney antioxidant status.

3.6.3 $N-3$ PUFAs and antioxidative status

Recent study shows that supplementation with fish oil show favorable change in antioxidant status (Popovic et al., 2014). EPA and DHA have beneficial effect in glomerular disease with attributed to their effect on the prooxidant and antioxidant status on the EFA status (Bouzidi et al., 2010; Das et al., 2001). On the other hand, PUFAs are very susceptible to oxidation as multiple double bonds are present in their structures. There are accumulated evidences suggesting that in patients on hemodialysis treatment oxidative mechanisms are deregulated and could result in increased peroxidation of plasma and tissue $n-3$ PUFA (Ikizler et al., 2013). So, there is a possibility that $n-3$ PUFAs may have potentially adverse effect on cardiovascular events in dialysis patients. However, this has not been shown in clinical studies that have examined dialysis patients (Hassan et al., 2010; Taziki et al., 2007). Patients with CKD exhibit increased lipid peroxidation in erythrocytes with a reduction of their LC-PUFA content (Peuchant et al., 1997). In the RCT, EPA supplementation (1.8 g/

day) during a 12-week intervention led to lower oxidized LDL by 38%. Two months of oral omega-3 (3 g/day) led to a decrease of indices of lipid peroxidation in patients following kidney transplantation (Khosroshahi et al., 2012). Recent evidence does not support the idea that $n-3$ PUFAs upregulate oxidative stress and further studies are needed to make more definitive conclusions (Hung et al., 2015).

3.7 Soybean, Flaxseed, and Fish Oil Effects on Blood Pressure

Hypertension is present in more than 80% of patients with CKD, leads to progression of kidney disease and contributes to cardiovascular events, such as heart attack and stroke. The NKF guidelines recommend a blood pressure goal of <130 mmHg for systolic blood pressure (SBP) and <80 mmHg for diastolic blood pressure (DBP) for CKD population (Harper and Jacobson, 2008). Soybean nuts consumption has no effect on blood pressure in adult population (Mohammadifard et al., 2015).

3.7.1 Flaxseed and hypertension

Evidence suggests that flaxseed and its components consumption show potential blood pressure-lowering effect (Caligiuri et al., 2014a,b; Cicero et al., 2010; Ursoniu et al., 2016). The effect on blood pressure could be achieved due to bioactive ingredients of dietary flaxseed ALA, lignans, fiber, or peptides or their synergistic effect. Results of published reviews suggest that dietary supplementation with whole flaxseed may reduce both SBP (by 1.77, Caligiuri et al., 2014a; and 2.85 mmHg, Ursoniu et al., 2016) and DBP (by 1.16, Caligiuri et al., 2014a; and 2.39 mmHg, Ursoniu et al., 2016). The magnitude of reduction in blood pressure reported in these metaanalyses is small; but even a 2-mmHg reduction in SBP may decline relative risk of stroke mortality by 10% and mortality from ischemic heart disease by 7% (Lewington et al., 2002). Based on these metaanalyses results, consumption of whole or ground flaxseed, but not oil and lignan extract, lead to significant reductions in SBP. DBP was significantly reduced with flaxseed powder and oil but not lignan extract. The magnitude of BP reduction was slightly higher among patients with mean baseline SBP >130 mmHg compared with participants with mean SBP <130 mmHg. Subgroup analyses on the basis of supplementations duration indicated that longer consumption of flaxseed (intervention duration >12 weeks) could significantly reduce DBP to a greater extent than interventions with durations of <12 weeks. As several studies used concentrated flaxseed extract (oil or lignans supplements) the dose-dependency analyses of the effect of flaxseed on blood pressure was not applicable. However, clinical trials show that consumption of 30–45 g of flaxseed per day can result in mild gastrointestinal discomfort, flatulence and bloating and incorporation of flaxseed into a variety of foods can improve adherence (Pruthi et al., 2012).

The biological mechanisms involved in antihypertensive effects of flaxseed are not entirely clear. However, it is known that dietary ALA reduce activity of soluble epoxide hydrolase

that produce proinflammatory oxylipins (Caligiuri et al., 2014a) and oxylipins are found to be responsible for loss of vasodilatation and progression of inflammation in arterial hypertension. The most abundant lignan found in flaxseed named SDG is a phytoestrogen known to be an angiotensin-converting enzyme (ACE) inhibitor (Prasad, 2013). The lignans converted to enterolignans in gastrointestinal tract may be antihypertensive through an antioxidative action as reactive oxygen species play a role in hypertension (Montezano and Touyz, 2012). Flaxseed peptides may also induce an antihypertensive due to high content of arginine converted in the vascular endothelium to nitric oxide (NO) that leads to vasodilatation (Udenigwe and Aluko, 2012). Flaxseed consumption has many advantages as an alternative or complementary strategy for antihypertensive treatment option of patients who cannot control their blood pressure with medication, cannot afford or do not have access to medication, or for patients who prefer a dietary approach. In patients already taking antihypertensive medication, flaxseed decreased the percentage of patients with uncontrolled hypertension by 17% (Rodriguez-Leyva et al., 2013). Even if some individuals do not exhibit a decrease in blood pressure with flaxseed, the addition to the diet is positive as it contains many nutrients that most individuals do not consume in adequate quantities (Caligiuri et al., 2014b).

Although the antihypertensive effect of flaxseed and flax oil consumption has been tested in clinical trials and potential beneficial effects are described for certain disorders (such as dyslipidemia and CVD), there are little literature data of flaxseed potential antihypertensive effects in CKD patients. The supplementation of diet with 30 g/day of seed mixture flax/sesame/pumpkin in weight ration 3:1:1 for 12 weeks showed antihypertensive effect in hemodialysis patients (Ristić-Medić et al., 2014). Dietary supplementation with seed mixture reduced both SBP and DBP by 11 and 6 mmHg, respectively. The significant reduction of blood pressure indicates that dietary consumption of flax or/and sesame or/and pumpkin could be beneficial in treatment of hypertension in hemodyalysis.

3.7.2 N − 3 PUFAs and hypertension

A metaanalysis concerning the dose response of therapy with fish oil on blood pressure showed that 5.6 g of $n-3$ PUFAs (supraphysiological dose) decrease blood pressure by 3.4/2.0 mmHg in hypertensive subjects. Supplementation with 2.4 g/day of fish oil during 8 weeks had no significant effect on blood pressure in patients on hemodialysis treatment (Svensson et al., 2004). However, several studies reported that therapy with fish oil reduced diastolic (7–15 mmHg) and/or SBP (16–30 mmHg) during treatment period from 8 to 52 weeks in hemodialysis patients, but only few studies are RCT (Mori et al., 2009; Schmitz et al., 2002; Svensson et al., 2004). A recent study showed that daily intake of 4 g of fish oil lowered SBP and DBP by 3.3–2.9 mmHg during 24 h ambulate measurement in patients with CKD (Mori et al., 2009). A proposed mechanism was that fish oil supplementation led to changes of the metabolism of the eicosanoids, hydroxyeicosatetraenoates and/or epoxyeicosatrienoates (McGiff and Quilley, 2001). Evidence from animal models and cell

culture studies also indicate that $n-3$ PUFA supplementation can lower blood pressure and proteinuria, potentially by $n-3$ PUFA vasorelaxation action with increase endothelium-derived releasing factor (Das, 2004) and by effects on TGF-beta, renin, fibronectin, and NO synthesis (Baggio et al., 2005). More, well designed studies are needed to determine the doses efficacy of fish oil rich in $n-3$ PUFA supplementation in patients with CKD.

3.8 The Effect of n – 3 PUFAs Supplementation on Thrombosis and Vascular Access Patency

Vascular access dysfunction is a common problem in patients undergoing hemodialysis treatment. Thrombosis occurs with high rates of all arteriovenous grafts (AVG) (in more than 50%) in the first year after placement (Irish et al., 2009). Maintaining a long-term functioning arteriovenous access is a major clinical challenge in management of dialysis patients (Bradley and Dixon, 2012). Fish oils have antithrombotic, antiproliferative, and platelet antiaggregatory effects and reduce intimal hyperplasia in autogenous grafts (Sarris et al., 1989). In addition, it has been proposed that $n-3$ PUFAs supplementation may improve arteriovenous fistula (AVF) and AVG patency in dialysis patients (Friedman, 2010; Jackson et al., 2011). Fish oil supplementation (3.2 g/day) may reduce the risk of AVF thrombosis in hemodialysis patients with a 60% higher graft patency rate in the first year (Schmitz et al., 2002). Possible mechanisms include inhibition of the inflammatory pathway and secretion of platelet-derived growth factor at the anastomosis site; 4 g fish oil up for 12 months showed no significant effect on proportion with graft thrombosis and/or radiological and/or surgical intervention to maintain graft patency comparing recipients of fish oil and placebo. However, fish oil treatment was associated with improved graft patency, lower rates of thrombosis and of failures (Lok et al., 2012). Thus, consumption of fish oil is still a promising dietary measure against and for prevention of thrombosis, but there was no significant difference in vascular access blood flow on native AVF and synthetic graft patency in chronic hemodialysis patients (El-Shinnawy et al., 2015). Currently, there is one large ongoing RCT designed to evaluate whether the use of aspirin (100 mg/day) and/or $n-3$ PUFAs (4 g/day) could improve the outcome of surgery to de novo creation of AVG (Palmer et al., 2015; Viecelli et al., 2015). The routine use of $n-3$ PUFAs supplementation in clinical practice could lead to a significant reduction in thrombosis and an increase in functionality of AVF. The results are not yet available. In the meanwhile recently metaanalysis RCT showed that fish oil supplementation reduced the risk of AVG events (He et al., 2016).

3.9 Soybean, Flaxseed, and Fish Oil Effects on Cardiovascular Events and Mortality

Dialysis patients have a high incidence of CVD as well as increased premature mortality (Shastri et al., 2012; Stenvinkel, 2010). In patients with high CKD mortality, mortality is mostly attributed to cardiovascular events, and may be related to chronic inflammation.

Adults with ESRD treated with hemodialysis experience mortality between 15% and 20% each year.

3.9.1 Soybeans and CVD

Soy protein–containing food is approved to be labeled as protective against CVD in 1999 by the US Food and Drug Administration (1999) and the American Heart Association released a similiar advisory shortly thereafter (Erdman, 2000). The UK Joint Health Claims proposed at least 25 g soy protein per day as part of diet low in saturated fat that can help reduce blood cholesterol. In 2012, the European Food Safety Authority concluded that a cause and effect relationship had not been established between the consumption of isolated soy protein and a reduction of plasma LDL-cholesterol levels (EFSA, 2012).

3.9.2 Flaxseed and CVD

Flaxseed and flaxseed oil could be beneficial in prevention of CVD in renal patients and consequently cardiovascular events and mortality. Antiinflammatory and lipid-lowering effects after dietary treatment with flaxseed and flaxseed oil in hemodialysis patients (Khalatbari Soltani et al., 2013; Lemos et al., 2012) indicate that flaxseed could be beneficial in prevention of CVD in dialysis patients. Erythrocyte DHA levels are important factors that determine risk of mortality in hemodialysis patients (Hamazaki et al., 2011; Terashima et al., 2014). Flaxseeds represent abundant dietary source of plant $n-3$ PUFA. ALA can be converted to EPA and DHA in vivo, however the efficiency of its metabolism could be low. The percentage of EPA and DHA in serum phospholipids of hemodialysis patients was increased after supplementation of their diet with milled flax/sesame/pumpkin mixture (Ristić-Medić et al., 2014) and as mentioned earlier, literature data suggest that $n-3$ status could be an important factor associated with mortality risk in hemodialysis patients.

3.9.3 N − 3 PUFAs and CVD

The beneficial effects of $n-3$ PUFAs supplementation on cardiovascular reduced mortality are an area of active investigation (Bessell et al., 2015; Tayyebi-Khosroshahi et al., 2012). Accumulated evidences from experimental and clinical trials suggest that LC-$n-3$ PUFAs may reduce sudden cardiac death incidence. Putative mechanisms include both the effects on eicosanoid production as well as on structure and function of cell membrane (Mozaffarian and Wu, 2011). An inverse relationship between dietary fish intake and erythrocyte DHA content and mortality has been observed in CKD (Friedman et al., 2008; Hamazaki et al., 2011; Kutner et al., 2002). Dialysis patients who reported fish consummation ("regular" fish-eaters during any one of two 3-day food diaries or during one 24-h food recall) have approximately 50% lower rate of mortality over 3 years. However, supplementation with 1.7 g $n-3$ PUFA in hemodialysis patients with established CVD had no improvement on the total number of cardiovascular events and primary end point of all-cause mortality in these

patients. As secondary outcome, this treatment with $n-3$ PUFA for 2 years significantly reduced the number of myocardial infarctions in these high-risk patients (Kutner et al., 2002).

The risk of mortality was 2.5 times higher in hemodialysis patients with a lower erythrocyte $n-3$ PUFA content expressed as omega-3 index compared to those with omega-3 index higher than 4.69% (Friedman et al., 2008). Risk of mortality for patients with the highest erythrocyte DHA levels (higher than 8.1%) was 57% lower at 5-years follow-up period (Hamazaki et al., 2011) and 48% at 10 years (Terashima et al., 2014) compared to those with lowest DHA tertile levels. In a cohort of Japanese hemodialysis patients' lowest quartile of serum omega-3/omega-6 ratio increases 5-year risk of cardiovascular events compared with highest quartile (Shoji et al., 2013). Similar results were obtained in the studies that analyzing the association between risk of sudden cardiac death and blood $n-3$ PUFAs levels in the United States (Friedman et al., 2013a,b). The risk of sudden cardiac death was lower in quartile groups with higher $n-3$ PUFAs levels compared to the first quartile group with the lowest $n-3$ PUFA content (Friedman et al., 2013a). Moreover, baseline serum albumin and 22:5 $n-3$ were inversely associated to the odds of sudden cardiac death (Friedman et al., 2013b). However, no association was observed between plasma phospholipids $n-3$ PUFA content and mortality (Huang et al., 2012). Differences in results may be attributed to the substantially different amount of fish intake in the study populations, methodological issues (food frequency questionnaires or 24-h food recall versus plasma vs. erythrocyte PUFA content) as well as study design. As levels of LC-$n-3$ PUFA (EPA, 22:5 $n-3$ and DHA) are modifiable by diets in patients on hemodialysis (An et al., 2012; Omrani et al., 2015; Peruničić-Peković et al., 2007, 2012; Rasic-Milutinovic et al., 2007), their risk of sudden cardiac death and possibly other cardiovascular events could be reduced by supplementing patients diet with fish oil. In a recent metaanalysis, fish oil intake significantly reduced the risk for cardiovascular events (He et al., 2016). Arrhythmias are often found in patients with high risk of sudden cardiac death.

It was demonstrated in experimental and clinical models that $n-3$ LC- PUFAs protect against malignant ventricular arrhythmias. The presence of arterial fibrillation in hemodialysis patients with CVD is linked to a lower level of plasma DHA (Kirkegaard et al., 2012). Thus, low DHA, low serum total cholesterol, increased SBP, and age all correlated independently with arterial fibrillation. Treatment strategy toward the serious supraventricular and ventricular arrhythmias in hemodialysis patients might be by increasing the $n-3$ PUFA intake. Supplementation with 1.7 g $n-3$ PUFA in hemodialysis patients did not significantly change the QTc (time taken for ventricular hyperpolarization and then repolarization) in these patients, but subgroup analyses linked to possible shortening effect of $n-3$ PUFA on QTc interval. Prolonged QTc interval was an independent risk factor of mortality in CRD patients (Hage et al., 2010). In the future, better-powered studies will need to be conducted to found out more about the relationship between fish or omega-3 supplements intake with $n-3$ PUFAs status in circulation and heart rate variability in hemodialysis patient with no arterial fibrillation on baseline.

3.10 Soybeans, Flaxseed, and n − 3 PUFAs in the Prevention of Polycystic Kidney Disease Progression

Polycystic kidney disease (PKD) is characterized by multiple fluid-filled benign cysts derived from the epithelial lining of the nephron characterized that contribute to massive kidney enlargement, structural damage, and loss of renal function at an early age (Mochizuki et al., 2013). PKD is the third leading cause of ESRD in autosomal PKD patients. Complications of PKD are hypertension at an early age and greater risk of CVD (Gevers and Drenth, 2013). *N* − 3 PUFAs influence the activation of transcription factors, expression of inflammatory genes, and the production of mediators regulating inflammation (Calder, 2006). Since these cellular functions are directly involved in PKD pathogenesis nutraceutical dietary modulation may be an effective means to attenuate PKD progression and severity (Fig. 11.5) (Gevers and Drenth, 2013; Maditz et al., 2013).

3.10.1 Soybeans and PKD

Dietary soy protein has been shown to have renal protective effects in various animal models of PKD (Fig. 11.5) . Additionally, research shows that soy protein consumption attenuates early stages of cyst pathogenesis as indicated by reduced epithelial cell proliferation

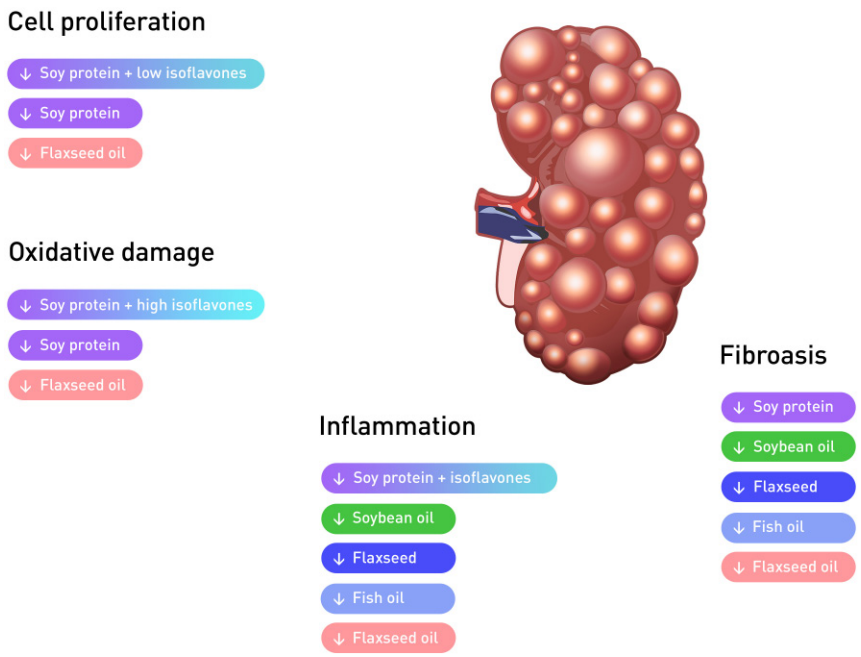


Figure 11.5: Summarized Impact of Soybean, Flax, and Fish Oil in the Pathogenesis of Kidney Cyst.

and apoptosis. Furthermore, in later stages the attenuation of cyst pathogenesis by soy consumption is also present; however, it is indicated with reduced oxidative damage, inflammation, and fibrosis (Maditz et al., 2013, 2014). Rats fed soy protein diet for 6 weeks had lower kidney weights, cyst area, low serum creatinine, serum urea, and higher creatinine clearance than rats fed casein diet (Aukema and Housini, 2001). Insulin growth factor-1 is considered as part of the mechanism of effects of soy diets. It could be concluded that dietary soy protein delays progression of PKD (Aukema and Housini, 2001). When offspring exposed with soy protein prenatally and during lactation reduced renal cyst changes and improved renal function was found (Cahill et al., 2007). Longer-duration feeding with soy protein had beneficial effect to renal fibrosis. It was reduced 22% after 1 week and 38% after 3 weeks soy diet. Soy protein also resulted in less cyst growth, but the diet effect was significantly only after 3 weeks of diet consumption. Dietary soy protein also partially ameliorated the suppression of prostaglandin E2 production observed in kidney diseases, which is suggested to be protective against disease progression in the early stages of PKD when inflammation is low (Fair et al., 2004).

3.10.2 Flaxseed and PKD

Dietary flaxseed rich in ALA and SDG has been investigated as dietary therapeutic option for PKD (Fig. 11.5) (Ogborn et al., 2006). Based on animal studies on PKD model, consumption of flaxseed oil (7 wt.%) resulted in lower renal cyst area, inflammation, lower renal epithelial cell proliferation, oxidized LDL tissue area, and fibrosis compared to corn oil diet. In female rats, possibly due to well-known estrogen influence on ALA conversation to EPA and DHA (Petrovic et al., 2014), greater renal benefits were indicated. In rats on diet supplemented with whole flaxseed (10 wt.%) for 8 weeks reduced renal cyst volume, inflammation, fibrosis, and improved kidney function were noticed (Ogborn et al., 2006). The results suggested that flaxseed might have dietary components in addition to ALA with potential effect on PKD progression and severity. Flaxseed is the richest source of SDG that shows antioestrogenic and antioxidant effects. It is already shown that antioxidants can prevent tissue injury in PKD, as results of a study on animal model indicate that tissue difference in vitamin E may be responsible for slower PKD progression in female rats. Han: SPRD-cy rats fed diet supplemented with SDG (20 mg/kg) for 12 weeks reduced renal inflammation in rats of both gender and lowered oxidized LDL renal tissue area in female rats (Ogborn et al., 2006). Evidences obtained from animal studies have several limitations when extrapolate effects to human. The main two reasons are that ALA conversion to EPA and DHA is more efficient in mice and rats than humans and diet therapy is typically implemented at more advanced disease stages in humans.

3.10.3 N − 3 PUFAs and PKD

Supplementation of $n-3$ PUFAs attenuated epithelial cell proliferation, oxidative damage, inflammation, and fibrosis (Deckelbaum and Torrejon, 2012). In this way dietary $n-3$

PUFAs have the potential to influence multiple steps in PKD pathogenesis. Currently, animals models demonstrated that EPA/DHA and EPA-enriched fish oil was beneficial, but DHA-rich algal oil (doses 16%–20% kcal of total energy intake) had adverse effects on PKD (Tou et al., 2015). However, rat model of autosomal recessive PKD showed that soy protein and fish oil supplemented rats had higher cyst obstruction and serum urea nitrogen concentration compared to diet groups (Maditz et al., 2014). Despite increasing renal tissue DHA content there were no significant effect on renal tissue inflammation and proliferation. Based on these results, dietary soy protein and/or $n-3$ PUFAs did not attenuate kidney disease progression in the female rat model of autosomal recessive PKD.

Only one human clinical study has been conducted on dietary $n-3$ PUFA using EPA supplementation on cyst pathogenesis (Higashihara et al., 2008). There was no efficacy of EPA capsules (2.4 g/day EPA) supplementation for duration of 2 years on kidney volume as measured by computerized tomography, or on 24-h urine creatinine clearance in patients with PKD. Study limitations were the small number of subjects and supplementation at the stage 2–3 CKD. This is in contrast to collectively animal studies that demonstrated beneficial effects of dietary $n-3$ PUFA supplementation on PKD (Lu et al., 2003; Maditz et al., 2013). It is suggested that increased renal cyclooxigenase-2 (COX-2) expression by diets containing EPA and DHA or DHA alone reflected a decrease in renal COX-2 activity. In animal models of renal cystic disease COX-2 expression is reduced, but activity is increased (Warford-Woolgar et al., 2006). Dietary DHA can regulate COX-2 through the transcription factor, NF kappa B and EPA is a direct substrate for COX-2. Thus, synthesis of the less potent 3-series eicosanoids through the COX pathway can reduce inflammatory responses that contribute to PKD progression. Animal studies showed that dietary $n-3$ PUFAs treatment regulates multiple steps in PKD cyst pathogenesis, first attenuated the early stages of cyst pathology of epithelial cell proliferation and oxidative damage as well as the later stages of inflammation and fibrosis but had inconsistent effects on renal function (Tou et al., 2015).

It could be concluded that renal beneficial effects differed depending on whether the type of dietary lipid sources rich in ALA, EPA, and/or DHA was consumed with limited to the early stages of PKD progression (Sankaran et al., 2007; Tou et al., 2015). A better understanding of the role of nutraceutical soybeans, flax and $n-3$ PUFA in PKD can contribute to the development of nutrition recommendations and diet-based therapies to reduce PKD progression and severity.

4 Conclusions

In conclusion, soybeans, flaxseed, and fish oil as nutraceuticals have important benefits for a variety of kidney disease-related complications. However, research in this area is still in its early stages and needs confirmation on experimental, epidemiological, biomarker, and clinical levels. Therapeutic foods ingredients show lipid-lowering effects and lower the overall risk

for CVD induced by dyslipidemia in renal disease. Evidence generated from studies using animal PKD models point to more preclinical and clinical studies investigating efficacy, safety, and to prove the therapeutic value of $n - 3$ PUFA supplementation for inhibiting PKD progression. Well-designed and controlled studies are required to discern that soybeans, flaxseeds, and fish oil supplementation might reduce the progression of chronic renal disease toward chronic renal failure and eliminate some clinical outcomes in renal disease with highlight of demonstrate the safety and tolerability of them in the long-term patients treatment.

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CHAPTER 12

Maca, A Nutraceutical From the Andean Highlands

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1 Introduction

A millenarian Peruvian plant growing at the highlands more than 4000 m altitude is currently of great interest worldwide. This is an interesting situation because around the 1980s, maca was declared in danger of extinction as a domesticated plant by the International Board for the Protection of Genetic Resources (Valerio and Gonzales, 2005). In fact, at that time, maca cultivation was focused on small lands and used only for self-consumption.

This plant grows in adverse environmental conditions characterized by low environmental pressure, extreme cold, high UV and cosmic radiation, hard winds, heavy rain, and low humidity. It is possible that this plant adapted to this adverse environment incorporating primary and secondary metabolites that allow its survival in such adverse conditions. These metabolites become important and useful for humans using this plant for consumption as food. In fact, the population consuming maca and living in the Central Peruvian highlands have higher scores in the Health-Related Quality of Life (HRQL) questionnaire than those living at the same place but nonconsuming maca (Gonzales, 2010).

After maca became in danger of extinction, cultivation was reactivated at the end of the 20th century due to the intervention of a pharmaceutical company publicizing maca as "Andean Viagra" or "Andean Gingseng." This increased the interest in international markets particularly in Asian ones. However, as maca does not have properties as clear as Viagra, the interest on maca dropped. Then, at the end of the 1990s, the amount of maca exportation was reduced dramatically (Vásquez-Apestegui, 2010).

Since 2000, scientists have been contributing evidence to demonstrate biological properties of maca and its importance as nutraceutical or functional food (Valentová and Ulrichová, 2003). In 2000, the first article showing that maca has two novel groups of compounds, macamides, and macaenes appeared. This study also showed that lipidic fractions of maca containing macamides increased sexual behavior in rodents (Zheng et al., 2000).

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In 2001 results of a clinical trial with gelatinized maca showing an increase in sperm count in healthy adult men were published (Gonzales et al., 2001a). Thereafter, it was demonstrated that gelatinized maca does not affect serum testosterone or estradiol levels (Gonzales et al., 2003a).

Many varieties or phenotypes of maca characterized by different external colors were reported (Valerio and Gonzales, 2005) and after were proved to have different biological properties (Gonzales et al., 2009).

This chapter was designed to discuss the reports about biological properties of maca acting as a nutraceutical.

2 A Crop Native From the Peruvian Highlands

Maca is a cultivated plant belonging to the Brassicaseae family, to the genus *Lepidium* and it is known as *Lepidium meyenii*, according to identification by Gerard Walpers. This author coined the name in specimen from the Peruvian Southern Andes. As maca is mainly found in the Peruvian Central Andes and to the fact that phenotypically looks different to the specimen showed by G. Walpers in 1843, it has been suggested that maca constitute a different species and it is named *Lepidium peruvianum* Chacon (Meissner et al., 2015b). However, specimen that the authors use as *L. meyenii* was obtained from Argentina, and there is no assurance that this specimen is *L. meyenii*.

Maca grows mainly above 4000 m altitude without fertilizers or pesticides (Gonzales, 2012). It is suggested that maca has protective genes that may act against pathogen microorganisms (Solis et al., 2007).

It is known that maca is cultivated at least since 600 years before Christ as first evidence of culture was observed in Óndores in the Peruvian central Andes (Matos-Mendieta, 1975). As pre-Hispanic Peruvian natives did not develop writing, it is not possible to obtain information about cultivation and consumption of maca from that period. Most of the information is transmitted from generation to generation through oral communication.

The first description about maca seems to be that from Cieza de Leon (1553), who indicated that natives in the central Andes consumed a crop based on roots growing in Bombon Lake (now Junin Lake in the Bombon plateau) different from maize. In this period, Yaros settled these lands (Brinckmann and Smith, 2004).

In 1549, it was described that natives from Chinchaycocha used maca to contribute as a tribute to the Spanish Encomendero (De la Puente and Janssen, 2014). In 1562, Ortíz de Zúñiga (1967) traveling to Leon de Huánuco describes that maca is used as barter among Yaros from Chinchaycocha. In 1628, Antonio Vasquez de Espinoza described that in the province of Chinchaycocha an edible root resembling the turnip called maca by natives

grew. This author described that the land in which maca is cultivated became sterile for at least 30 years. He also describes that despite the climate, the place is very populated. This description of maca seems to be earlier than that from Bernabe Cobo, who wrote the book *History of the New World* in 1653 (Cobo, 1956).

3 The Tradition Behind Maca

Traditionally, maca is cultivated without use of synthetic fertilizers or pesticides. In the Peruvian central Andes, maca is cultivated near the summer season between November and December each year and harvested during June and July. After harvesting, maca hypocotyls are dried naturally for several weeks. Fresh maca is characterized because it has high water content. However, scientific study showed that over the course of 3 weeks, despite the evidence of dehydration, the total starch content or the alpha and beta-amylase activities were maintained (Rondán-Sanabria et al., 2012).

Maca is traditionally used because of its nutritional characteristics and fertility-enhancing properties (Gonzales, 2010). These properties have been demonstrated scientifically since 2000 (Canales et al., 2000; Gonzales et al., 2001a,b). Both properties have been described since the 16th century (Cobo, 1956; Vasquez de Espinosa, 1948).

Up-to-date maca is consumed after a natural drying process (Gonzales, 2010). It is probable that this procedure may release nonuseful volatile compounds. Recently, a study showed that the traditional drying process is fundamental for bioactive metabolites to be formed since they are absent in fresh maca (Esparza et al., 2015).

As is well known among farmers, maca depletes the nutrients of the land. For such a reason is a common practice in the Peruvian central Andes, to leave the land in rest for up to 5 years, to allow recovery after harvesting maca.

4 The Science Behind Maca

4.1 Phenotypes of Maca and Differential Properties

Maca is originally native from Peru in South America. Recent data show that maca is also cultivated in China using Peruvian seeds (Zhang et al., 2015). However, several differences in the content of mineral elements between maca from Peru and China have been reported. In fact, sodium and nickel contents were higher in Chinese maca than in Peruvian (Zhang et al., 2015). These minerals may be toxic after consumption.

Maca has different secondary metabolites and benzyl glucosinolates have been claimed to be the chemical marker. However, glucosinolates may be metabolized in the plant, as well as in the organism. In such a sense, glucosinolates are not suitable as chemical markers for maca (Gonzales et al., 2007).

Instead, macamides (nonpolar, long chain fatty acid *N*-benzylalkamides) are present in dry maca hypocotyls as distinctive of the plant because it is not present in any other plant. The amount of macamides measured in dried maca samples varies between 0.0016% and 0.0123% (McCollom et al., 2005). The macamides analysis of several maca products commercially available showed similarity in the qualitative pattern but significant differences in the concentration (0.15%–0.84%). These data suggest that daily intakes vary from 1.52 to 14.88 mg (Ganzera et al., 2002).

In the hypocotyls of *L. meyenii* a novel compound has been described: macaridine, a benzylated derivative of 1,2-dihydro-*N*-hydroxypyridine, together with macamides (benzylated alkamides): (1) *N*-benzyl-5-oxo-6E,8E-octadecadienamide, (2) *N*-benzylhexadecanamide, and the acyclic keto acid, (3) 5-oxo-6E,8E-octadecadienoic acid (Muhammad et al., 2002), (4) *N*-benzyl-9-oxo-12Z-octadecenamide, (5) *N*-benzyl-9-oxo-12Z,15Z-octadecadienamide, (6) *N*-benzyl-13-oxo-9E,11E-octadecadienamide, (7) *N*-benzyl-15Z-tetracosenamide, and (8) *N*-(m-methoxybenzyl)hexadecanamide. These compounds are exclusive for *L. meyenii* and could be used as markers for authentication and standardization (Zhao et al., 2005). More recently, liquid chromatography-ultraviolet detection-tandem mass spectrometry (LC-UV-MS/MS) and partial least squares discriminant analysis (PLS-DA) have been used to measure macamides to differentiate maca samples. For this, five macamides were selected as chemical markers to identify maca (Pan et al., 2016).

Two new imidazole alkaloids (lepidiline A and lepidiline B) have been isolated from a hypocotyl extract of *L. meyenii* and were identified as (1) 1,3-dibenzyl-4,5-dimethylimidazolium chloride and (2) 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride, respectively (Cui et al., 2003).

Recent studies reveal the dynamic of macamides production. Macamides are absent in fresh undamaged hypocotyls but production increases during drying process (Esparza et al., 2015). Isothiocyanate produced from glucosinolates increases benzylamine products during the drying process, which joined fatty acids produces macamides (Esparza et al., 2015). At this time, 19 macamides have been described (Wu et al., 2013).

As referred to earlier, maca is present in nature in different phenotypes distinguished by external colors ranging from white to black (Valerio and Gonzales, 2005). To date, most of the scientific research has been performed with yellow, black, and red maca (Gonzales et al., 2014). Black maca shows the best results on spermatogenesis, memory and fatigue, whereas red maca reverses benign prostatic hyperplasia (BPH) and osteoporosis (Gonzales et al., 2014).

Although the composition of secondary metabolites may have the same pattern, differences between colors seem to be due to different proportions of the secondary metabolites. In fact, the lead-colored, yellow and violet maca hypocotyls were rich in glucosinolates, macaene, and macamides, respectively (Clément et al., 2010a).

Table 12.1: Maca samples.

Compound	Yellow	Red	Black
Formic acid	6.41	6.05	4.52
Adenine	4.39	4.97	4.12
Fumaric acid	22.16	25.98	18.78
Uridine	6.67	7.70	8.35
Sucrose	585.48	582.85	590.88
Glucotropaeolin	49.98	54.71	53.69
Macamides	41.23	45.05	37.72
Choline	263.34	281.97	256.68
GABA	78.93	96.52	96.24
Malic acid	109.96	118.03	91.99
Glutamine	78.83	83.99	80.66
Proline	1203.19	1190.66	834.27
Fatty acids	410.41	419.05	370.51
Alanine	151.06	165.00	174.05
Valine	82.42	97.58	95.70
Phytosterols	26.06	27.95	24.82

It is important to develop methods that allow differentiating maca from different colors. Recently, it has been reported that HPLC measurements and DNA techniques provide a "fingerprint" to identify individual maca phenotypes (Meissner et al., 2015a).

NMR profiling combined with chemometric analysis was applied to identify yellow, pink, violet, and lead-colored maca (Zhao et al., 2012). A total of 16 metabolites were identified by NMR analysis. Most of the metabolites vary according to planting sites. Differences with colors were observed in one place of planting for formic acid, sucrose, glucotropaeolin, glutamine, and alanine. In another place of planting difference by colors were observed for GABA, malic acid, and proline.

Table 12.1 shows metabolite levels in dry maca hypocotyls obtained from Junin, Peru (values in the table are the relative intensities of the characteristic peaks of the compounds to the internal reference TSP. Proton number contributing to the peak is not taken into consideration).

According to Table 12.1, less proline is observed in black maca than in red or yellow maca. In other plants, high levels of proline enhance cold tolerance (Peng et al., 2015; Yuan et al., 2015). It is possible that black maca may not grow well in lower temperature conditions.

4.2 Biological Properties

4.2.1 Maca and the immune system

Scientific evidence has demonstrated a role for maca on immune system. Maca is a cruciferous and it is well known that cruciferous are antiinflammatory foods (Tilg, 2015).

In a population living permanently at 4200 m altitude, people traditionally consuming maca showed lower serum interleukin 6 (IL-6) levels than those nonconsuming maca. In addition, low serum IL-6 levels were associated with better response in the sitting down and standing up test 5 times in a chair without using hands or arms (Gonzales et al., 2013a).

Oral administration of red maca extracts increased serum interferon gamma in a dose-dependent fashion without affecting serum IL-4 in ovariectomized rats (Leiva-Revilla et al., 2014). Interferon gamma is a compound responsible of TH1 immune response (Schoenborn and Wilson, 2007), whereas IL-4 is a marker of TH-2 immune response. With another model of study, Gasco (2014) demonstrated that treatment with red maca extracts in rats with benign prostatic hyperplasia induced by testosterone enanthate resulted in increase in interferon gamma in prostate tissue.

TH1 response is involved in intracellular infections (Matucci et al., 2014). In such sense, red maca may be useful as coadjuvant therapy for these infections or as coadjuvants for vaccine production. These include bacterial, parasites, and viruses intracellular infections.

Maca hypocotyls also contain two new flavolignans, one of them (tricin 4′-O-[threo-β-guaiacyl-(7″-O-methyl)-glyceryl)ether and Lepidiline B were modestly active for inhibiting nitrite production in macrophage (tricin 4′-O-[threo-β-guaiacyl-(7″-O-methyl)-glyceryl)ether and Lepidiline B, and tricin are selectively active against HL-60 cells with IC50 values of 40.4, 52, and 52.1 uM, respectively (Bai et al., 2015).

Similarly, methanolic extracts of maca have potent inhibitors of influenza viruses (type A and B) infecting Madin-Darby canine kidney (MDCK) cells (Del Valle Mendoza et al., 2014).

4.2.2 Maca as sexual enhancement product

The use of herbal supplements to enhance sexual functions is increasing worldwide. Uncontrolled availability of these products could be of public health concern (Corazza et al., 2014). Maca has been described as a plant with effects as a sexual behavior enhancer in animals and humans (Balick and Lee, 2002). In male rats, maca produced a small effect on sexual behavior during acute and short-term administration but not during long-term (21 days) treatment (Lentz et al., 2007). Hexanic maca extract containing the most nonpolar compounds but not methanolic extract administered subacutely improved most effectively sexual behavior in sexually inexperienced male rats (Cicero et al., 2002).

In another study, both acute and chronic (15 days) oral administration of maca extract significantly improved sexual behavior in male rats, in an effect that is independent from its action on spontaneous locomotor activity (Cicero et al., 2001).

In human beings, oral consumption of gelatinized maca increased sexual desire at 8 and 12 weeks of treatment, and this effect is not due to changes in either Hamilton scores for depression or anxiety and the effect is observed without changes in serum testosterone and estradiol levels (Gonzales et al., 2002). Treatment with maca extract for 14 days in trained

male cyclists improved sexual desire (Stone et al., 2009). In conclusion, treatment with maca improved sexual desire in men and maca extracts could be better than gelatinized flour.

According to a systematic review, although several studies showed an effect of maca on sexual dysfunction in men and women (Shin et al., 2010) and on erectile dysfunction (ED) in men (Ernst et al., 2011; McKay, 2004), the evidence is still insufficient suggesting the need for more controlled studies.

One clinical trial on mild erectile dysfunction showed a small but significant improvement during maca treatment on subjective perception of general and sexual well-being in adult men (Zenico et al., 2009).

A double-blind, randomized, parallel trial with maca in women with a selective-serotonin reuptake inhibitor (SSRI)-induced sexual dysfunction showed that maca was well tolerated and it increased sexual desire (Dording et al., 2008). Maca also reversed sexual dysfunction in postmenopausal women (Brooks et al., 2008). These effects are observed independent of estrogen or androgen activity (Brooks et al., 2008).

4.2.3 Maca and fertility

4.2.3.1 Males

Most of the studies on the effect of maca on sperm quality have been done in healthy rats and mice (Gonzales et al., 2001b, 2003b). Extract of black maca orally administered and in less proportion extract of yellow maca but not extract of red maca increased sperm count in male rats (Gonzales et al., 2006b).

Chronic treatment for 84 days to male rats showed that extracts of yellow and black maca but not red maca increased epididymal sperm count without affecting daily sperm production. These results suggest that maca acts as a modulator of sperm count (Gasco et al., 2007a).

Studies on the acute effect of extract of black maca in male rats suggest a first action at the epididymal level, then at vas deferens level, and finally at the testis level (Gonzales et al., 2006a).

Treatment with maca also resulted in improvement in sperm count in animals with experimental damage of spermatogenesis. For instance, maca reversed the deleterious effect on sperm count produced by lead acetate (Rubio et al., 2006a,b) and malathion (Bustos-Obregon et al., 2005), or acute exposure to high altitude (Gonzales et al., 2004).

Treatment of healthy men with gelatinized maca during 4 months improved motile sperm count almost double of pretreatment value (Gonzales et al. 2001a). Maca improved sperm production and sperm motility without affecting LH, FSH, PRL, T, or E2 (Gonzales et al., 2001a). In a double blind, randomized, placebo-controlled study in 20 healthy volunteers aged 20–40 years, maca showed fertility enhancing properties (Melnikovova et al., 2015). Clinical trials in men with infertility treated with maca are needed.

The interest in maca has been extended to farmers as well. Several studies are focused on the impact of oxidative stress on sperm production and quality in livestock (Clément et al., 2012). Maca supplementation during 10 weeks improves sperm quantity and quality of bulls to a certain degree, without effect on mating behavior (Clément et al., 2010b).

Although most of the studies do not find effect of maca on serum testosterone levels in rats, mice or men, a recent study showed that feeding hydroalcoholic extract powder of *L. meyenii* (maca) increased serum T levels and enhanced the steroidogenic ability of Leydig cells in male rats (Ohta et al., 2015). This is surprising since changes in T levels occurred without changes in prostate weight, serum LH concentration, and number of Leydig cells (Ohta et al., 2015). The report that maca may have testosterone assay-interference increasing falsely levels of T may explain this finding (Srikugan et al., 2011).

In another experiment in male rats, red maca was unable to affect serum testosterone levels (Gasco et al., 2007b). Moreover, serum T or E_2 levels in adult male rats were not affected by treatment with extracts of yellow, red, or black maca (Gonzales et al., 2005).

In healthy men and women, serum levels of luteinizing hormone, follicle-stimulating hormone, prolactin, 17-alpha hydroxyprogesterone, testosterone and 17-beta estradiol were measured before and at 2, 4, 8, and 12 weeks of treatment with placebo or gelatinized maca (1.5 or 3.0 g per day). Compared with placebo, these hormones remained unchanged during the period of study (Gonzales et al., 2003a).

Maca extracts (obtained with different solvents: methanol, ethanol, hexane, and chloroform) do not exert direct androgenic activities (Bogani et al., 2006).

Studies of fractions of *L. meyenii* (black maca) showed that ethyl acetate fraction has the best effect on sperm production (Yucra et al., 2008).

4.2.3.2 Females

Studies about the effect of maca administration in females particularly at reproductive age are scarcer. In one study, female Sprague-Dawley rats feed containing 5, 25, or 50% maca powder for 7 weeks. During the pro-oestrus stage serum luteinizing hormone (LH) and follicle stimulating hormone (FSH), increased 4.5-fold and 19-fold with 50% maca powder, respectively, without changes in serum growth hormone (GH), prolactin, adrenocorticotropic hormone (ACTH), and thyroid-stimulating hormone (TSH) (Uchiyama et al., 2014).

Other authors suggest that maca acts as a toner of hormonal processes in adult female rats since its administration at dose of 7.5 g/kg for 90 days, increased progesterone and a steady estradiol level, without affecting serum FSH, LH and TSH levels (Meissner et al., 2006a).

Oral administration of extract of yellow maca to pregnant rats increased litter size without affecting number of implantation ova suggesting that maca is acting improving embryo survival (Ruiz-Luna et al., 2005).

4.2.4 Maca on endurance capacity and antifatigue

The effect of maca as an energizer stimulant is known from traditional medicine described by chroniclers of the Spaniard conquest to Peru (Ruiz, 1952). Experimental studies using the forced swimming test demonstrated that black maca extract delays fatigue, an effect that seems to be mediated by lower lactate production (Gonzales et al., 2014). Extract of black, but not red, maca have more effects as a fatigue delayer in the swimming endurance capacity (Zevallos-Concha et al., 2016).

Irradiation of black maca improved this effect (Zevallos-Concha et al., 2016). *N*-benzyloleamide, a macamide, has been suggested as a compound responsible of the retarding exercise-induced fatigue (Yang et al., 2015).

Three randomized clinical trials controlled with placebo have demonstrated favorable effects of maca on endurance (Gonzales et al., 2009; Gonzales-Arimborgo et al., 2016; Stone et al., 2009).

4.2.5 Maca and mental health

Several properties of maca seem to be related with brain functions including sexual behavior as described earlier. A pentane extract of maca administered intravenously to rats prior to and following middle cerebral artery occlusion showed a neuroprotectant effect (Pino-Figueroa et al., 2010).

Pregelatinized organic maca showed antidepressant-like and sedative effects in ovariectomized rats (Meissner et al., 2006a). Petroleum ether extract of maca (250 and 500 mg/kg) in the chronic unpredictable mild stress (CUMS) model of depression in mice showed antidepressant-like effects and also activated both noradrenergic and dopaminergic systems without changes of the serotonergic system, oxidative stress in the mouse brain was also attenuated. These effects accompanied a reduction in serum corticoid levels (Ai et al., 2014). In another animal model, three phenotypes (yellow, black, and red maca) demonstrated antidepressant activity (Rubio et al., 2006a,b).

This is important because the use of maca in cases of patients using selective serotonin reuptake inhibitors (Dording et al., 2008) could be allowed because maca does not affect the serotonergic system. The effect of maca on sexual desire in these subjects seems to be due to factors different from those affecting serotonergic system.

Another property described for maca is on memory and learning. Extracts of maca improve memory and learning in different experimental models, including memory impairment induced by ethanol (Rubio et al., 2011b), ovariectomy (Rubio et al., 2011a) or scopolamine (Rubio et al., 2007). Extract of black maca was better than yellow and red maca in ovariectomized mice (Rubio et al., 2006a,b). Black maca decreased malondialdehyde (MDA), and acetylcholinesterase (Ache) levels in ovariectomized mice, without changes in monoamine oxidase (MAO) levels (Rubio et al., 2011a).

4.2.6 Maca in ovariectomized rats

In many studies, ovariectomized rats have been used to mimic the impact of postmenopause on several health variables. Ovariectomy reduced weight, diameter, and width of the femoral bone in rats but treatment with estradiol, or extract of black or red maca reduced these effects (Gonzales et al., 2010).

Treatment during 7 weeks with maca in ovariectomized rats resulted in similar serum triglyceride, LDL-cholesterol, and alkaline phosphatase values to controls without ovariectomy (sham operated group) (Wang et al., 2009).

A treatment with maca in ovariectomized rats showed a slight increase in serum E2 levels and a reduction in serum testosterone levels at 12 weeks, whereas serum FSH was slightly higher in maca groups than in the sham group. At week 29, maca treated animals had similar E2 levels than the sham group and higher E2 and lower FSH levels related with ovariectomized group (Zhang et al., 2014). Treatment of ovariectomized Sprague Dawley rats with alcoholic extract of maca for 28 weeks showed that higher doses of maca prevented estrogen deficient bone loss (Zhang et al., 2006).

4.2.7 Maca and menopause

Maca is a known cruciferous. Extracts of cruciferous vegetables, such as broccoli and linseed, may change the metabolism of estrogens, and antioxidants reversing altered epigenetic DNA methylation. This in turn may reduce the risk of breast cancer or its recurrence (Comhaire and Depypere, 2015). Analysis of randomized clinical trials (RCT) that tested the effects of maca on menopausal symptoms in healthy perimenopausal (Meissner et al., 2006d), early postmenopausal (Meissner et al., 2005, 2006b,c), and late postmenopausal women showed favorable effects. However, a systematic review considers that data are still limited to obtain convincing conclusions (Lee et al., 2011). Then, although maca is recognized in the scientific literature to reduce vasomotor symptoms (Depypere and Comhaire, 2014), further evidences are still needed.

With respect to maca effects on other parameters associated with menopause, a 6-week maca treatment in postmenopausal Hong Kong Chinese women decreased diastolic blood pressure and depression without changes in estradiol, FSH, TSH, sex hormone binding globulin (SHBG), glycemia, lipid profiles, and serum cytokine levels (Stojanovska et al., 2015).

4.2.8 Maca and benign prostatic hyperplasia (BPH)

BPH appears generally at 40 years affecting quality of life (Shrivastava and Gupta, 2012). Medications, as well as surgical procedures to treat BPH are not free from side effects, phytotherapy has been considered as an alternative for treatment (Shrivastava and Gupta, 2012).

Extract of red maca reversed BPH but this effect was not achieved by the yellow or black varieties (Gonzales et al., 2005). A similar effect was observed when boiled aqueous extract

or hydroalcoholic extract of red maca was used (Gonzales et al., 2007). The effect of red maca extracts was observed in a dose-response fashion (Gonzales et al., 2007).

A better effect was observed when red maca was administered from day 1 to 7 than from day 7 to 14. (Gonzales et al., 2012). At day 7, red maca was acting at the prostate stromal level, whereas at days 14 and 21 it was acting at the prostate acinar area (Gonzales et al., 2008).

4.2.9 Maca and blood pressure

L. meyenii Walp (maca) inhibited angiotensin I-converting enzyme (ACE) becoming a potential tool for treating hypertension (Ranilla et al., 2010). The high levels of potassium in hypocotyls of maca are also important as an antihypertensive food (Valerio and Gonzales, 2005).

4.2.10 Maca and lipid and glucose metabolism

Maca administered for 2 weeks to rats with high-sucrose diet (HSD) significantly improved glucose tolerance and reduced glycemia. In addition, maca reduced serum levels of very low-density lipoproteins (VLDL) and low-density lipoprotein (LDL), total cholesterol, and triacylglycerol (Vecera et al., 2007). These actions seem to be associated with an increased activity of superoxide dismutase (SOD) in the liver, glutathione peroxidase in blood, and glutathione in liver (Vecera et al., 2007).

Recent findings showed that maca might reduce glycemia in streptozotocin-induced diabetes through an improvement in insulin resistance (IR). Red maca increased glucose consumption at a concentration of 80–100 μg/mL and black maca increased glucose consumption at a lower concentration of 40–60 μg/mL (99.69 ± 7.98% and 105.23 ± 13.35%, respectively) (P <0.01 vs. control) in HepG2 cells with induced insulin resistance. This effect of maca extracts was not observed in HepG2 cells without IR. In conclusion, extracts of maca reduced glycemia in rats with diabetes mellitus (DM); through an improvement in the insulin resistance as observed in IR-HepG2 cells (unpublished data). The detailed molecular mechanism still needs to be explored in the future (Fig. 12.1).

4.2.11 Maca and photoprotection

Maca grows more than 4000 m, where elevated solar and cosmic radiation occurs. UV radiation may affect life adversely, but maca grows without problems in these conditions suggesting that it may counteract the effect of UV radiation. This quality has been proved in animals submitted to UV radiation.

Maca extract obtained from hypocotyls protect the skin of rats against UVA, UVB, or UVC irradiations (Gonzales-Castañeda and Gonzales, 2008). Hydroalcoholic extracts of three phenotypes of maca leaves (yellow, red, and black maca) applied topically to dorsal skin of young-adult mice prior to exposure to UVA/UVB radiation have UVA/UVB absorptive properties and antioxidant activity, being the highest with red maca than with others. Maca

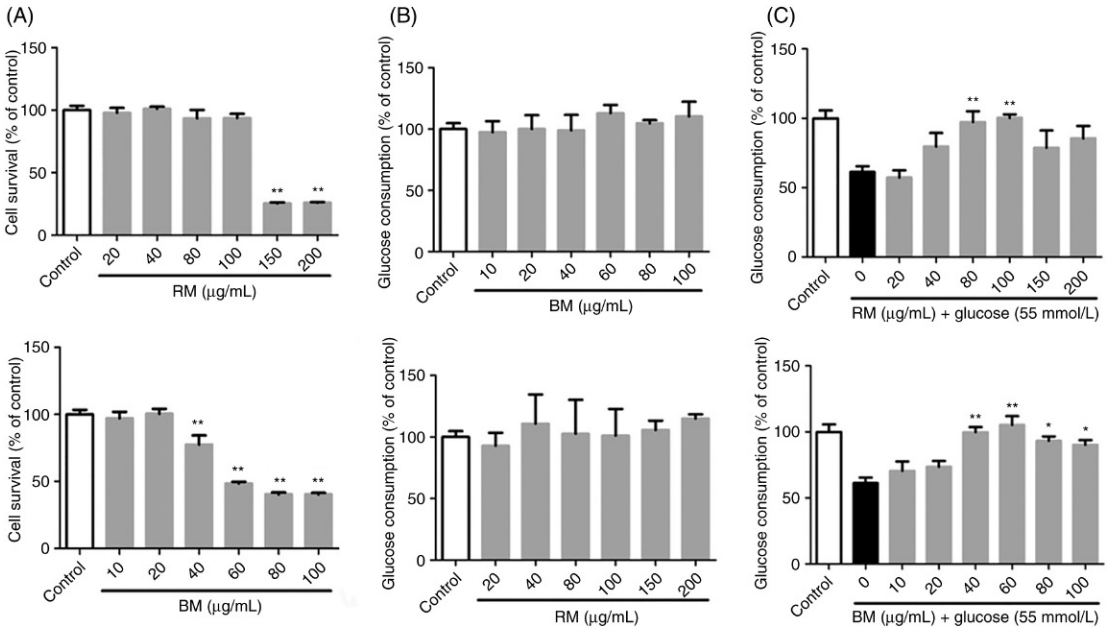


Figure 12.1: Cell survival (A) of HepG2 cells under basal (A) or under insulin resistance (C) and cell survival (B) of HepG2 cells treated with different amounts of red maca (upper) or black maca (bottom). The HepG2 cells were treated at high glucose concentration (55 mmol/L) to establish the insulin resistance cell model. The glucose consumption was measured by glucose oxidase and peroxidase (GOD-POD) assay. Data are mean ±standard deviation. $*P < 0.05$; $**P < 0.01$ with respect to control. *Data presented as poster in ENDO 2015, San Diego, USA by Gustavo F. Gonzales, Carmen Maldonado, and Lijia Xu.*

prevented the effect of UVB on skin in part attributable to high antioxidant activity and the inhibition of lipid peroxidation (Gonzales-Castañeda et al., 2011).

4.2.12 Safety

Since medicinal plant consumption is increasing notably worldwide, concerns about safety are rising. The same happens with consumption of *L. meyenii* (maca) (APSSA, 2004). One paper showed that maca hypocotyls contain (1R,3S)-1-methyl-1,2,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA) (Piacente et al., 2002). Although the authors suggest that MTCA may be harmful, other studies showed that MTCA is a natural constituent of several fruits and foods, including maca and no toxicity observed once consumed (Gonzales and Gonzales-Castañeda, 2009). Interestingly, metabolomics study with different phenotypes of hypocotyls of maca did not show the presence of MTCA (Zhao et al., 2012).

An in vitro study with methanolic and aqueous extracts of maca showed no cytotoxicity in hepatocytes primary cultures (Valentová et al., 2006). Maca extract has estrogenic activity

similar to silymarin in human breast cancer MCF-7 cells (Valentová et al., 2006). Silymarin derived from the milk thistle plant (*Silybum marianum*), is a flavonoid complex with anticancer and chemopreventive properties (Jiang et al., 2015).

In a study in 29 Peruvian patients with functional dyspepsia, treatment with spray-dried extract of black maca did not produce changes on gastric mucosa (Benites Goñi et al., 2014).

Experimental studies have proven that short- and long-term consumption (84 days) of maca have no toxicity under in vivo conditions (Gasco et al., 2007b; Gonzales et al., 2014). In summary, maca extract in doses up to 5 g/kg (corresponding to the consumption of 770 g hypocotyls in a man of 70 kg) was innocuous (Chung et al., 2005).

4.2.13 Quality control

As maca is a food and is not free of microorganisms contamination, gamma irradiation is a plausible alternative to prevent contaminating agents. However, concerns have been raised about the biological activities after irradiation. For this, a recent study was performed with red and black maca before and after irradiation. Results showed that irradiation of maca samples with 8 kGy using Co60 as radioisotope prevents the presence of microorganisms in the extracts of black or red maca without diminishing its biological properties (Zevallos-Concha et al., 2016).

A DNA-barcoding approach based on the internal transcribed spacer (ITP) sequences of maca samples have been developed to determine adulterants (Chen et al., 2015a).

5 The Future of Maca

5.1 Biotechnology in the Study of Maca

After aqueous or hydroalcoholic preparation of maca samples, the liquor residue is normally discarded. Using chemical and enzymatic methods, dietary fibers were obtained. Fibers obtained using these methods have good functional properties, as swelling capacity, water-holding capacity, oil-holding capacity, glucose adsorption capacity, and glucose retardation index (Chen et al., 2015b). Water-soluble polysaccharides obtained from maca aqueous extract composed of rhamnose, arabinose, glucose, and galactose showed high capability of scavenging hydroxyl free radical (52.9%) and superoxide radical (85.8%) at 2 mg/mL. (Zha et al., 2014).

Carcass fat composition of bulls fed a forage-based diet could be lower with maize silage compared to grass silage. As an attempt to increase carcass fat composition of maize-silage food, several supplements, including maca, were assessed. However, results were negative because maca supplementation given to bulls does not improve carcass fat properties of maize silage based-system (Staerfl et al., 2011).

5.2 Mixture of Plants

Several studies over the past years attempted to demonstrate the effect of maca administered with other plants trying to increase effectiveness on health. Administration of Peruvian *L. meyenii* (black maca) with African *Fagara tessmannii* (Rutaceae) resulted in reduced sperm count in testis, epididymis, and vas deferens with doses of maca of 0.5 and 5 mg/kg, but it was counteracted at high doses of maca (50 mg/kg) (Lembè et al., 2014).

The mixture of *L. meyenii* (black maca) with the Amazonian fruit *Myrciaria dubia* (Camu camu) has a better effect on spermatogenesis than administered alone (Gonzales et al., 2013c). Similarly, a mixture of black maca with *Smallanthus sonchifolius* (yacon) improved glycemia and sperm count in diabetic mice and improved the antioxidant activity in respect with maca alone (Gonzales et al., 2013a).

Combinations of maca and silymarin have been described as useful in subjects with metabolic syndrome (Valentová et al., 2008).

In summary, a mixture of plants, including *L. meyenii*, could be useful to improve well being.

5.3 Maca and the Endocannabinoid System

An outstanding characteristic of *L. meyenii* is the fact that there is no active compound identified that has solely the same effect as maca as a whole. Many studies have tried to identify and isolate an active compound, in such travails macamides were discovered (Zheng et al., 2000), these fatty acid *N*-benzylalkamides are unique and therefore have been baptized after maca.

These compounds modulate the endocannabinoid system (ECB) through the inhibition of fatty amidic acid hydrolase (FAAH), an enzyme which main function is to degrade anandamide. (Wu et al., 2013).

The ECB system is a widely expressed signaling pathways that modulate a vast range of functions, such as the release of neurotransmitters, sensation of pain, metabolism and blood pressure (Maccarrone et al., 2015).

In this section, we discuss possible mechanisms of action of *L. meyenii* through the endocannabinoid system and how research on this subject has been prompting maca to a new whole level: novel drug manufacturing.

5.3.1 The endocannabinoid system

The study of the endocannabinoid system (ECS) began in 1988 when Devane et al. found in rat's brain a receptor for delta-9-tetrahidrocannabinol, the active principle of *Cannabis sativa.* Later on, the endogenous ligand for this receptor (cannabinoid receptor 1 or CB1)

was described and named anandamide (*N*-arachidonoylethanolamine; Devane et al., 1992). This receptor is widely expressed in mammalian tissues while the cannabinoid receptor 2 is found primarily in the immune system (although it has been also found in the nervous system; Cabral et al., 2008; Van Sickle et al., 2005).

The other constituents of the ECB system are the enzymes responsible for its synthesis and degradation; for anandamide, mainly acyltransferases and for degradation, the FAAH.

5.3.2 FAAH and L. meyenii

The ability of macamides to inhibit fatty acidic amide hydrolase was characterized by Wu et al. (2013), who conducted a series of in vitro assays to measure the inhibition capacity of 11 macamides. They concluded that macamides are in fact FAAH inhibitors but the power of inhibition differs from one macamide to another, and even though not particularly high, a constant diet, including maca, could render a cumulative effect that might impact on the endocannabinoid system and such effect ought to be studied in the future.

N-3-methoxybenzyl-linoleamide, a macamide, was a time-dependent and dose-dependent FAAH inhibitor through an irreversible or slowly reversible mechanism (Almukadi et al. 2013). FAAH inhibitors are currently being studied as therapeutic agents for the treatment of different pathological scenarios, such as hypertension (Baranowska-Kuczko et al., 2016; Godlewski et al., 2010), inflammatory pain (Booker et al., 2012), visceral pain (Sakin et al., 2015), and it has also been showed to enhance the antitumorigenic effects of anandamide in cancer cells (Ravi et al., 2014). Therefore *L. meyenii's* macamides and their FAAH inhibition properties open the doors for renewed efforts seeking to use maca as the base for new drug formulations.

6 Concluding Remarks

The use of molecular docking approach has revealed that no compound present in *L. meyenii* (maca) is able to bind to the human estrogen receptor (ERα and ERβ) (Powers and Setzer, 2015). Other study using SERMS on the contrary found that maca might act on estrogen receptor (Gonzales et al., 2013b). Further studies are required to clarify the role of maca on estrogen receptor.

An interaction of plant-derived *N*-alkylamides (NAAs) and the mammalian endocannabinoid system (ECS) was found in recent years. For this reason, several studies have focused to determine the role of *L. meyenii* on ECS.

Five macamides derivatives of oleic, linoleic, and linolenic acids and benzylamine or 3-methoxybenzylamine were FAAH (Wu et al., 2013). *N*-3-methoxybenzyl-linoleamide, one of the most active macamides, has significant time-dependent and dose-dependent FAAH inhibitory activity (Almukadi et al., 2013).

N-benzyl-(9Z,12Z)-octadecadienamide isolated from *L. meyenii* (maca) showed submicromolar and selective binding affinity for the cannabinoid CB1 receptor (Ki = 0.48 μM), weak FAAH inhibition (IC50 = 4 μM) and a potent inhibition of anandamide cellular uptake (IC50 = 0.67 μM) (Hajdu et al., 2014).

It is suggested that maca might act on the central nervous system to provide analgesic, antiinflammatory, or neuroprotective effects, by modulating the release of neurotransmitters through the ECS (Almukadi et al., 2013). This is an attractive hypothesis to be worked in the next future.

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CHAPTER 13

Phytochemical and Pharmacological Properties of Secondary Metabolites in Berries

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1 Introduction

Chronic diseases, such as cancer, can reduce their effects with the consumption of fruits and vegetables rich in bioactive compounds (Hjartåker et al., 2014; Wang et al., 2014). Research has implicated the bioactive compounds as phytochemicals having beneficial health effects (Stoner, 2009). Biologically active phytochemicals found in berries are formed by a wide variety of important compounds, such as berries (*Rubus* sp.) blackberry, blueberry (*Vaccinium myrtillus*), blackcurrant (*R. rugrum*), blueberries (*V. corymbosum*), chokeberry (*Aronia melanocarpa*), cranberry (*V. macrocarpon*), pear (*Myrica* sp.), raspberry (*R. ideaus*), black raspberry (*R. occidentalis*), and strawberry (*Fragaria ananassa*) are important sources of bioactive compounds that can be consumed in the diet as well as fresh and processed (Seeram, 2008a). Berries are among the fruits rich in natural compounds, and this is considered functional for its high content of compounds, such as minerals, vitamins, dietary fibers, and especially polyphenols and anthocyanins considered as a great source of phytochemicals in the berry with beneficial effects on cancer through different mechanisms of action and different metabolic pathways. These phytochemicals found in berries have undergone extensive research because of their antioxidant properties, promotion of health improvements, furtherance of consumption and acceptability of these berries (Afrin et al., 2016). It is important to study the compounds from natural sources with effects against cancer and the side effects generated by the use of chemotherapy to fight cancer, such as fatigue, mouth dryness, nausea, constipation, anorexia, pain, anxiety, and vomiting, which are very exhausting for patients with these chronic degenerative

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diseases (Flis et al., 2012). The anticancer effects generated by the consumption of bioactive compounds of berries is made through various complementary and overlapping mechanisms of action, including the induction of enzymes that metabolize, modulation of gene expression, and their effects on cell proliferation, apoptosis, and subcellular signaling pathways (Seeram, 2008b). Blackberries and raspberries are important sources of anthocyanins with antioxidant properties, chemopreventives associated with beneficial health effects; Bowen-Forbes et al. (2010) showed that the black raspberry has biological activity against cancer of the esophagus, colon, and oral cancers. Other phytochemicals used as chemopreventive found in strawberry and black raspberry have been identified as anthocyanins, gallic acid, and ellagic acid, which have cancer chemopreventive activities (Marra and Boland, 1995). Flavonoids, tannins, and phenolic acids are part of the phenolic compounds in various fruits have been the subject of interest for research as related to the beneficial health effects. The objective of this review was to relate the berries as a source of bioactive compounds as chemopreventive in the treatment of cancer and gather current knowledge related to berries.

2 Taxonomic Problems

Berries are consumed for their taste, nutrition, and convenience worldwide. Berries in botanical terms are classified as berries at all and true berries (seeds and edible pulp of one ovary). The consumption of berries is related to their content of bioactive compounds with antioxidant capacity and its beneficial effects in diseases, such as cancer, berries are consumed as fresh fruit. Compounds with phytochemical effects studied in berries, such as carotenoids, provitamin A, vitamin C and E, folic acid, calcium and selenium, simple phenols and complexes, and phytosterols, tannins, and anthocyanins are the most active compounds (Ferreira et al., 2008). Anthocyanins found in berries are cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvinidin. Stoner and Seeram (2011) reported that anthocyanins could range between 2 and 768 mg/100 g of fresh weight, although it has been reported that black chokeberries could reach up to 1458 mg/100 g of fresh weight and raspberries 2199 mg/100 g of fresh weight (Bowen-Forbes et al., 2010). A number of articles have been published on the bioavailability and metabolism of phenolic compounds. Bioavailability differs greatly among the various polyphenols and the most abundant polyphenols in our diet are not necessarily those with the best bioavailability profile. A thorough knowledge of the bioavailability of the hundreds of polyphenols in the diet will help us identify those that are most likely to be protective of health (D Archivio et al., 2007; Manach et al., 2004). The production and consumption of berries have increased, so it is important to assess these variables in berries. Different varieties of berries are strawberries, raspberries, blackberries, blueberries, cranberries, currants, grapes, gooseberries, and elderberries. Most of the berries consumed are strawberry and raspberry, and the leading producer of berries is the United

States. Japan is a leader in the production of supplements using berries. Berries are a very versatile fruit that can be processed as frozen fruit puree, nuts, yogurt, jam, jelly, or juice (Seeram, 2008a).

3 Berry Fruits, Bioactives, and Bioavailability

A number of articles reviewed showed that the main bioactive compounds that determine the nutritional and health benefits of berries quality are polyphenols, anthocyanins, and flavonoids (Forbes et al., 2010; Namiesnik et al., 2014) (Table 13.1). Adams et al. (2010) discussed varied phytonutrients fat-soluble/lipophilic to water-soluble compounds/hydrophilic. Most blot assays of hydrogen atoms are based on the kinetics, and involve a competitive reaction scheme in which an antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds, electron transfers based assays the ability of an antioxidant in reducing an oxidant, which changes color when reduced is measured (Costantino et al., 1994). The berries that showed high scavenging activity to superoxide radicals were blackberries, red and black currants, blueberries, and black and red raspberries (Nile and Park, 2014). Peroxyl radicals absorb more oxygen radicals that peroxyl radicals in strawberry, blackberry without thorns, and red and black raspberry (Wang and Lin, 2000). Raspberries, strawberries, and blueberries have anticancer effects and antiinflammatory inactivating mutagenesis benzo pyrene and blackberries suppression is for mutagenesis of 2-amino anthracene, and found that the magnitude of the effect varies with the variety of berries (Kulczyński and

Table 13.1: Bioactive and biological properties of berries.

Berries	Phytochemicals	Biological Properties	References
Blackberry	Polyphenols, cyaniding-3-O-glucoside, high tannin	Fights free radical damage, antibacterial, anticancer	Yi et al. (2005)
Cranberry	Phenolic compounds	Antibacterial removes fats from lymphatic system and promotes cardiovascular health	Afrin et al. (2016)
Blueberry	β-Carotene, lutein, zeaxanthin	Anticancer, antiinflammatory, antidiabetic, and so on	Nile and Park (2014)
Raspberry	Galic acid, ellagic acid, and lutein	Anticancer, prevents free radical damage	Koponen et al. (2007)
Bilberry	High anthocyanins, flavonols, carotenoid, lutein, and zeaxanthin	Antiinflammatory and improves circulation	Cocetta et al. (2012)

Source: Based on Adams, L.S., Phung, S., Yee, N., Seeram, N.P., Li, L., Chen, S. 2010. Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. Cancer Res. 70, 3594–3605.

Gramza-Michałowska, 2016). Biological compounds of the berries extract that inhibits the cytochrome P450, system inactivating carcinogens in ways capable of binding with covalent DNA. Ultraviolet radicals induce the active compounds of the berries to inhibit mutagenesis (God et al., 2007). In vitro studies have demonstrated the activity of various berry extracts against different aspects of the tumorigenic process and have shown that diets rich in fruits and vegetables protect against cancer (Bobinaitė et al., 2012; Kulczyński and Gramza-Michałowska, 2016; Tulio et al., 2008). The effect against cancer is performed by several complementary mechanisms that include the induction of enzymes that metabolize; modulation of gene expression; and its effects on cell proliferation, apoptosis, and subcellular signaling pathways and all this in the function of the bioactive compounds from berries (Nile and Park, 2014). Bioactive compounds that have biological effects against cancer of the esophagus, colon, and oral cancers are cyanidin-3-glucoside and cyanidin-3-rutinoside found in the black raspberry (Tulio et al., 2008).

4 Metabolic Syndrome

4.1 Traditional Uses of Medicinal Berry Plants Against Cancer

Beyond providing essential nutrients and minerals the food we eat affects our body. The pharmacological effects of dietary bioactive compounds significantly alter the natural activity of therapeutic agents by modulating the biochemical pathways (Pan et al., 2009). In observation, these bioactive compounds respond to acute exposures and have successful variation in the responses of the human body at a dose of nutrients, offering an extraordinary opportunity to match the dietary guidelines for biology of the individual, prevention of disease and aging, to name just a few. The therapeutic potential and personalized nutrition phytochemicals are the future of research. However, science is way too promising to start with very slow results that could seriously compromise the trust of the population and reduce the rate of growth of the cultivated field (Bouchard and Ordovas, 2011), such as blackberries, blueberries, strawberries, raspberries, and blueberries, as well as the "berries" more atypical, like black currants, blueberries, cranberries, and cloudberries (Seeram, 2008b). Berries are consumed as part of a Western diet of processed fresh ways. The antiarcinogenic properties of berries are evidence, although the mechanisms are still not fully understood but may include free radicals, induction of enzymes involved in the metabolism of xenobiotics, the regulation of gene expression, modulation of pathways cell signaling, including those involved in the repair of DNA damage, cell proliferation, apoptosis, and invasion (Brown et al., 2012). The anticancer activities of bioactive compounds in berries have been evaluated in many in vitro studies, focusing on proliferation and apoptosis using extracts of mixed berries, fractionated berry extract, or components of purified/commercial berries in a range of colon cell lines. They represent different stages of human progression of colorectal cancer. Cellular processes are modulated by phenols found in berries above or below the

main regulatory proteins involved in cell signaling pathways that control proliferation, differentiation, and apoptosis, potentially resulting in anticancer activity (Ramos, 2008). The most sensitive modulation of nontumorigenic cells are affected by different ways of regulating the use of compounds with biological activity cancer cell lines (Zhao et al., 2004). However, the effectiveness of bioactive compounds changes with dose, type of cell used, exposition, the type of compound, isolated compound or mixture (Larrosa et al., 2006a). Ordovas and Mooser (2004) recently reviewed studies examining diet/genotype interactions involved in chronic risk. Kornman et al. (2004) approached one nutrigenomic treatment of inflammation and cancer. The function of nutritional compounds is one of the main objectives of modern food science and nutrition at the molecular level (its interaction with genes, proteins, and effect on metabolites), for which they have created strategies to manipulate functions through the diet.

4.2 In Vitro Validation of Berries Against Different Types of Cancer Cell Lines

The activities of various berry extracts have been demonstrated in several in vitro studies against several aspects of cancer. On the other hand, to prevent the initiation of cancer by enhancing genomic stability, prevention of carcinogens, or suppress the growth of cancer cells, these compounds with biological activity would qualify as a chemopreventive compound or a fruit (Watson, 2011). The phytochemical effect on cancer processes is checked by means of cell culture systems. In vitro studies are a potential tool; however, care should be taken with the interpretation of data and the concentration of the compounds used for in vitro studies may be greater than those provided in vivo, in addition to the compounds used for these studies in vitro and in vivo may be different (Heber et al., 2011). The summary presented below includes reports of in vitro studies for the last 10 years where both berry extracts, as well as purified compounds from them have been tested (Table 13.2).

5 Clinical Efficacy and Mechanism of Action Berries

When the cells acquire characteristics to divide without control, the result is metastasis of the cancer process (Fig. 13.1). The genetic and epigenetic changes are part of the process of conversion of normal cells to malignant. Cancer cell genotypes are an exhibition of six essential alterations in cell physiology that together dictate malignant growth: (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory signals, (3) evasion of programmed cell death (apoptosis), (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. Berries and their components may act along this point to alter the sequence of cancer (Duthie, 2007; Routray and Orsat, 2011; Seeram, 2006).

Table 13.2: Anticarcinogenic effects of berries in vitro/in vivo models.

Cell Line	Tissues	Compound Used	Observations	References
KB, CAL-27 MCF-7 HT-29 HCT116 LNCaP	Oral Breast Colon Colon Prostate	Berry extract (blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry) in methanol containing 0.1% HCl	Antiproliferative activity 25–200 μg/mL With increasing concentration of berry extract, increasing inhibition of cell proliferation	Seeram et al. (2006)
HT-29 SW48	Colon Colon	Blueberry extract in methanol with 50% DMSO	Citotoxic effects; cells showed a decrease in G1 phase with simultaneous increasing in the percentage of the cells in the S phase; induction of cell cycle arrest; apoptosis	Flis et al. (2012)
SEM, RS4;11, and REH	Leukemia-derived cell lines	Extracts from blackberries, blueberries, red and black grapes, raspberries, and strawberry powder in methanol containing 0.1% HCl	Apoptosis was accompanied by an arrest in the G2/M phase of the cell cycle; for SEM y REH cells, an S phase arrest was also observed with strawberry or blackberry extract	Zunino et al. (2010)
AGS LoVo MCF-7	Stomach Colon Breast	Extract of raspberries in water	Kill of cancer cells	God et al. (2010)
SEM and RS4;11	Leukemia-derived cell lines	Purified strawberry components; Pelargonidin (Pg), pelargonidin-3-glucoside (Pg3g), and cyanidin-3-glucoside (Cy3g), quercetin, kaempferol, ellagic acid (EA), kaempferol-3-(6′-coumaroyl)-glucoside (Ka3cg), and glucose ester of *p*-coumaric acid (Pcage)	Apoptosis; growth arrest in the S phase	Zunino et al. (2009)
HepG2	Liver	Purified pigeonberry components; betacyanins and betaxanthins	Citotoxicity	Khan et al. (2012)
HL60	Leukemia	Strawberry, raspberry, and blueberry leaves; extract with methanol, HCl, and NaOH	Citotoxicity	Skupień et al. (2006)
Caco-2	Colorectal	Chokeberry juice digested in the presence of food matrix	Antiproliferative activity	Stanisavljević et al. (2015)

Table 13.2: Anticarcinogenic effects of berries in vitro/in vivo models. (*cont.*)

Cell Line	Tissues	Compound Used	Observations	References
HeLa Fem X LS-174 MCF-7 PC-3	Cervix Melanoma Colon Breast Prostate	Fresh-pressed juices of red raspberry, black raspberry, black currant, red currant, and bilberry	Antiproliferative activity in dose-dependent manner	Konić-Ristić et al. (2011)
Caco-2	Colon	Chokeberry juice subjected to in vitro gastric and pancreatic digestion	Antiproliferative activity; 2 or 5% of digested chokeberry juice resulted in 40 and 70% inhibition; with 5% of digested chokeberry juice, there was a 2-fold increase of cells in the G2/M phase	Bermúdez-Soto et al. (2007)
A549	Lung	Mulberry extract with 0.1% HCl in methanol; anthocyanin fractions: cyanidin 3-rutinoside and cyaniding, 3-glucoside	Inhibitory effect on the migration and invasion in a dose-dependent manner	Chen et al. (2006)
Cl 41	Mouse epidermal	Blackberry extraction with methanol	Down-regulation of VEGF and COX-2 expression	Lu et al. (2006)
MCF-7 SF-268 NCI-H460 HCT-116 AGS	Breast CNS Lung Colon Gastric	Raspeberries (Jamaica and Michigan-grown species) extract with hexane, ethylacetate, and methanol	Antiproliferative activity; COX inhibitory activity	Bowen-Forbes et al. (2010)
HeLa, SiHa, C-33A	Cervical	Black raspberry ethanol extract	Inhibition of growth; apoptosis; extract concentration of 25, 50, 100, or 200 µg/mL for 1, 3, and 5 days; inhibition in a dose-dependent and time-dependent manner	Zhang et al. (2011)
B16F10 A2780 HeLa	Murine melanoma Ovarian Cervical	Anthocyanin-rich fractions in acidified methanol obtained from two commercially available juices (blueberry and blackcurrant juices)	Antiproliferative; cell viability decreased in a dose-dependent manner	Diaconeasa et al. (2015)
DU145	Prostate	A crude fraction, an anthocyanin-enriched fraction, and a proanthocyanidin-enriched fraction from blueberries	Decrease in the activity the activity of MMP (MMP-2 and MMP-9); the proanthocyanidin-enriched fraction was the most effective at inhibiting MMP; no induction of either necrotic or apoptotic cell death was noted	Matchett et al. (2005)

(*Continued*)

Table 13.2: Anticarcinogenic effects of berries in vitro/in vivo models. (*cont.*)

Cell Line	Tissues	Compound Used	Observations	References
HCT-116, Caco-2, SW480	Colon cancer	Anthocyanins of blackberry	Inhibit proliferation; suppress DNMT1 and DNMT3B proteins; suppress downstream of Wnt pathway, induce apoptosis	Wang et al. (2013)
LoVo cells	Colon cancer	Red raspberry	Inhibit cancer cell growth; induce apoptosis	Afrin et al. (2016)
HT-29 cell	Colon cancer	Strawberry	Organically grown strawberry extract shows higher antiproliferative activity	Olsson et al. (2006)
HCT-116, SW480, SW620 cell	Colon cancer	Cranberry	Enhance antiproliferative activity	Seeram et al. (2004)

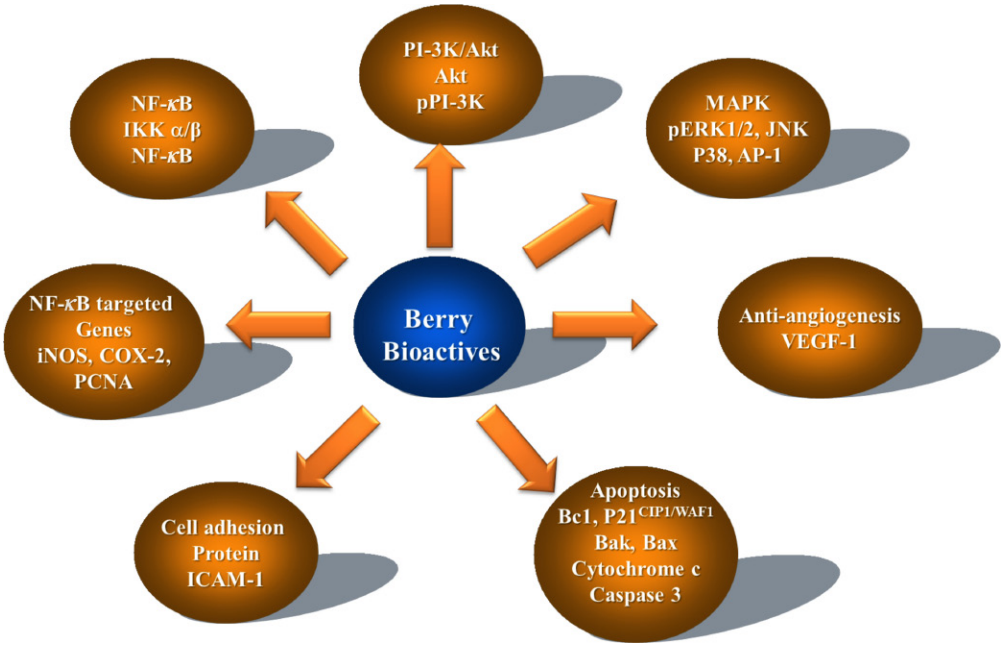


Figure 13.1: The Effects of Berry Bioactive on Cellular Functions and Signal Transduction Pathways. All genes shown are downregulated by berry bioactives except for the intercellular adhesion molecule.

Nevertheless, through various complementary and overlapping mechanisms of action berry extracts and phytochemicals, individual berries may exert anticancer effects. The free radical scavengers, including antioxidant effects, as well as acting secondly through antioxidant actions that protect DNA from damage, regulation of enzymes are important in the metabolism of xenobiotics and carcinogens, modulation of nuclear receptors, gene expression, and subcellular signaling pathways proliferation, angiogenesis, and apoptosis (Heber et al., 2011; Nile and Park, 2014). On the other hand, more than one mechanism is performed at the same time, as explained later.

Seeram et al. (2006) mention that the induction of apoptosis or cell cycle arrest can be an excellent method to inhibit promotion and progression of carcinogenesis and to eliminate genetically damaged cells, restarted, or neoplastic. During the development of cancer the function of the induction of apoptosis is lost. The most common pathways for apoptosis are the extrinsic and intrinsic. Extrinsic depends on ligand binding to cell surface receptors "death" with the activation of caspase-8 and is spread by direct cleavage of the executors caspases 3, 6, and 7, while intrinsic is induced by factors including DNA damage and involves mitochondrial function. Moreover, lysosomes and autophagy activation mechanism can be initiators of apoptosis (De Rada et al., 2004). Mitochondria play an important role in apoptosis (Brown et al., 2012) as the induction of apoptosis is measured by the reactive oxygen species (Juan et al., 2012). Proteases that degrade proteins during crucial apoptosis are cytochrome c and caspases. Caspase-3 is an important point of different pathway of caspase-dependent apoptosis. The apoptosisi via mitochondrial is activated caspase-9 and -3. These proteases can be active by ellagic acid. Various caspases target proteins distributed throughout the cell bind, resulting in the morphological changes that are typical of apoptosis (Larrosa et al., 2006b).

Decrease in cell proliferation is dependent on cell cycle arrest. Kinases cyclins and CDKs, which in turn, are controlled by CDK inhibitors (CDKIs), which regulate cell cycle control. Therefore, it is a fine adjustment, including regulating the activation or deactivation of the cell cycle. Larrosa et al. (2006b) found that a decrease in the protein expression of cyclin A might account for an arrest in S phase after treatment with ellagic acid, a compound present in berries.

In the carcinogenic process mutagenesis is an essential event. The compounds of berry extracts are activators of carcinogenic agents and transformation carcinogenic (Duthie, 2007), which inhibit the cytochrome P450 system responsible for converting carcinogenic compounds in forms capable of covalent binding to DNA (God et al., 2010). Moreover, phytochemical berries may induce sensitivity of tumor cells to chemotherapeutic agents by inhibiting the pathways that lead to drug resistance. Nitric oxide production is another mechanism when berry extracts are used. This may be part of the mechanism of early

mitochondrial membrane depolarization leading to cell death after treatment with fruit extracts (Zunino et al., 2010).

Oxidative stress and inflammation play a central role in the pathogenesis of chronic diseases, or electrons donating hydrogen to free radicals occur through the activity of anthocyanin in the berries. They can also act as metal chelators through the inhibition of free radicals catalyzed by transition metals. The antioxidant response element is a regulatory element found in the promoters of many genes encoding phase II drug metabolism/detoxification and antioxidant enzymes stress regulated by nuclear factor (erythroid-derived 2)-like 2(Nrf2). Cancer prevention is signaled via Nrf2-SE. Indeed, Saw et al. (2014) reported that the berry constituents quercetin, kaempferol, and pterostilbene activated the Nrf2-ARE signaling pathway and exhibited synergistic antioxidative stress activity at appropriate concentrations. Antioxidants can protect cell mutagenesis in the initiation stage of carcinogenesis; its role in the destruction of the transformed cells is less clear. Boivin et al. (2007) as well as Liu et al. (2002) found no correlation between antioxidant activity and the ability to inhibit cancer cell replication, measured antioxidant activity and antiproliferative activities of berries. Suppression of transcription factor nuclear factor-kappa B (NF-κB) is one of the mechanisms (Costa et al., 2013). Transcription factors AP-1 and NFAT are another oncogenic signal (Lu et al., 2006). For this reason, great efforts are done in the evaluation of berry extracts on these pathways. A mechanistic link between inflammation and cancer is provided in NF-κB. It may be connected to the cell resistance of apoptosis, as well as to develop the tumor angiogenesis and invasiveness (Karin, 2006). So, it is a target for chemopreventive and chemotherapeutic approaches. Genes constitutively overexpressed as cyclooxygenase-2 (COX-2) are found in every premalignant and malignant condition. Therefore, the modulation of angiogenesis by berries is suggested to be associated with downregulation of VEGF and COX-2 (Stoner et al., 2007). Both, COX-2 and VEGF are targets of NF-κB, AP-1, and NFAT pathways.

Their signaling transforms a wide range of extracellular stimuli into intracellular events. MAPK signaling pathway is an important upstream regulator of transcriptional factor activities. In this way, this pathway controls the activities of downstream transcription factors involved in carcinogenesis. It has been reported (Fig. 13.2) that anthocyanin extracts inhibit MAPK constitutive activation, which plays a major role in cell growth and proliferation (Duthie, 2007; Nandakumar et al., 2008).

Mammalian cell survival signaling and a disregulation of PI3K/Akt pathway have been implicated in various cancers. It regulates the cell cycle. The serine-threonine kinase Akt is a key downstream effector of PI3K, which, in response to PI3K activation phosphorylates, regulates the activity of a number of molecular targets. Activated kinases are most frequently in human cancer as constitutively active Akt can promote unregulated cell

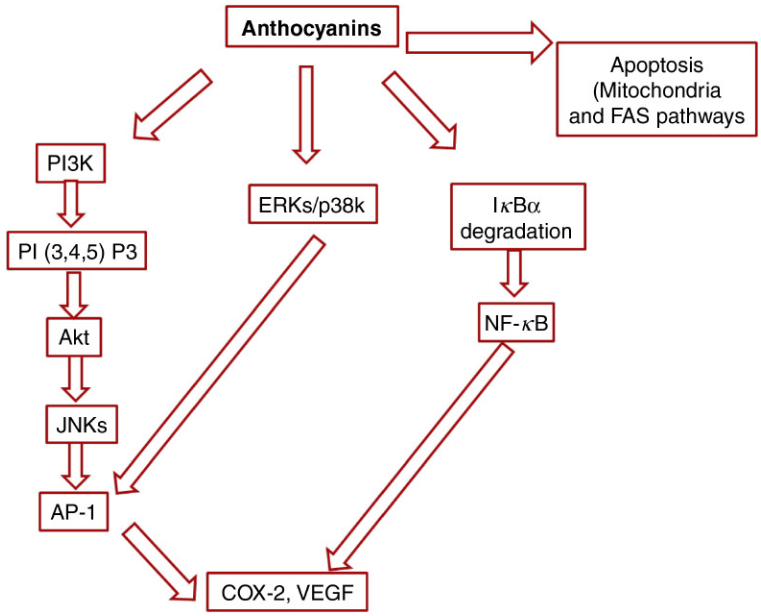


Figure 13.2: Schematic Illustration of Known Molecular Mechanisms That may be Involved in the Chemopreventive Mechanisms of Anthocyanins.

proliferation (Hanahan and Weinberg, 2011; Nandakumar et al., 2008). Berry extracts may exert their chemopreventive effects through targeting this pathway (Stoner and Seeram, 2011).

The presence of specific inflammatory cells and inflammatory mediators is marked by cancer-associated inflammation. Cytokine have an important role in tumor development and progression, such as TNF-α and interleukin (IL)-10. The expression of these cytokinines is increased in tumor necreosis from fat in rodents' obesity. The promotion of angiogenesis and metastasis is related to cancer through TNFα (Khandekar et al., 2011). Paul et al. (2010) reported that pterostilbene, mucosal levels of the proinflammatory cytokines, tumor necrosis factor-α, interleukin (IL)-1β, and IL-4 decreased a stilbene present in blueberries. In this study, reduced expression of inflammatory markers and nuclear staining for phospho-p65 in the colon tumors from animals fed with pterostilbene is shown, a key molecule in the NF-κB pathway.

The process of metastasis (the spread of malignant cancer cells from the place it first arose—primary location—to secondary sites in the body) depends on specific changes in the tumor: increased motility, surface adhesion properties, and increased extracellular protease activity to facilitate cell movement and invasiveness (Duthie, 2007). Cells that metastasize are basically of the same kind as those in the original tumor. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases capable of

degrading ECM components. Matrix metalloproteinases (MMP) have an important role in tumor development and metastatic spread of cancer (Davies, 2014). The metastatic spread of cancer is the invasion through the basement membrane and proteolytic degradation of the extracellular matrix proteins and is one of the earliest events. MMP are important targets of cancer therapy in many human cancers, given the clear implications of MMPs. The recognition that MMPs facilitate tumor cell growth, invasion, and metastasis of cancer has led to the development of MMP inhibitors as cancer therapeutic agents (Seeram, 2006). It has been reported that berries (raspberry, blackberry, cranberry) inhibit the activity of MMP (Heber et al., 2011). Also, Matchett et al. (2005) found that flavonoids from blueberries decrease MMP activity.

New blood vessels are formed from preexisting vessels by the angiogenesis process. Angiogenesis triggered tumor growth and metastasis depends on chemical signals from tumor cells. Angiogenesis is a determining factor in the transition of tumors from benign to malignant. Secreting growth factors, such as VEGF to induce capillary growth into the tumor allowing tumor expansion and spread, is controlled by the proliferation of new vessels (Bogenrieder and Herlyn, 2003; Nishida et al., 2006). Angiogenesis is essentially a four-step process: (1) basement membrane in tissues is injured locally, (2) endothelial cells activated by angiogenic factors migrate, (3) endothelial cells proliferate and stabilize, and (4) angiogenic factors continue to influence the angiogenic process. Different proteins have been identified as angiogenic activators and inhibitors. The expression levels of angiogenic factors reveal the hostility of tumor cells (Nishida et al., 2006). It has been found that berry extracts, such as wild blueberry, bilberry, cranberry, elderberry, raspberry seed, and strawberry are able to inhibit angiogenesis via altered VEGR expression and invasiveness (Duthie, 2007). Consumption of berries may contribute to the reduction of cancer through partially understood mechanisms. Further studies need to be conducted to elucidate these mechanisms and take advantage of the knowledge.

6 Cancer and Oxidative Species

The conversion of cellular macromolecules to specific reactive oxygen species during normal cellular metabolism is the cause of most chronic diseases, including cardiovascular disease and many types of cancer (Stoner et al., 2008). There are five recognized types of reactive oxygen species (ROS); that is, singlet oxygen ($'O_2$), superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and nitric oxide (NO^{-}). Although some types are more injurious to cells than others, there is no question that, collectively, these ROS cause significant oxidative damage to cellular DNA, proteins, and lipids leading to the development of chronic diseases, including cancer (Ames and Gold, 1991). Berry bioactives have many roles in cancer prevention, inhibition of the formation of carcinogen-induced DNA adducts, enhancement of DNA repair, inhibition of carcinogen-induced tumorigenesis

in animals, and modulation of signaling pathways involved with cellular proliferation, apoptosis, inflammation, angiogenesis, and cell cycle arrest (Stoner et al., 2008). Cells against the oxidative damage caused by free radicals are protected by phytochemicals, such as flavonoids and other phenolics, may have antioxidant activity. Measuring the superoxide scavenging activity other studies have assessed the antioxidant activity of berries, including black and red raspberries (Nile and Park, 2014). Extracts of berries of several cultivars of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably high scavenging activity toward chemically generated superoxide radicals. Numerous in vitro studies have demonstrated activity of various berry extracts against different aspects of the tumorigenic process and proved that the diets high in fruits and vegetables protect against cancer (Jimenez-Garcia et al., 2013a). The anticancer effect is induced by berry bioactive compounds through various complementary and overlapping mechanisms of action, including the induction of metabolizing enzymes; modulation of gene expression; and their effects on cell proliferation, apoptosis, and subcellular signaling pathways (Bobinaitė and Viškelis, 2013). In the pathogenesis of chronic diseases play a central role the oxidative stress, such as obesity, diabetes, cancer, and atherosclerosis (Costa et al., 2013). Free radicals are produced in organisms as a result of several metabolic activities and have been associated with chronic diseases (Benherlal and Arumughan, 2007). Each polyphenol, such as anthocyanin, has different free radical scavenging activities depending on their chemical structure (position, number, and types of substitutions) (Costa et al., 2013; Wallace, 2011). The properties occur through the donation of electrons or hydrogen to free radicals by chemical activities of anthocyanins. They also can act as metal chelators, inhibiting the formation of free radicals catalyzed by transition metals (Rice-Evans et al., 1997). On the other hand, tropical red–blackberries contain cyanidin-3-glucoside (Celli et al., 2011; Rufino et al., 2011), which had one of the highest oxygen radical absorbance capacity (ORAC) (Wang et al., 1997). In addition, the levels glutathione and the activity of natural antioxidant enzymes enhanced intake berries seed extract, such as glutathione S-transferase, superoxide dismutase, and catalase (Arun et al., 2011). The ability of different berry juices (blackberry, strawberry, raspberry, cranberry, and blueberry) had the highest antioxidant capacity against superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), and singlet oxygen ($'O_2$) (Wang and Jiao, 2000).

6.1 Antioxidants Compounds From Natural Origin

Bioactive phytochemicals, the most prominent of which are phenolic in nature, are content in the berries (Stoner, 2009) (Fig. 13.3); the phytochemicals in berries fall into several structural and chemical classes, including phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (anthocyanins, flavanols, flavonols), condensed tannins (proanthocyanins), hydrolysable tannins (ellagitannins and gallotannins), stilbenoids, lignans, triterpenes, and sterols. The anthocyanins and ellagitannins that are most prevalent compounds in berries are

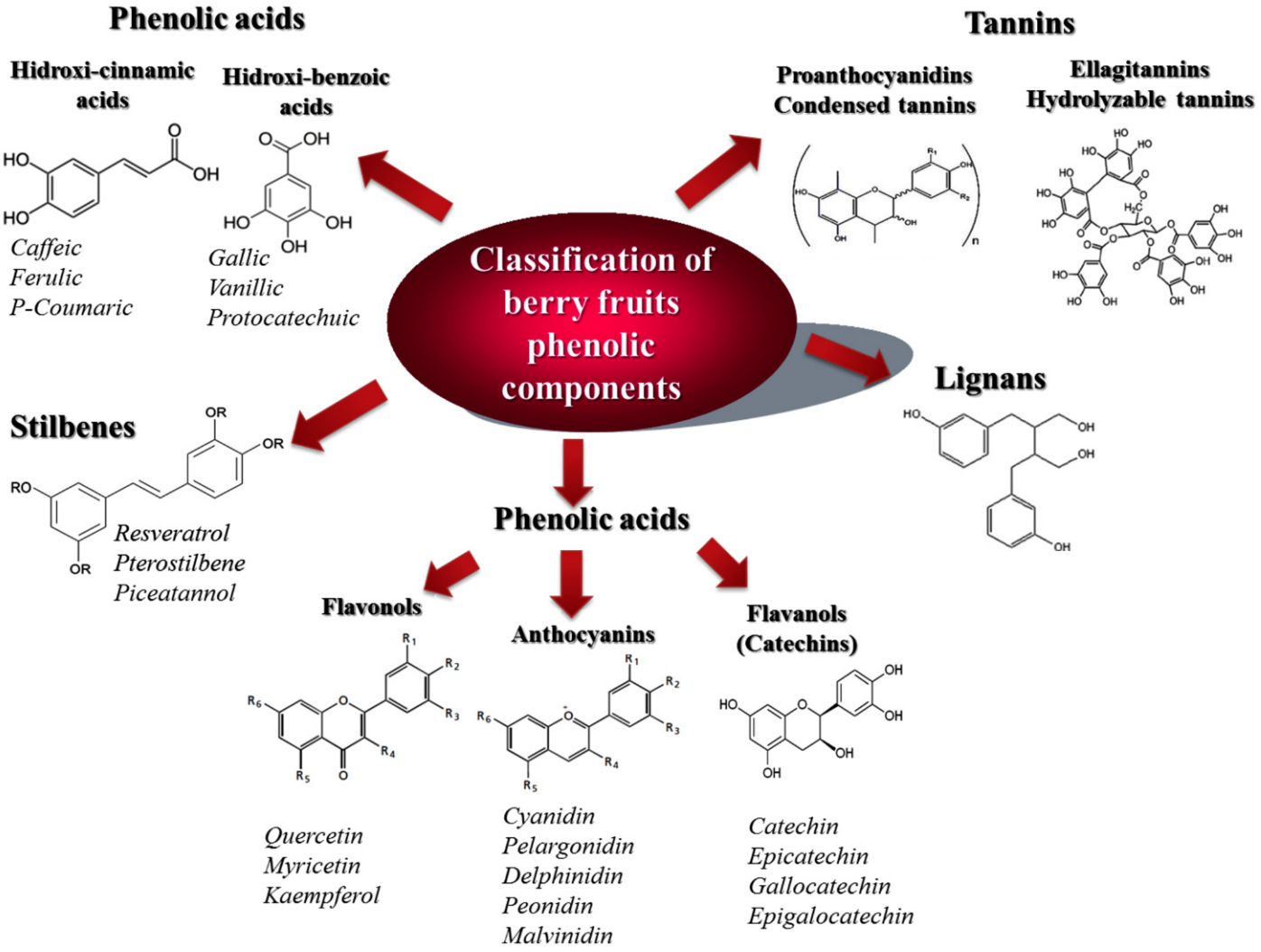


Figure 13.3: Bioactive Compounds in Berries.

responsible for much of their antioxidant activity (Gary David et al., 2008). Plants produce an important and diverse assortment of organic compounds; some of them do not appear to be involved in their development. These compounds are traditionally referred to as secondary metabolites. Secondary metabolites play an important role in plant adaptation and defense under different stress conditions, such as drought, UV radiation, pathogens, and plagues (Jimenez-Garcia et al., 2013b).

6.1.1 Plant-derived food extracts with identified antioxidants properties

The biological activities of individual compounds may not be able to accurately implicate the observed activities for the whole extract (Roleira et al., 2015). Diaconeasa et al. (2015) reported that the structure-degrading enzyme that enables cancerous cells to spread and invade other tissues downregulates by blueberry flavonoids. On the other hand, the growth of tumor cells is inhibited, also blueberry extracts, and have anticarcinogenic properties. Also, Stanisavljević et al. (2015) showed that chokeberry juice was subjected to in vitro gastric digestion in the presence of the food matrix to determine the changes in polyphenol content and antioxidant activity. The addition of food matrix immediately decreased the total phenolic content, anthocyanin content, DPPH scavenging activity as well as total reducing power by 36, 90, 45, and 44%, respectively. The effect reduction of proliferative rate by approximately 25% was determined on caco-2 cells proliferation. Konić-Ristić et al. (2011) demonstrated total phenolics, total anthocyanins, radical scavenging activity, and a proliferative activity against human cancer cell lines evaluated in fresh pressed juices. Total phenolic content ranged from 133.0 to 260.3 mg of gallic acid equivalents/100 g of fresh weight, for red currant and black currant, respectively. Bilberry juice contained the highest amount of total anthocyanins (0.18%).

6.1.2 Antioxidants compounds from synthetic origin

Free radicals and the chronic diseases associated with the aging process, antiviral, and anticancer activity antioxidants are thought to help protect the body against the damaging effects of these. Fresh dark fruited berries (raspberry, blueberry, gooseberries, blackberries, strawberries) having high natural antioxidant content relative to vegetables, such as anthocyanins, other flavonoids and phenolic compounds, vitamins C and E, which exhibit antioxidant activity (Stanisavljević et al., 2015). Extracts of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably high scavenging (Skupień et al., 2006). The berry species belonging to the *Rubus* and *Ribes* genus have very high amounts of antioxidant compounds. For example, the anthocyanin content in black current cultivars (*R. nigrum* L.) ranged between 128 and 420 mg/100 g fruit, for blackberries *(R. hybrid*) 250 mg/100 g fruit, and for black raspberry to 630 mg/100 g fruit. The antioxidant capacity of anthocyanidin is one of their most significant biological and human health properties (Maro et al., 2013; Namiesnik et al., 2014). Vitamin C, several anthocyanins, ellagitannins, and some minor proanthocyanidins-like tannin could cclassify

as the dominant antioxidants in raspberry. Among berry species ellagitannins are only represented in cloudberry and raspberry (between 1 or 2 g per 100 g dry weight and to a minor extent in strawberry (around 100 mg per 100 g). The analyses of raspberry, strawberry, and blueberry indicate that they are rich source of flavonoids, ellagic acid, and tannins are a naturally occurring constituent of many species and has shown promising antimutagenic and anticarcinogenic activity against chemical-induced cancers (Hurst et al., 2010; Vinson et al., 2008). Ellagic acid is a compound that is known to have significant contribution to human health. It is formed by oxidation and dimerization of gallic acid (Namiesnik et al., 2013). Hosseinian and Beta (2007) reported that higher amounts of delphinidin 3-glucoside, malvidin-3-glucoside, and malvidin-3-galactoside were showed the Saskatoon berry and wild blueberry in Canada. Céspedes et al. (2010) detected gentisic acid, ferulic acid, gallic acid, *p*-coumaric acid, sinapic acid, 4-hydroxybenzoic acid, delphinidin, cyanidin, vanillic acid, delphinidin gallate, gallocatechin gallate, quercetin, rutin, myricetin, catechin, epicatechin, and several glycosides of anthocyanidins in Chilean wild blackberry. Delphinidin, cyanidin, ellagic acid, myricetin, quercetin, and their glycoside derivatives were the most abundant components of strawberries from Spain (Huang et al., 2012; Pallauf et al., 2008). Resveratrol (3,40,5-trihydroxy-*trans*-stilbene is a phytochemical found in several dietary sources, such as grapes, berries, peanuts, as well as red wine. An impressive body of experimental findings reveals multiple cellular targets of resveratrol affecting cellular proliferation and growth, apoptosis, inflammation, invasion, angiogenesis, and metastasis (Bishayee et al., 2010). Grape and cranberry juices contain primarily *trans*-resveratrol, whereas processed juice products contain higher proportions of *cis*-resveratrol (Stanisavljević et al., 2015).

6.1.3 Flavonoids, carotenoids, anthocyanins

Berries contain powerful antioxidants, and other bioactive compounds, such as phenolic compounds, organic acids, tannins, anthocyanins, and flavonoids. The chemical structure of phenolic compounds is characterized by one or more aromatic rings with hydroxyl groups. They are classified by structural characteristics in five major groups: phenolic acids, stilbenes, flavonoids (flavonols or catechins, flavonols, flavones, flavonones, isoflavonoids), tannins, and lignans (Jimenez-Garcia et al., 2013a). Subgroups of flavonoids that are commonly found in nature are anthocyanins. They are widely distributed in fruits and vegetables, such as blueberries, blackberries, raspberries, strawberries, blackcurrants, elderberries, grapes, and cranberries. Lower risk for certain cancers, urinary tract health, improved memory, and normal aging have been associated with anthocyanins (de Castro and Anderson, 2015). Cyanidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, isolated from strawberry extracts showed antiproliferative activities in cervical, colon, prostate, and oral cavity cancer cell lines (Zhang et al., 2008); cyanidin-3-glucoside and delphinidin-3-glucoside, isolated from grape skin inhibited the growth of breast cancer cells (Fernandes et al., 2010). Briefly, (+)-catechin and (–)-epicatechin protect liver cancer cells

against *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine, and benzo(a)pyrene induced DNA damage (Delgado et al., 2008); catechin hydrate, possesses anticancer effects in breast cancer cells through TP53/caspase-mediated apoptosis (Alshatwi, 2010); sigmoidin A was cytotoxic to mouse melanoma cells, and this effect was significantly attenuated by glutathione suggesting a prooxidative mechanism of cell death (Habtemariam and Dagne, 2010). An ellagic acid derivative makes the consumption of strawberries and other berries (raspberries, blackberries, and cranberries) suitable for potential health benefits. Ellagic acid comprises 51% of the total phenolic compounds, and it can exist as the free form, as glucosides, or linked as ellagitannins esterified with glucose. Ellagic acid is a phenolic compound that is known as a potent anticarcinogen, antiviral, and antibacterial bioactive compound (Wang et al., 2014). Ellagic acid, a phenolic compound widely found in fruits and vegetables, exerts antiangiogenesis effects in breast cancer and also significantly inhibited breast cancer cells growth (Wang et al., 2012). When isolated from strawberry extracts, they showed antiproliferative activities in cervical, colon, prostate, and oral cavity cancer cells (Zhang et al., 2008). Gallic acid is a potent antioxidant found in berries, black tea, and red wine. It inhibits cell proliferation and cell death in prostate cancer cells (Gach et al., 2015). Gallic acid demonstrated a therapeutic potential for controlling tumor metastasis in stomach adenocarcinoma, by inhibition of NF-jB activity and downregulation of PI3 K/AKT/small GTPase signals (Ho et al., 2010). Acid 1,4-lactone 3-O-gallate, chebulanin, chebulagic acid, mallotusinin, geraniin, and isocorilagin isolated derivated gallic acid from *Phyllanthus emblica* fruit showed antiproliferative activities against breast cancer cells (Liu et al., 2012; Luo et al., 2011). Raspberries contain quercetin glucuronide; it is a potent antioxidant that has additional important biologic effects and potential anticancer properties (Zunino et al., 2010). In fruits rich in anthocyanins, tannins stabilize anthocyanins by binding to them to form copolymers. Most fruits contain condensed tannins. Hydrolysable tannins (derivatives of gallic and ellagic acids) are less frequently encountered and have been found in strawberries, raspberries, and blackberries (Wang et al., 2014). Hydroxycinnamic acids constitute another major group of antioxidant compounds, with inhibitory activity in the proliferation of cancer cell lines. The effectiveness of hydroxycinnamic acids, such as caffeic, chlorogenic, sinapic, ferulic, 3-hydroxycinnamic, and 4-hydroxycinnamic acids, as DNA-cleaning agents in the presence of CuII ions, shows that compounds bearing o-hydroxy or 3,5-dimethoxy groups on phenolic rings, were remarkably more effective at causing DNA damage than the compounds bearing no such groups, caffeic acid being the most active among the acids examined (Fan et al., 2009). 3,4,5-Trihydroxyphenyl-acrylic acid and the glucose ester of (E)-*p*-coumaric acid, isolated from strawberry extracts, showed antiproliferative activities in cervical, colon, prostate, and oral cavity cancer cell lines (Zhang et al., 2008). The radiation sensitizing properties of ferulic acid was also evaluated in vitro, using cervical cancer cells. The results showed an enhancement of oxidative DNA damage and apoptotic morphological changes in ferulic acid plus radiation treated cells (Karthikeyan et al., 2011).

7 Types of Cancer With Potential to be Treated by Medicinal Plants

7.1 Breast Cancer

Breast cancer is the most widespread and leading cancer; it is the second most prevalent cancer among women around the world (Roleira et al., 2015). The cell growth effect of raspberry extracts depended on the concentrations and cell line. The most pronounced effects, that is, the lowest EC_{50} values, were obtained in the breast adenocarcinoma cell line (MCF7), reaching 34.8 and 60.3 μg/mL. Raspberry extracts exhibited substantial cytotoxicity toward the most sensitive tumor cell line and they were from 5- to 20-fold more toxic (Četojević-Simin et al., 2015). Ellagitannins, which may inhibit some mutagenic properties, include geraniin, mallotusinic acid, pedunculagin, agrimoniin, and epigallocatechin gallate. The main examples of inhibited substances are Trp-P-1 (3-amino-1,4-dimethyl-5H pyrido[4,3-*b*]indole), (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine), and *N*-OH-Trp-*P*-2 (3-hydroxyamino-1-methyl-5H-pyrido[4,3-*b*]indole). Ellagitannins limit the mutagenic properties of some chemical compounds may prevent or delay the lengthy carcinogenesis process and inhibit different type cancers (Aqil et al., 2012). Linoleic acid is converted to arachidonic, which can promote inflammatory processes that stimulate cancer cell growth. Also, resveratrol, in an amount easily obtained by supplementation, inhibited the growth of breast cancer cells, and blocked the growth promoting effects of linoleic acid (Stanisavljević et al., 2015). On the other hand, extract of strawberry can induce cytotoxicity in cancer cells, irrespective of origin, in a concentration and time-dependent manner. Treatment of mice bearing breast adenocarcinoma with extract of strawberry blocked the proliferation of tumor cells in a time-dependent manner and resulted in extended life span. Histological and immunohistochemically studies suggest that extract of strawberry treatment affected tumor cell proliferation by activating apoptosis and did not result in any side effects (Stoner et al., 2006). Kedzierska et al. (2012) have demonstrated that the increased generation of free radicals in blood platelets is able to promote changes of platelet aggregation that may contribute to thrombotic complications in breast cancer patients, not only before the surgery, but also during chemotherapy. To reduce severe side effects of treatment various components of diet have been used. Dietary compounds, including the polyphenol rich extract from black chokeberries can reduce oxidative damages and changes of hemostasis (Kedzierska et al., 2010; Kulling and Rawel, 2008; Valko et al., 2006). The effect of chokeberry extract on the oxidative/nitrative stress and hemostatic activity of plasma in whole blood have provided information about an important role of this extract in the defense against oxidative stress-induced changes of hemostatic properties of plasma in patients with breast cancer (Bijak et al., 2011; Malinowska et al., 2012) and distinctly reduce the changes of hemostasis in plasma isolated from patients, but the molecular mechanism(s) of this effect is still unknown. Kędzierska et al. (2013) suggest that the black chokeberries extract decreases the level of homocysteine (which may stimulate thrombosis and oxidative stress) in plasma from different patients, including breast cancer patients. Cyanidin-3-glucoside was found to be the

major anthocyanin in all the berries except *R. rosifolius* and *R. idaeus* cv. *Heritage*, in which pelargonidin-3-glucoside and cyanidin-3-glucosylrutinoside were the major anthocyanins. *R. idaeus* cv. *Golden* was void of anthocyanins. The extracts of *R. racemosus* inhibited breast cancer cells by 31%. The extract of *R. idaeus* cv. *Heritage* resulted in inhibition of breast cancer cells by 17% (Bowen-Forbes et al., 2010). Recent studies reveal the possible benefits of delphinidin in preventing tumor development and malignant progression through inhibition of several major pathways involved in breast oncogenesis. Cyanidin 3-glucoside inhibits ethanol-mediated migration/invasion in cells expressing high levels of ErbB2 and inhibited ethanol stimulated phosphorylation of ErbB2, cSrc, FAK, and p130Cas in breast cancer BT474, MDA-MB-231, and MCF-7 cells (de Castro and Anderson, 2015; Xu et al., 2010). In the cell experiments, we also evaluated other anthocyanin-rich extracts, including blueberry and cranberry suppressed proliferation of MCF7 human breast cancer cells and HT-29 (Hogan et al., 2010). Also isolated and identified were 20 pure compounds from cranberries, including ursolic acid, quercetin, and 3,5,7,3′,4′-pentahydroxyflavonol-3-O-D-glucopyranoside and showed that these compounds have potent antiproliferative activities against breast MCF7 cancer cell growth (Seeram, 2008a).

7.2 Lung Cancer

Lung cancer was uncommon at the start of 20th century; however, it has now reached almost epidemic magnitude. Lung cancer is responsible for most of cancer deaths in developed countries and rising at alarming rates in the developing countries would climb further to more than 3 million per year by the end of 2015 (Diaconeasa et al., 2015). Anthocyanins have been shown to inhibit the development of tumors induced in mice following the subcutaneous injection of lung tumor cells. Ding et al. (2006) showed that cyanidin 3-glucoside could inhibit the proliferation, migration, and invasion of A549 lung tumor cells (Ding et al., 2006). It was found that cyanidin 3-glucoside exhibited antiproliferative activity in a concentration range of 10.4–62.2 μg/mL (Dai et al., 2009). Dietary quercetin, which is the most abundant flavonol in many berry fruits, has been shown to reduce adenoma of the lung. Mechanisms for action of these compounds include interactions with multiple targets in intracellular signal transduction pathways (Zunino et al., 2010). Furthermore, kaempferol-7-O-*b*-D-glucoside and quercetin glucuronides (isolated from rabbit serum) induced G2/M arrest in lung cancer cells (Liu et al., 2008). Arrest in the S phase of the cell cycle was observed in SEM cells treated with strawberry extract and REH treated with strawberry and blackberry. Both blackberries and strawberries predominantly contain hydrolysable ellagitannins, which release ellagic acid (Seeram, 2008a). Therefore, the increase in the percent of cells in S phase after treatment with the blackberry and strawberry extracts is likely at least partially due to the presence of ellagitannins and hydrolyzed ellagic acid (Zunino et al., 2010). Quercetin, found in several varieties of berries and black currents, at concentrations up to 70–80 mg/kg dry wt., was found to inhibit growth and stimulate apoptosis of lung cancer

cells (Stoner et al., 2008). Survival of a lung cancer cell line (NCI-H209) was decreased in a concentration-dependent manner after treatment with quercetin glucuronide. Quercetin treatment resulted in an increase in cell cycle arrest with the cells accumulating in the S and G2/M phases of the cell cycle and in subG0 while undergoing apoptosis was associated with decreases in mitochondrial membrane potential and in the antiapoptotic protein, Bcl-2, and increases in the proapoptotic proteins, p21CIP1/WAF1, Bak, Bax, cytochrome c, and caspase 3. Nguyen et al. (2004) treated A549 human lung epithelial cells with quercetin and found a similar dose-dependent reduction in cell viability and induction of apoptosis. Most of the reduction in cell viability at the two highest doses (43.5 and 58 M) was attributed to apoptosis. In addition, quercetin treatment led to phosphorylation of the extracellular signal-related kinase, ERK 1/2, and ERK 1/2 activation was accompanied by phosphorylation of the mitogen-activated kinase, MEK (Stoner et al., 2008). As well, resveratrol could induce apoptosis in a variety of cancer types, including induced hepatocellular carcinoma (HCC), breast cancer, lung cancer, colon cancer, and prostate cancer through the intrinsic pathway of cell apoptosis, that is, some sort of stimulus disrupts the mitochondria membrane potential. This leads to the release of cytochrome c activating the caspases, which result in a cleavage of PARP and eventually, DNA fragmentation (Han et al., 2015). Thus, the whole process of the intrinsic apoptosis pathway finally causes cell apoptosis. Treating ASTC-a-1 cells of human lung adenocarcinoma with 100 μM resveratrol-induced cell apoptosis specifically by intrinsic pathway as proved by the activation of caspase 3 and 9 but not caspase 8 detected by fluorometric assay (Khandekar et al., 2011). Anthocyanins have been shown to inhibit the development of tumors induced in mice following the subcutaneous injection of lung tumor cells. Isoliquiritigenin is a simple chalcone derivative and found in licorice, often used in Chinese medicine, and vegetables, such as shallot and bean sprouts. The isoliquiritigenin-inhibited cell proliferation in the A549 human lung cancer cell line and induced cell cycle arrest at the G2/M phase, and this induction was associated with the enhanced expression of p21CIP1/WAF1 (de Castro and Anderson, 2015).

7.3 Digestive System Cancer

Stomach cancer is the fourth most common type of cancer despite a major decrease in the incidence and deaths over several decades. The frequency is particularly high in Europe, and parts of central and South America, while it is about twice as high among men as among women (Stoner et al., 2007). Berry extracts retain their biological activity after digestion and fermentation despite considerable structural modification and this strongly suggests that breakdown products and individual colonic metabolites can modulate cellular processes associated with colon cancer. Beyond activity related to the inhibition of the initiation of carcinogenesis, DNA damage, and mutation, the berry phytochemicals also exhibited activity related to metastasis (Coates et al., 2007). Berry constituents can significantly affect many pathways, leading to the development of esophageal adenocarcinoma. These phytochemical

have been shown to reduce DNA damage, increase DNA repair gene expression, and reduce oxidative stress, inflammation, and angiogenesis. Blueberry and blackberry have been employed in the prevention of squamous cell carcinoma and esophageal adenocarcinoma (Aiyer et al., 2011). A recent study indicated that diets rich in anthocyanins prevented the development of esophageal tumors, reducing tumor numbers by 42%–47%, suggesting that the anthocyanins in lyophilized berries are important for their chemopreventive activity. The role of these anthocyanins in the prevention of esophageal tumors in vivo, however, has not been determined (de Castro and Anderson, 2015). Regarding in vitro studies, treatment with black raspberry derived anthocyanins suppressed cell proliferation and increased apoptosis in colon cancer HCT-116, caco-2, and SW480 cells. This effect was partly mediated by mRNA regulation of β-catenin and c-Myc genes. Wang et al. (2013) found that anthocyanins demethylated tumor suppressor genes, such as CDKN2A, SFRP2, SFRP5, and WIF1, through the inhibition of DNA methyl transferase 1 (DNMT1) and DNMT3B in colon cancer cells. The blueberry (*V. corymbosum* L., family: *Aricaceae*) is rich in polyphenols, such as anthocyanins, flavonols, tannins, and phenolic acids, which show potential to prevent cancer through their biological activities (Afrin et al., 2016). In the last decade, the blueberry has become more famous for its nutritional value and human benefits. Blueberry extract was reported to inhibit human colon cancer HT-29 and HCT-116 cell proliferation at high concentrations (Seeram, 2006) and human colon cancer caco-2 cells growth at low concentrations (McDougall et al., 2008). Strawberry fruit crude extracts and purified compounds have been reported to inhibit the growth of human colon cancer HT-29 and HCT-116 cells (Lizarraga et al., 2011). Polyphenol-rich extracts also exhibited greater antiproliferative activity in caco-2 cells (McDougall et al., 2008). The anticarcinogenic effects of kaempferol have been described in HT-29 cells due to the induction of cell cycle arrest at G1 and G2/M phase as well as suppression of the activity of CDK2, CDK4, cyclin D1, cyclin E, cyclin, A Cdc25C, Cdc2, cyclin B1, retinoblastoma protein (Rb), and Wnt signaling pathway (Cho and Park, 2013). Bilberry phenolic extracts were reported to induce antiproliferative effect in HT-29 cells by increasing the expression of the p21WAF1 pathway (Afrin et al., 2016). Esselen et al. (2011) found that bilberry extracts significantly suppressed the DNA strand breaking effects of camptothecin and doxorubicin (topoisomerase poisons applied during chemotherapy) in HT-29 cells. The antiproliferative activity of cranberry extracts and various fractions were observed against a series of colon cancer cell lines including HCT-116, SW480, and SW620. The total polyphenol fraction was the most active fraction against all cell lines compared to other cranberry extracts (Afrin et al., 2016).

7.4 Skin Cancer

Skin cancer is currently the most common type of human cancer in Americans, and its incidence is increasing at an astonishing rate. Epidemiologic evidence and molecular studies suggest that nonmelanoma skin cancer is related to excessive exposure to the UV

radiation in sunlight. Most skin cancers comprise basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and cutaneous malignant melanoma (CMM), together responsible for about 98% of all skin cancers (de Vries et al., 2012). Cyclooxygenase (COX), a rate-limiting enzyme for the oxidative conversion of arachidonic acids to prostaglandins, has two main isoforms: COX-1 and COX-2. COX-1 is a constitutively expressed isoform, and COX-2 is an inducible isoform (Wang et al., 1992). Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone is one of the major flavonoids found in various foods, including berries and grapes, as well as red wine (Nishino, 1995). A recent study showed that myricetin strongly inhibited tumor promoter–induced neoplastic cell transformation by direct inhibition of mitogen-activated protein kinase (MAPK)/ERK kinase (MEK) kinase activity. Moreover, its inhibitory effect was more potent than resveratrol, which is a well-known chemopreventive agent with anticancer effects (Stoner, 2009). These accumulated data provide evidence that myricetin is a potent anticancer agent, but the underlying mechanism and target(s) in skin cancer are unclear. Here, we report that myricetin is an ATP-competitive inhibitor of Fyn and subsequently suppresses UVB-induced COX-2 expression in JB6 P^+ mouse skin epidermal cells and in mouse dorsal skin. In a mouse skin tumorigenesis model, myricetin strongly suppressed UVB-induced incidence of mouse skin tumors (Jung et al., 2008). The choice of berry formulation (powder, extract, and anthocyanin) for specific chemoprevention studies depends, in part, on the target tissue. For example, black raspberry gels applied topically to oral lesions optimize the delivery of anthocyanins to target tissues (Shumway et al., 2008), and the topical application of an alcohol-water extract of black raspberry powder to mouse skin inhibits UVB-induced skin tumorigenesis (Stoner, 2009). Using the SKH-1 hairless mouse, Yun et al. (2009) investigated the photochemopreventive effect of delphinidin, a major anthocyanidin present in many pigmented fruits and berries, on UVB-induced biomarkers of skin cancer development. Topical application of delphinidin (1 mg/application) to mouse skin inhibited apoptosis and markers of DNA damage, such as cyclobutane pyrimidine dimers, and 8-OHdG. Topical application of anthocyanin- and tannin-rich berries and pomegranate extracts (2 mg/mouse) to the skin of CD-1 mice significantly inhibited TPA-mediated increases in skin edema and hyperplasia, ornithine decarboxylase (ODC) activity, and protein expression of both ODC and COX-2 (Wang and Stoner, 2008).

7.5 Other Cancer Types

Some berry extracts showed good in vitro and in vivo activities against other tumor cell lines. For example, flavonoids and lignans were reported active against cervical cancer cell lines, and flavonoids isolated from low bush blueberry on human prostate cancer cells. This regulatory effect was directed at the activity of MMP-2 and MMP-9. These activities may be one of several targets of the potential antiproliferative effects associated with flavonoid-containing fractions isolated from low bush blueberries (Matchett et al., 2005). There are many reports describing the in vivo activities of resveratrol and related stilbenes

on the prevention of skin esophageal, lung, and mammary tumorigenesis (Rimando and Suh, 2008). In another report, the administration of resveratrol (0.2 mg/kg/day) to male F344 rats inhibited the formation of aberrant crypt foci preneoplastic lesions of the colonic mucosa (Bishayee et al., 2010). A study by Seeram (2006) showed that blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit the growth of human oral (KB and CAL27), breast (MCF-7), colon (HT29, HCt 116), and prostate (LNCaP) cancer cell lines in a dose-dependent manner. Berry extracts, including strawberry and raspberry, were evaluated for their effects on cell viability and expression of markers of cell proliferation and apoptosis in human HT29 colon cancer cells. Wu et al. (2007) concluded that the berry extracts inhibited cancer cell proliferation mainly via the p21WAF1 (a member of the cyclin kinase inhibitors) pathway. Other studies showed that the blueberry proanthocyanidins had a greater effect on the androgen-dependent growth of prostate cancer cells (Schmidt et al., 2006). Finally, and anthocyanin-rich preparation, from berries (wild blueberry, bilberry, cranberry, elderberry, raspberry, and strawberry), was found to reduce vascular endothelial growth factor (VEGF) expression in a spontaneously immortalized human keratinocyte cell line (HaCaT) and inhibit endothelial tube formation in a Matrigel assay (Bagchi et al., 2004).

8 Epidemiological Studies of Cancer

Epidemiological studies showed that populations with fruit- and vegetable-rich diets (bioactive compounds rich) have a lower rate of cancer and other diseases, such as cardiovascular disease and chronic disease (Liu, 2013). Phytochemicals are defined as bioactive nutrient plant chemicals in fruits, vegetables, grains, and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases (Liu, 2004). Actually, obesity is frequently correlated with a large number of cancers, and is among those most responsive to dietary modification (Tulipani et al., 2008). Shih et al. (2005) initially demonstrated the ability of anthocyanins to induce phase II antioxidant and detoxifying enzymes in cultured cells. Treatment of rat liver clone 9 cells with 50 μM anthocyanins (Wang and Stoner, 2008) and noncancerous breast cells with 10–20 μg/mL anthocyanins (Singletary et al., 2007) enhanced their antioxidant capacity by activating glutathione-related enzymes as well as the activity of NAD(P)H: quinone reductase. Epidemiological studies in humans have not provided convincing evidence of the anticancer effects of anthocyanins unlike the in vivo animal model studies, For example, a case-control study involving 805 subjects with oral and pharyngeal cancer and 2081 hospital controls without neoplasia was conducted in Italy to examine the relationship between anthocyanidin intake and cancer risk (Rossi et al., 2007). The role of six principal classes of flavonoids, including the anthocyanidins, on prostate cancer risk was studied using data from a multicentric case-control study (Bosetti et al., 2006). Supplementation of anthocyanins in the diet of cancer patients receiving chemotherapy did not result in increased inhibition of tumor development

when compared to chemotherapy alone (Bode et al., 1998). In Germany, individuals who consumed an anthocyanin/polyphenolic-rich fruit juice reduced oxidative DNA damage and had a significant increase in reduced glutathione when compared to controls (Weisel et al., 2006). Anthocyanin intake did not show a reduction of cancer risk in humans, although epidemiological studies suggest that anthocyanin intake may reduce certain parameters of oxidative damage.

9 Conclusions

The cancer chemotherapeutic agents, such as phytochemicals in berries, have been shown to have modulatory effects along the pathway cancer. Nevertheless, investigations are deepening on the knowledge of the effect of the bioactive compounds found in these fruits and the functioning of these on the organism. Furthermore, several articles show the possibility of positive correlates with antiproliferative activity antioxidant activity, showing that oxidative mechanisms may be involved in the proliferation of cancer cells. Nonetheless, the presence of higher antioxidant capacity and cytotoxic activity usually is related to the high amount of bioactive compounds in berries. The mechanism of the observed cytotoxic effects normally related to cell cycle arrest followed by apoptosis. Future research should consider these problems to provide numerous opportunities in the research of berries used in the food industry and medicine and provide a wide range of health benefits.

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CHAPTER 14

Functional Foods and Chemoprevention in Cancer

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1 Functional Foods and Bioactive Compounds

Functional foods are natural or processed foods that contain biologically active substances (bioactive compounds or bioactives) in addition to nutritive compounds. The International Life Science Institute (ILSI) describes functional foods as having a proven beneficial effect, beyond basic nutrition, on one or more specific functions in the organism, to improve health and wellness and/or decrease the risk of disease (Herrera et al., 2014). According to the American Dietetic Association (ADA), functional foods are: "vegetables, fruits, plants, or seeds that provide a beneficial effect on human health, when they are consumed in a dairy and varied diet in effective doses, including fortified or enriched products." The term, "phytochemical" is a recent evolution of the term "functional foods," and refers to the naturally occurring compounds of plants with protective or preventive disease properties (Hasler and Brown, 2009).

Phytochemicals are nonessential nutrients and therefore, in contrast to vitamins and minerals, no recommended intake exists. However, the European Prospective Investigation into Cancer and Nutrition (González et al., 2004) suggest that a daily consumption of 500 g of fruits and vegetables can decrease the incidence of digestive tract cancers by up to 25%. Additionally, WHO (2004) statement indicates that eating 5 portions or 400 g of vegetables and fruits each day, decreases the risk of noncommunicable diseases and helps ensure an adequate daily intake of dietary fiber. Indeed, their consumption benefits many aspects of human health and plays an active role in cancer prevention. Phytochemicals have effects on gene modulation and display antioxidant, antiinflammatory, anticarcinogenic, inmunomodulative, and antimicrobial properties (Heber, 2004).

The combined or synergic effect of dietary phytochemicals is reportedly responsible for their antioxidant and anticarcinogenic properties. Such effects are provided by a high

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fruit and vegetable diet that is varied in color. This is due to the fact that many different phytochemicals are responsible for the color and odor of plant foods (Heber, 2004).

1.1 Bioactive Compounds With Chemopreventive Action

Chemoprevention acts to prevent, decrease, control, or reverse cancer through the administration of one or more natural or synthetic bioactives, by slowing down the rate of carcinogenesis. This concept continues to gain strength as an alternative or complementary cancer treatment for its accessibility and cost-benefit. Including bioactives in the daily diet is the most sustainable way to decrease long-term cancer risk and/or positively contribute to cancer treatment due to the availability and affordability of bioactives, compared to other cancer treatments, such as chemotherapy and radiotherapy (Tsao et al., 2004).

Chemopreventive agents can be classified into two broad categories based on their mechanism of action:

1. The first category is the antiinitiation agents or "blocking agents," which inhibit carcinogenesis by preventing carcinogens or their precursors from reaching or reacting with critical targets (e.g., DNA, RNA, proteins).
2. The second category is the antipromotion or "suppressive agents," which act against tumor promoters by suppressing the expression of a neoplasia in cells that would otherwise become malignant due to previous exposure to certain doses of a carcinogen (Steward and Brown, 2013).

Certain chemopreventive agents may exhibit several different simultaneous mechanisms of action. While the exact mechanisms by which dietary bioactives elicit their anticancer effects are unknown, various mechanisms have been proposed. Some of which are:

- *Antioxidant functio*n (*A*): equilibrate the oxidation-reduction mechanisms of the organism and prevent cellular aging.
- *Apoptosis promotion* (*APOP*): induce cellular apoptosis.
- *Tumor growth reduction* (*RCC*): interfere in the process of cell growth by decreasing the rate of cell proliferation or decreasing tumor size.
- *Immune system* (*SI*): support immune system through the administration of minerals and vitamins.
- *Antiinflammatory* (*AINF*): stimulate the synthesis of prostaglandins, thromboxanes, and leukotrienes (PGE1, TXA1) with antiinflammatory and antithrombotic action.
- *Antiangiogenic* (*AANG*): arrest the formation of new blood vessels around tumors to slow tumor growth and metastasis.
- *Detoxification* (*DTX*): induce the expression of cytochrome p450.
- *Bioinactivation of carcinogen substances* (*BISC*): inactivate or eliminate carcinogens, prior to cell damage.

Table 14.1: Bioactive compounds in food and their action mechanisms.

Bioactive Compounds	Anticancer Mechanism	Food Sources	Type of Cancer Prevention	References
Lycopene	A, RCC, SI, APOP	Tomato, watermelon, grapefruit, and pink guava	Breast, prostate, and colon cancer	Chen et al. (2013)
Fiber (inulin and lignans)	DTX	Wheat, rye bread, oat flakes, whole wheat bread (whole grain bread), corn tortillas, fruit and the peel of vegetables, and beans and lentils	Colon cancer	Buck et al. (2010); Kim (2010)
Indol and glucosinolates,	A, APOP, BISC	Broccoli, cauliflower, chard, spinach, green peppers, and col	Skin, breast, ovary, and prostate cancer	Tse and Eslick (2014)
β-Carotene	A, RCC	Mango, carrot, red and yellow peppers, Italian squash, and local cabbage	Mouth, esophagus, and lung cancer	Männistö et al. (2010); Jeon et al. (2011)
Genistein	A, RCC, AANG	Beans, soybeans, lentils, and peas	Breast and prostate cancer	Hong et al. (2012)
Resveratrol, ellagic acid, anthocyanins, quercetin	A, A, APOP, RCC	Red grapes, cherries, strawberries, blueberries, red apples, purple grapes, mulberries, and red plums	Ovary, breast, prostate, colon, skin, and liver cancer	Woo and Kim (2013)
Gingerol, curcumin	A, SI, AINF	Curry, turmeric, ginger, and rosemary	Skin and lung cancer	He et al. (2011)
PUFAs	RCC, APOP, AANG, SI	Nuts, almonds, olive oil, avocado, salmon, and tuna	Breast and prostate cancer	Fu et al. (2015)
Poliphenols, epilocatequin-3-gallate	A, SI, RCC, AANG, APOP	Coffee, black chocolate, and green tea	Cancer in general	Thomasset et al. (2009)

A, Antioxidant activity; AANG, antiangiogenic; AINF, antiinflammatory; APOP, promotion of apoptosis; BISC, bioinactivation of carcinogenic substances; DTX, detoxification; RCC, reduction of its growth; SI. immune system.

As is shown in Table 14.1, one bioactive compound, which can be found in different foods, is capable of performing different functions simultaneously in one cell.

1.2 Compounds That Inhibit Carcinogens

Compounds that inhibit carcinogens primarily act to prevent the formation of nitrosamines. Nitrosamines are formed from the naturally occurring reaction between amines and nitrites, which can be added to food or produced by bacterial nitrate reduction. Nitrosamines and other *N*-nitroso compounds can exist in contaminated foods or be produced in the processing, storage, or preparation of foods and in vivo. The

Table 14.2: Blocking agents' categories (antiinitiators).

Mechanism	Bioactive Compounds	Food Sources
Inhibition of citochrome P450	Isoticionates Ellagic acid	Cruciferous (broccoli, cabbage, cauliflower, and Brussels sprouts) Green tea
Induction of phase N enzymes	Isoticionates Poliphenols	Cruciferous (broccoli, cabbage, cauliflower, and Brussels sprouts) Green tea, curcumin, legumes, red wine, red fruits and vegetables, cocoa, olive oil, nuts, onions, and garlic
Electrofilic kidnappers	Ellagic acid	Green tea

Source: Cimino, S., Sortino, G., Favilla, V., Castelli, T., Madonia, M., Sansalone, S., et al. 2012. Polyphenols: Key issues involved in chemoprevention of prostate cancer. Oxid. Med. Cell. Longev. 2012, 1–8.

compounds have been declared carcinogenic in experimental studies and animal models, but there is insufficient evidence of their effects in low doses in humans. Ascorbic acid efficiently inhibits the in vitro and in vivo formation of nitrosamines. Alphatocopherol and certain phenols can inhibit the formation of *N*-nitroso compounds (Agudo and González, 2002).

1.2.1 Blocking agents

Chemical carcinogens act via common mechanisms. All are ultimately converted, by either nonenzymatic (direct action) or metabolic activation, into highly reactive, positively charged electrophiles, which interact directly with target DNA, altering its chemistry and structure. This is believed to be a key step in the initiation of carcinogenesis in the cell. Table 14.2 lists the categories of blocking agents, their mechanism of action, the bioactive blocking compound, and certain foods in which they can be found.

1.2.2 Tumor suppressor compounds

Tumor suppressor compounds inhibit tumor promoters when they are administrated after the ingestion of a carcinogen. Many epidemiological studies do not consider the importance of the sequence of food consumption, yet it is known that in a meal containing a suppressive compound and a carcinogen, consuming the protective substance first has a greater protective effect than if the carcinogen was consumed first (Tanaka et al., 2016). Table 14.3 lists nine classes of compounds that inhibit tumor growth, their mechanism of action, the bioactive tumor suppressor compounds, and foods in which can be found.

2 Bioactive Compounds With Anticancer Activity

Some of the principal dietary phytochemicals registered in clinical trials as having one or more anticancer activities are discussed in this section and have been classified according to their chemical characteristics.

Table 14.3: Suppress agents' categories (antipromotion/antiprogression).

Mechanism	Bioactive Compounds	Food Sources
Inhibition of arachidonic acid metabolism	Quercetin	Apples, grapes, onions, mangoes, oats, cherries, pears, and broccoli
	Curcumin	Curcuma
Differentiation induction	Retinoid	Eggs, milk, green leaf vegetables, carrots, liver, and yellow-orange vegetables
	Calcium	Milk and its derivatives, almonds, sesame seeds, and broccoli
	Vitamin D	Fish, eggs, mushrooms, and dairy products
Inhibition of oxidative damage to DNA	Selenium	Nuts, legumes, oatmeal, chicken, fish, eggs, and garlic

Source: Cimino, S., Sortino, G., Favilla, V., Castelli, T., Madonia, M., Sansalone, S., et al. 2012. Polyphenols: Key issues involved in chemoprevention of prostate cancer. Oxid. Med. Cell. Longev. 2012, 1–8.

2.1 Carotenoids

These compounds have protective functions, particularly in breast cancer. Several studies in vitro confirm that high carotenoid consumption has a chemopreventive effect and a protective effect against cancer metastasis (Fernández, 2010).

2.1.1 β-Carotene

β-Carotene is a retinoid and liposoluble antioxidant associated with decreasing DNA damage caused by oxidative processes (Fernández, 2010). Evidence shows that the molecule modulates mammalian cell proliferation, differentiation, and apoptosis (Jeon et al., 2011). Various studies have demonstrated an inverse correlation between serum β-carotene levels and cancer patients. A low-fat, high β-carotene diet slowed down the risk of early death in women with breast cancer; however, clinical evidence concerning β-carotene and breast cancer has shown contradictory results (Kellen et al., 2009).

Epidemiological studies suggest that β-carotene supplements have no positive effect against cancer and adverse effects on lung cancer in cigarette smokers and asbestos laborers. The association between lung cancer risk and β-carotene ingestion has been analyzed using primary databases from seven cohort studies in North America and Europe. A follow-up 7−16 years after the study, found 3,155 lung cancer diagnoses among the 399,765 participants. β-Carotene ingestion showed no association with the risk of lung cancer (Männistö et al., 2010).

According to Jeon et al. (2011), β-carotene supplements had no preventative effect on cancer prevention. Similarly, Druesne-Pecollo et al. (2010) showed no effect of β-carotene supplementation on the incidence of pancreatic, colon, prostate, breast, and skin cancer. In lung and stomach cancer, β-carotene supplementation (20−30 mg per day) elevated cancer incidence. The authors concluded that β-carotene supplementation has no positive effect on

cancer incidence and it is not recommended. In contrast, β-carotene supplementation (50 mg, alternate days) did not increase the relative risk of total, lung, prostate, or colon cancer among 22,071 male physicians monitored over approximately 13 years (Cook et al., 2000). Thus, high doses of a single antioxidant vitamin do not indicate a viable chemopreventive strategy. However, the age of the participants appears to play a crucial role in the effects obtained. A primary prevention trial evaluated the effect of the supplementation with β-carotene (15 mg), selenium (50 μg), and vitamin E (30 mg), over 10 years in 29,584 adults of both sexes. Interestingly, they found a decrease in mortality caused by cancer, especially in stomach cancer (Qiao et al., 2009).

2.1.2 Lycopene

Lycopene is an open-chain polyisoprenoid containing 40 carbon atoms and 11 conjugated double bonds. The carotenoid is found in red fruits and vegetables, such as guavas, watermelon, grapefruit, red peppers, and, in particular, tomatoes. In addition to its antioxidant properties, nonoxidative mechanisms have been proposed for its chemopreventive roles, such as regulation of intercellular gap junction communications, immune and hormonal systems, and metabolic pathways of xenobiotics. In breast tissue cells, lycopene can interfere with IGF-1, decreasing cell proliferation and promoting apoptosis. A combination of lycopene, vitamin E, selenium, and soy isoflavones inhibited cell proliferation and induced apoptosis in breast cancer (Tomita et al., 2010).

Lycopene ingestion (principally from tomato products) has demonstrated a lower risk in human cancer. Lycopene administration in in vivo studies inhibited the incidence and multiplicity of lung adenomas and carcinomas in rat models (Palozza et al., 2011). According to Canene-Adams et al. (2010), supplementation with 15 mg of lycopene twice a day for 3 weeks reduced the size of prostate tumors by 45%, in comparison with the nonsupplemented group. Furthermore, prostate-specific antigen plasma concentrations decreased 18% in the supplemented group, compared to 14% in the control group. The authors concluded that a daily 30 mg lycopene supplementation might be sufficient to modulate prostate cancer clinical markers and treat this disease. According to Chen et al. (2013), 15 mg of daily lycopene supplementation decreased lipid and protein peroxidation. The same dose elevated antioxidant enzymes, such as superoxide dismutase (SOD). A dose of up to 30 mg per day for 2−4 weeks, decreased urinary 8-hydroxy-2-deoxyguanosine, a marker of oxidative stress.

2.2 Phenolics and Polyphenols

2.2.1 Resveratrol

Resveratrol is a polyphenolic phytoalexin found in red wine and grapes. In several studies a reduction has been found in oesophageal, intestinal, and colon tumors by inducing apoptosis in prostate cancer cells. Resveratrol has low toxicity and has the capacity to target multiple

signaling molecules that promote cancer cell survival and tumor growth. It modulates numerous intracellular cancer targets, which affect cell growth, apoptosis, inflammation, angiogenesis, invasion, and metastasis. It is also able to potentiate apoptotic effects of chemotherapeutic agents, gamma radiation, and cytokine effects (Cimino et al., 2012).

Treatment of HaCat cells (a model for human skin keratinocytes) with resveratrol (25 and 100 μM) prior to ultraviolet (UV) radiation potentiated UVB-induced apoptosis and also induced autophagy, thus decreasing the odds of damaged cells escaping programmed cell death to initiate malignant transformation (Vitale et al., 2013). Resveratrol also had a chemopreventive effect in human oral cancer cell metastasis (Lin et al., 2015). In mice with lung metastatic cancer, a 2.5 and 10 mg/kg dose of resveratrol decreased tumor volume (42%), tumor weight (46%), and metastatic potential by 56% (Athar et al., 2010).

There are few studies concerning dietary resveratrol and cancer in humans, due to the very low bioavailability of oral resveratrol. However, several studies have associated red wine consumption with a decreased cancer risk. The resveratrol content in wine varies, depending on the wine making process and grape variety. Red wines contain the highest amounts of resveratrol (maximum 12.59 mg/L) (Andlauer et al., 2010; Walle et al., 2008). Chao et al. (2010) reported that moderate wine consumption (one or more glasses of wine per day) was inversely associated with lung cancer risk. In another study, consumption of one to three glasses of wine per day was reported to have potential preventive effects against oral cancer; however, four glasses of wine per day increased cancer risk (Varoni et al., 2015).

2.2.2 Curcumin

Curcumin is a yellow polyphenol derived from the rhizome of *Curcuma longa*, also called turmeric, and is well known for its high antioxidant activity (Cimino et al., 2012). Curcumin and other chemically related curcuminoids represent 3%−5% of turmeric.

Curcumin has antitumor activity and suppresses cancer initiation, progression, and promotion. It also inhibits cancer cell growth and promotes apoptosis. Curcumin has synergic chemopreventive effects with other polyphenolic compounds, such as genistein and green tea polyphenols, and increases the efficacy of anticancer medicines (Cimino et al., 2012). Its anticancer properties are attributed to its effect on multiple targets, such as transcription factors, growth regulators, adhesion molecules, apoptosis genes, angiogenesis regulators, and cell signaling molecules (Cimino et al., 2012).

It has been observed that curcumin induces depletion of cellular reduced glutathione (GSH), increasing the concentrations of radical oxygen species (ROS) and promoting GSH elimination in the cytosol and mitochondria. Curcumin also induces cell release of cytochrome c, and activation of capsase-3 (a marker of apoptosis) and poly(adenosine diphosphate ribose) polymerase (PARP). However, the curcumin-induced ROS increase also liberated apoptosis inductor factor (AIF) and endonuclease G (EndoG). Curcumin causes

apoptosis of ROS-activated cells, which implicates that it suppresses gene tumor p53. Many studies suggest that p53 is a potential mediator for ROS and is dependent on apoptosis (Chung et al., 2011).

According to Shehzad et al. (2010), consumption of 180 mg of curcumin for 4 months showed no long-term toxicity in humans. The Pan American Health Organisation (PAHO) recommends a daily maximum curcumin ingestion of 0−1 mg/kg with no adverse effects (WHO, 2000). In in vitro colon cancer cells, curcumin has been demonstrated to inhibit cell proliferation. Asok et al. (2012) studied the antiproliferative effect of curcumin demonstrated in breast cancer cells and suggested that curcumin was a potential anticancer agent. According to He et al. (2011), curcumin administration decreases serum concentrations of tumor necrosis factor-alpha (TNF-α), increases apoptosis in cancer cells, and increases the expression of p53 in tumor tissues, modulating apoptosis.

Curcumin protects against DNA damage. In an occidental population exposed to arsenic, curcumin exerted an antagonistic role against ROS and lipid peroxidation (He et al., 2011). After 3 months of curcumin administration, arsenic-induced DNA damage, ROS, and lipid peroxidation were decreased and antioxidant activity increased (He et al., 2011). A meta-analysis evaluated the efficiency of purified curcumin supplementation (80−1500 mg per day, for 4−8 weeks) on enzymes, such as SOD and GSH, which are used as oxidative stress markers in blood. In seven studies, it was found that SOD blood concentrations increased after curcumin supplementation. In another study, there were no significant effects of administrating curcumin for less than 6 weeks, but SOD increased after 6 weeks of curcumin supplementation (Biswas et al., 2010).

2.2.3 Flavonoids

Flavonoids are natural polyphenolic pigments that protect fruits and vegetables from antioxidant damage caused by UV and pollution, for example. These compounds can be found in onions, grapes, citrus fruits, apples, cherries, red wine, green tea, soy products, green leaves, and in some fungi and seaweed (Ochoa and Ayala, 2004). Flavonoids have antioxidant activity and also regulate cancer cell growth and induce detoxification enzymes, such as cytochrome p450 (Cabrera and Mach, 2012).

The average ingestion of flavonoids is 23 mg/day, with quercetin the predominant flavonoid at 16 mg/day (Cimino et al., 2012). This ingestion value exceeds that of some common antioxidants, such as β-carotene (2−3 mg/day) and vitamin E (7−10 mg/day). This is because flavonoids have particularly important antioxidant potential in the human diet. Citrus flavonoids and proanthocyanidins from grape seeds improve the absorption of vitamin C, protecting it from oxidation and improving its function. Hence, they are involved in collagen maintenance, which participates in tissue formation, preventing ROS damage, inhibiting enzymatic action and preventing liberation of inflammatory agents (Meiyanto et al., 2012).

In humans, flavonoid contribution to the antioxidant defense mechanism may be substantial, because daily flavonoid consumption may be as much as 50−800 mg (Ochoa and Ayala, 2004); this average consumption is high compared vitamin C (70 mg), vitamin E (10 mg), and carotenoids (2 mg) consumption. Flavonoids with the highest antioxidant power are catechin, apigenin, quercetin, genistein, naringenin, and cyanidin-3-glucoside (C3G) (available in red wine and cherries) (Cimino et al., 2012).

Flavonoids have been well studied because of their anticancer properties and are used in cancer therapies. The precise mechanisms responsible for flavonoid antitumor effects are not fully known. However, induction of cell cycle arrest and apoptosis, tumor suppression, increased levels of cyclin-dependent kinase (CDK) inhibitors (such as p53, p21, and p27), and inhibition of cyclin D1, E and CDK4 expression may contribute to their anticarcinogenic activities. Clinical studies show a dose-dependent association between flavonoids and their anticancer effects; however, an overdose may lead to secondary effects (Hui et al., 2013).

In vitro and in vivo studies have demonstrated that flavonoids can reduce inflammation by 78%. Apigenin showed chemopreventive activity toward prostate cancer, decreasing cancer cell migration and inducing in vitro apoptosis; it also extended the lifetime of the animals with prostate cancer and protected against UV damage, which may be useful in the treatment of skin cancer (Álvarez, 2010). According to Zhang (2014), Chinese women with ovarian cancer who drank at least one cup of green tea per day lived longer than patients who did not. In an epidemiological study published in 2010 involving a cohort of 36,177 men and 40,484 oriental women 45−75 years of age with an 11-year follow-up, a proportional inverse association was found between flavonoid (isoflavone) ingestion and the risk of lung cancer in nonsmokers (men and women); this association was not observed in smokers or exsmokers (García et al., 2012).

As evidenced in in vitro and in vivo studies, flavonoids show potential chemopreventive effects against several types of cancer. However, contradictory results are found in epidemiological studies. Hence, based on the results of these studies, no specific dietary flavonoid intake has been recommended to treat cancer patients.

2.2.3.1 Quercetin

Quercetin is a flavonoid found in high concentrations in fruits and vegetables, particularly in onions. It is the most abundant and common flavonoid in the human diet and has excellent antioxidant activity. The anticancer activity of quercetin, however, is attributed to its inhibition of tumor cell growth and proliferation (Celis, 2014).

Several studies have demonstrated the biochemical effects of quercetin in mammalian cells, including increased cyclic adenosine monophosphate (AMP), cyclic guanosine monophosphate (cGMP), and phosphodiesterase concentrations, inhibition of enzymatic activities, such as protein kinase C and protein tyrosine kinase, and interaction with type II

oestrogen sites. These biological effects can explain its inhibitory effect on tumor cells, and its capacity to arrest tumor cells in the G1 phase, or less frequently, in the G2 phase of the cell cycle (Fernández, 2011). Colorectal tumor risks could be decreased by polyphenol-rich diets that inhibit cell growth. The survival of in vitro colon adenocarcinoma cells (HT29) was assessed after treatment with apple polyphenols for 24 h (He et al., 2015). The treated cells showed a 1.6−2.1-fold increment in GSH-S-transferase 1, GSH-S-transferase 2, and GSH-S-microsomal transferase expression (He et al., 2015).

In in vivo studies, quercetin administration decreased breast carcinoma size and invasion in chemically treated female Wistar rats, increasing serum antioxidant levels and decreasing tumor markers, thereby exerting a chemopreventive effect (Santhalakshmi and Ramakrishna, 2015). Quercetin has also been shown to prevent neoplastic hepatic lesions and suppress the tumorigenic activity of various carcinogens in rats with colon cancer (Celis, 2014).

Flavones, flavanones, isoflavones, and quercetin have an inverse association with smoking-cancer risk. Quercetin showed the most significant reduction in lung cancer risk. The quercetin doses evaluated were: >4.7 versus <1.8 mg per day, 32.79 versus 6.49 mg per day, 13.20 versus 3.34 mg per day; these doses depended on the region and food source, as well as maturation stage of the fruit (Woo and Kim, 2013).

2.2.4 Anthocyanins

Flavonoids have subgroups, anthocyanins are one of them and they give red, purple, and blue colors to fruits and vegetables, such as blackberries, blueberries, raspberries, red grapes, and cranberries. Due to the diversity of glycosylation and acylation, there are at least 300 naturally occurring anthocyanins. The most important are pelargonidin, delphinidin, cyanidin, petunidin, peonidin, and malvidin (Ortíz et al., 2011).

According to Albrecht et al. (2014) extracts of pomegranate inhibited prostate cancer cell proliferation, both hormone sensible and hormone resistant. In vivo studies demonstrated anthocyanins had antiinflammatory activity in intestinal myofibroblast cells (CCD-18Co), decreasing ROS generation and TNF-α, NF-κβ, interleukin-6 (IL-6), and IL-1β expression at 25 μg/mL concentration (León et al., 2014). Paller et al. (2013) evaluated pomegranate extract (1−3 g) in 104 men presenting high prostate-specific antigen levels, an early indication of disease recurrence, after initial treatment for localized prostate cancer. Pomegranate extract treatment was associated with at least a 6-month increase in prostate-specific antigen doubling time without adverse effects.

Bode et al. (2009) evaluated anthocyanin supplementation in cancer patients who received chemotherapy. A combined administration of anthocyanins (50 mg), cysteine (40 mg), and GSH (200 mg) did not decrease or inhibit tumor growth in comparison to those patients who received only chemotherapy. However, in a study with 25 patients who consumed 60 g/day

of a blackberry anthocyanin-rich powder, over 2−4 weeks, tumor diameter decreased and apoptosis increased (Wang et al., 2007). Similarly, Thomasset et al. (2009) evaluated 25 patients with colorectal cancer consuming 0.5−2 g/day of a blueberry anthocyanin extract and found an improvement in cell inflammation and angiogenesis.

2.2.5 Genistein

Genistein is a polyphenol phytoestrogen found in legumes, such as beans, soy, lentils, and peas. It has anticancer properties with specific mechanisms, such as tyrosinase inhibition, which results in decreased cancer cell growth and antioxidant capacity against ROS (Chen et al., 2010).

In vitro studies in human cells showed genistein exerted both proliferative (oestrogenic) and antiproliferative (antioestrogenic) effects. In breast cancer, it has been observed that high genistein concentration ($>10^{-5}$) inhibited cell growth while low concentrations ($10^{-6}-10^{-8}$) stimulated it. Genistein competed with [^{3}H] oestradiol binding to the oestrogen receptor, will 50% inhibition at 5×10^{-7} M and stimulated oestrogen-responsive pS2 mRNA expression, a specific marker of similar oestrogen activity mediated by ER42 (Chen et al., 2010).

Genistein and other phytoestrogens inhibit cancer cell growth by interfering with tyrosine kinase activity and DNA topoisomerases, which are essential for mitogenic signaling. In in vivo studies, genistein inhibited angiogenesis, showed antioxidant activity by protecting cells from ROS damage, which initiates carcinogenesis, and decreased cancer risk by 25% (Grace et al., 2014).

According to Hong et al. (2012), genistein administration at 40−100 mg decreased prostate hyperplasia (HPB) (65.43 ± 17.05 ng/mL), compared to the control group (86.96 ± 37.75 ng/mL; $P = 0.032$). Epidemiological studies have demonstrated an inverse association between genistein consumption and breast, prostate, and endometrial cancer risk. A mixture of soy isoflavones consisting of 74% genistein and 21% daidzein was found to decrease prostate adenocarcinomas (Grace et al., 2014).

Several studies show an association between phytoestrogen consumption and decreased prostate cancer risk. A significantly lower prostate cancer risk was associated with a high consumption of genistein and daidzein; meanwhile, total isoflavone consumption showed no positive association (He et al., 2015).

2.3 Lignans

Lignans (secoisolariciresinol, enterodiol, enterolactone lariciresinol) are bioactive compounds found in many fruits, vegetables, and cereals (Boluda et al., 2005).

In vitro studies showed dietary flaxseed lignan inhibited breast cancer cell growth. The lignan was converted to enterolactone and enterodiol in vivo; these lignin-derived compounds inhibited cell growth in colon tumor cancer cells (Mali et al., 2012).

Animal studies support the potential chemopreventive effect of lignans. Several studies have demonstrated foods rich in linseed lignans inhibit breast and colon cancer in animals and decrease melanoma metastasis in mice (Thompson, 2012; Thompson et al., 2010).

In a clinical study involving postmenopausal women, consumption of a high dietary intake of total lignans and a high exposure to enterolignans, it has been found a significant decrease in invasive breast cancer risk (Touillaud et al., 2007). Epidemiological studies have found lariciresinol consumption and breast cancer risk have an inverse association (Seibold et al., 2014). In the same way, many other studies have found an inverse relationship between lignan dietary consumption and breast cancer risk. The most common doses studied are ≥1036, ≥9900, and ≥236 μg/day from lignan vegetable sources and ≥1289, ≥896, and ≥670 μg/day from enterolignans (Buck et al., 2010).

2.4 Glucosinolates

Glucosinolates are a group of phytochemicals that can be found in cruciferous vegetables. There is a subgroup formed by the hydrolysis of glucosinolates, which are called isothiocyanates. Sulforaphane [1-isothiocyanato-4-(methylsulphinyl)butane] (SFN) is one of the most studied isothiocyanates. It is considered as a chemopreventive agent able to induce expression of enzymes, such as phase 2 detoxification and/or antioxidant enzymes to remove carcinogens. SFN and other isothiocyanates have been found to induce cell cycle arrest and apoptosis in cancer cells, blocking cancer development. (Brennan et al., 2005).

Cruciferous consumption has been associated with a decrease in lung, stomach, colon, and rectum cancer risk. The benefits of these types of vegetables, such as broccoli, cabbage, cauliflower, and Brussels sprouts, is attributed to their high concentration of glucosinolates, principally the chemopreventive effect of indole-3-carbinol. The highly reactive isothiocyanates, derived from glucosinolate hydrolysis, also decrease activation of carcinogens and increase their detoxification (Brennan et al., 2005).

In vitro studies in human prostate cancer cells have shown digestion of the mitochondria by autophagosomes, which delayed activation of intrinsic caspase pathways using a dose of 40 μM SFN (Naponelli et al., 2015). The autophagy was a protective mechanism against apoptosis induced by SFN. On the other hand, cells treated with a 20 μM SFN inhibited the mitochrondrial respiratory chain enzymes causing ROS generation, which initiated cell apoptosis and a protective autophagic response in prostate cancer cell lines (Ho et al., 2011).

The Netherlands Cohort Study on Diet and Cancer (an epidemiological study) found that women who consumed high intakes of cruciferous vegetables have lower risk of colon cancer (but not rectal cancer) (Voorrips et al., 2000). Several studies report that cruciferous consumption is inversely associated with colon cancer risk: in particular, broccoli, due to its high glucosinolate content. A portion of raw broccoli (1 cup) can contain up to 60 mg of

glucoraphanin, a type of glucosinolate. The stalk of broccoli contains 20−100 times more glucoraphanin than a head of broccoli. Evidence suggests that the chemopreventive effect can be achieved by consuming 1−2 servings of cruciferous vegetables a day, equivalent to 0.5−1.5 cups/day, depending on whether it is raw or cooked (Tse and Eslick, 2014).

2.5 Long-Chain Polyunsaturated Fatty Acids (PUFAs)

These dietary components are involved in multiple physiological processes. For example, they play a structural role in the phospholipids of cell membranes and are substrates for the synthesis of various physiological mediators. Omega-3 (ω-3) and omega-6 (ω-6) are essential long-chain polyunsaturated fatty acids (PUFAs) for humans due to lacking the enzymes necessary for their biosynthesis (Valenzuela et al., 2011b).

PUFA consumption, in the form of fish or fish oil supplements, has proved its beneficial effect in several epidemiological and experimental studies concerning cardiovascular disease. Among the fatty acids, ω-3 PUFAs have the most potent immunomodulatory and antiinflammatory activities, ω-3 fatty acids from fish oils include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and they are more biologically potent than α-linolenic acid (α-LA, 18:3, ω-3). There have been several clinical trials evaluating the benefits of dietary supplementation with fish oils on a number of autoimmune and inflammatory diseases in humans, such as Crohn's disease, ulcerative colitis, lupus erythematosus, arthritis, and other autoimmune diseases. Many of the placebo-controlled trials of fish oil in chronic inflammatory diseases reveal a significant benefit, including decreased disease activity and a lowered use of antiinflammatory drugs. For a cancer patient, weight loss is common and the presence of cachexia in advanced stages is associated with a lower quality of life and a short survival period. The ω-3 PUFA modulate various effector pathways in cancer processes, which have been used to decrease weight loss, promote weight gain, and increase the survival of patients with cachexia associated with cancer. However, its effect depends on the various bioactive structures, according to the food source and their bioavailability, dose, and local concentration in the tissues (Fu et al., 2015).

EPA and DHA have the duality of exerting little harm to healthy cells and substantial harm to cancer cells. A certain specificity of their anticancer effect has been suggested due to their direct action on cancer cells, decreasing their proliferative capacity and facilitating their self-destruction (apoptosis), among other effects (Valenzuela et al., 2011a). EPA and DHA are more potent anticancer bioactives than α-LA. On ingestion of α-LA, part of the molecule is used by the cells to synthesise EPA and DHA, but ingestion of foods already containing EPA and DHA significantly accelerates the bioavailability and metabolic use of these fatty acids by cancer cells (Valenzuela et al., 2011b).

In a clinical phase II study that included 25 patients with metastatic breast cancer, patients were given 600 mg of DHA from seaweed, 3 times a day for 7–10 days before chemotherapy

and for 5 months of chemotherapy. A higher survival was reported in patients who showed plasma DHA level of 2.5%, in comparison to the control group (Fernández, 2011).

Clinical studies involving administered ω-3 PUFAs in cancer patients vary with respect to the administrated dose (1.4−4 g/day) and administrated form (capsules or as a part of complements or supplements) and the duration of the intervention. However, during the interpretation of the results, two relevant aspects should be considered: the bioavailability or bioactivity of the ω-3 form and dose, and the effective local concentration in the tissues. In this regard, fish consumption tends to be more effective at increasing serum EPA and DHA levels compared to fish oil. This is probably due to the intramolecular distribution of fatty acids (Pérez et al., 2013).

The National Health and Nutrition Examination Study (NHANES) III study reported combined EPA and DHA consumption from 0−<1 g/day of the α-LA consumption in the American population (HHS, 2004). In contrast, 4 g/day is consumed in Greenland, corresponding to 2.7%−6.3% of the daily energy. Traditional Japanese consume between 1% and 2% of the daily energy as ω-3, that is, 2−4 g/day (Kim et al., 2010).

A meta-analysis of prospective studies on ω-3 and prostate cancer risk, with a follow-up of 2–16 years, identified a negative association between prostate cancer risk and a dose of 0.5 g of α-LA and EPA daily dietary enhancement (Zheng et al., 2013). A dose of 0.1 g/day dietary ω-3 consumption was associated with a 5% less breast cancer risk according to dose-response studies (Zheng et al., 2013). Case-control studies in Japan (Kimura et al., 2007) and Scotland (Theodoratou et al., 2007) have demonstrated the protective role of LC-PUFA ω-3 against cancer, noting a trade-off between the risk of developing this disease and the increased consumption of LC-PUFA ω-3, being this significant association for distal cancer. In Jamaica, men undergoing prostate biopsy and having elevated prostate-specific antigen levels showed a positive correlation between α-LA and DHA levels in erythrocyte membrane phospholipids and a smaller tumor volume in the prostate (Ritch et al., 2007).

The current epidemiological literature establishes an association between LC-PUFA ω-3 intake and protection against cancer effects. However, not all the results are fully conclusive, suggesting that further studies are required. The discrepancies could be due to variations both in the quantity and source of ω-3 PUFA (of terrestrial or marine origin; α-LA, EPA, or DHA) used. Many studies are based on data from dietary intake surveys (consumer surveys) or from estimates based on national consumption, which poorly correlates with direct AG measurements in individuals. Another aspect to consider is that the ratio of ω-6/ω-3 consumption may be more important than the absolute intake of LC-PUFA. In a study involving *Pten* mice (animals immune competent and genetically predisposed to develop prostate cancer), in which prostate specific indicators were assessed, it was established that when the ratio of ω-6/ω-3 consumption was less than 5, there was a significant decrease in prostate cancer progression (Berquin et al., 2007).

3 Conclusions

A large number of bioactive compounds with beneficial activities against cancer have been identified. However, clinical studies have not yet defined the doses required for a chemopreventive effect and long-term toxicological complications are unknown. The consumption of a varied diet, considered from a functional rather than a nutritional strategy, is a dependable tool for cancer prevention and treatment. As the aforementioned clinical studies demonstrate, numerous bioactive compounds have a potential effect on cancer, which could be an important and readily accessible tool to design and customize dietary guidelines for complex disease prevention and treatment.

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CHAPTER 15

Epigenetic Nutraceuticals in Cancer Treatment

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1 Introduction

Cancer emerges in a multistep process in which genetic and epigenetic alterations accumulate in a sequential order. The comprising biological capabilities acquired during the cancerogenesis, known as eight hallmarks of cancer, include sustained proliferation, evading growth suppressors, apoptosis avoidance, replicative immortality, neovascularization, invading and metastatic capabilities, deregulating cellular energetics, and avoiding the immune destruction (Hanahan and Weinberg, 2011). Dietary components and caloric intake can play a role in the oncogenesis by modulating the hallmarks of cancer. Thus, poor diet and adverse lifestyle factors, such as low levels of physical activity, obesity, and smoking, are major risk factors for developing certain types of cancer.

Both genetic and epigenetic alterations have been linked to all the hallmarks of cancer. Epigenetic modifications are defined as alterations in gene expression that occur without changes in DNA sequence, and the key mechanisms include DNA methylation, histone modification, and noncoding RNAs. Epigenetics has the potential to explain many features of cancer: cancer is associated with lifestyle factors (e.g., smoking, diet, body mass index), as well as epigenetic changes that can be modified by environmental factors, nutrition, and genotoxic factors. The association of cancer and age corresponds to epigenetic changes that occur in aging. The reversibility and changeability of epigenetic changes are likely to be a source of phenotype changes by which metastatic phenotype emerges from the primary tumor. In has been demonstrated that changes in the E-cadherine promoter DNA methylation status is a key event in obtaining a motile phenotype during the migration of metastatic cells to the new site, and in phenotype changes as they become established as a metastases (Kudo et al., 2004). The expressions of genes involved in the angiogenic switch are also thought to have an epigenetic etiology. The potential reversibility of epigenetic changes is utilized for the development of targeted epigenetic therapy and for cancer chemoprevention. Moreover, aberrant epigenetic silencing of tumor suppressor genes in cancer could potentially

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be reversed by bioactive phytochemicals (Supic et al., 2013, 2016b). Despite a number of unresolved questions, there is a promising future for personalized dietary recommendations in cancer prevention and treatment. Complete revealing of epigenetic mechanisms and its regulation will lead to the chemoprevention of cancer with natural bioactive compounds, expansion of epigenetic therapy based on known nutraceuticals, as well as finding novel natural bioactive compounds.

1.1 DNA Methylation

The most extensively studied and characterized epigenetic modification in mammals is DNA methylation. DNA methylation is a covalent, postreplicative modification, which includes the addition of methyl group at the 5-carbon cytosine (5mC). This process is catalyzed by the family of enzymes DNA methyl transferases (DNMTs), and the methyl-group donor is S-adenosyl-methionine (SAM). Generally, DNA methylation is a negative regulator of gene transcription and leads to gene expression silencing. Apart from the fundamental role in protection from potentially harmful elements in the genome, such as inserted viral sequences and transposons, DNA methylation has a key role in a number of physiological processes in mammalian cell, such as X chromosome inactivation and imprinting of genes with monoallelic expression (Herman and Baylin, 2003).

The CpG nucleotides are nonrandomly distributed throughout the mammalian genome. Whereas solitary CpG dinucleotides out of gene promoter are infrequent and randomly distributed, the long stretches of CpG dinucleotides, length from 0.5 to 4 kb, referred as CpG islands, are clustered in the promoters of a great majority of genes. Solitary CpG dinucleotides are generally methylated, while CpG-islands are usually unmethylated in normal cell frequently are associated with the transcription start site. Hypermethylation of CpG islands in the promoters of tumor suppressor genes cause their transcriptional silencing and malignant transformation (Herman and Baylin, 2003).

DNA methyltransferases are enzymes responsible for controlling the process of DNA methylation, and act as methylation "writers." In higher eukaryotes, three classes of active DNMTs have been identified: DNMT1, DNMT3A, and DNMT3B. Even though all three DNMTs types recognize hemimethylated DNA, DNMT1 is responsible for maintenance of existing DNA methylation pattern after replication, while DNMT3A and DNMT3B are involved in active de novo DNA methylation (Herman and Baylin, 2003). Overexpression of DNMTs genes have been reported in various cancers (Girault et al., 2003; Robaina et al., 2015; Supic et al., 2016a; Yu et al., 2015).

Methyl cytosine binding proteins (MBPs) bind to DNA methylated sites and act as methylation "readers." These include MBD1, MBD2, MBD3, and MeCP2 proteins, which binding to methylated DNA recruits histone-modifying machinery (Dawson and

Kouzarides, 2012). Therefore, the process of aberrant DNA methylation is usually accompanied by aberrant histone modifications, which regulate chromatin remodeling.

DNA methylation marks can be removed from the set of demethylating enzymes referred as ten–eleven translocation dioxygenases proteins (TET 1-3) (Ahuja et al., 2016). TETs convert 5-methyl cytosine to 5-xydroxylmethyl cytosine, process found to be very important during developmental process and cancerogenesis (Baylin and Jones, 2011). Numerous mutations have been identified in DNMTs, TETs, and MBPs genes, which were associated with developmental problems and cancer (Hamidi et al., 2015; Ley et al., 2010).

Pattern of DNA methylation is generally stable in somatic cells, but can undergo changes during development and cancerogenesis and show tumor-type specificity (Esteller, 2007). Global hypomethylation and regional hypermethylation, also known as methylation paradox, is one of the characteristics of cancer cells. Global DNA hypomethylation could activate repetitive elements, latent viruses or proto-oncogenes, while promoters hypermethylation results in transcriptional silencing of tumor suppressor genes (Herman and Baylin, 2003). Aberrant methylation has been reported in a number of cancer types (Belinsky et al., 2002; Fotouhi et al., 2014; Palmisano et al., 2000; Supic et al., 2009, 2011b,c), in early phases of cancerogenesis (Ahuja et al., 2016), and in the surgical margins without pathohistological changes (Supic et al., 2011c). Accumulating evidence indicates that dietary bioactive compounds could have an important role in cancer chemoprevention by affecting DNA methylation.

1.2 Histone Modifications

Histone proteins have an important role and dynamic function in regulation of chromatin structure and gene expression. The main covalent modifications of histones include acetylation, methylation, phosphorilation, and ubiquitination. All these modifications lead to fine chromatin remodeling, which consequently results in gene expression regulation through changes in the DNA accessibility to transcription factors.

Accumulating evidence demonstrate that histone modifications cannot be interpreted independently, but in combination with other epigenetic modifications and within a context. Chromatin remodeled by various posttranslational histone modification results in almost an endless pattern combination for gene expression regulation, while the each histone in the histone octamer, H2A, H2B, H3, or H4 can be modified. As previously mentioned, methylation of cytosine within CpG islands is associated with MBPs, recruitment of HDACs and histone methyltransferases (HMTs), and histone modifications. Different combinations of histone modifications and histone variants are referred to as histone code. The most studied and best understood posttranslational histone modifications are histone acetylation and histone methylation.

Histone acetylation is the major type of histone modifications. Histone acetyl transferases (HATs) are enzymes that catalyze acetylation of lysine residues (K) of histone tails. Acetylation neutralizes the lysines positive charge and weakens the interaction between negatively charged DNA and histones, which leads to chromatin decondensation. Subsequently, histone tails are released from the DNA, allowing the assembly of transcription-initiation complex and the activation of gene transcription (Audia and Campbell, 2016; Dawson and Kouzarides, 2012).

Histone deacetylases (HDACs) catalyze the removal of acetyl groups, which lead to chromatin condensation and negative regulation of gene transcriptional activity. In the deacetylated state, lysine is positively charged, which allows the histone tails to tightly interact with the negatively charged DNA. This leads to the low accessibility of gene promoters to transcription factors and other regulatory molecules, which results in gene expression repression. To date, all identified HDACs are categorized into four classes, referred as class I, II, III (sirtuins: SIRT1-7), and IV. Both histone acetylation "writers"—HATs—and histone acetylation "erasers," HDACs are required for optimal regulation of gene expression (Audia and Campbell, 2016). Aberrant expression of HDACs and HATs has been reported in a variety of cancers (Avvakumov and Cote, 2007; Barneda-Zahonero and Parra, 2012; Fraga et al., 2005; Muller et al., 2013; Ouaissi et al., 2014; Poyet et al., 2014; Ropero and Esteller, 2007; Schneider et al., 2011; Theocharis et al., 2011). Reversibility of chromatin acetylations is recognized in designing novel pharmacological agents, which target HATs and/or HDACs. In addition, the utility of natural HDACs and HATs inhibitors are widely being investigated as cancer therapeutic compounds.

Histone methylation is the process by which methyl groups are covalently added to lysine (K) and arginine (R) residues on histone tails, which does affect electrostatic interactions between histone tails and DNA. However, histones chemical properties are changed, which will change the affinity of transcription factors and regulatory molecules. This complex, dynamic process is catalyzed by enzyme HMTs, while histone lysine demethylases, such as LSD1 and the Jumonji-domain (JMJD)-containing family, demethylate histones. The effect of histone methylation can be either gene activation or repression, depending on the modification target, amino acid, and its position in the histone tail. Thus, methylation H3K4, H3K36, H3K79, H3R17, and H4R3 leads to transcriptional activation, while H3K9, H3K27, H4K20, and H4K59 methylation leads to transcriptional repression (Dawson and Kouzarides, 2012; Ellis et al., 2009). The degree of methylation varies among lysine and arginine residues. While lysine residues can be mono-, di-, or trimethylated, arginine residues can be only mono- and dimethylated. Aberrant histone methylation and trimethylation of histone H4 is a common hallmark of human cancers (Fraga et al., 2005).

Histone phosphorilation is essential for vital cellular processes, such as mitosis, DNA repair, apoptosis, replication, and transcription (Banerjee and Chakravarti, 2011). Phosphorilation

of serin, threonine, and tyrosine residues have been reported in all histones and changes histone charges. Histone phosphorilation is controlled by phosphatases and protein kinases, such as the tyrosine kinase JAK2 that specifically phosphorilate H3Y41 (Dawson and Kouzarides, 2012). Aberrant histone phosphorylation has been observed in a number of cancer types, such as breast, prostate, and colorectal cancer (Jenuwein and Allis, 2001; Kouzarides, 2007).

Histone ubiquitination of H2A and H2B histones is well characterized (Weake and Workman, 2008), and it has been documented that monoubiquination of H2B is required for methylation of histone H3 at lysine K4 and K79 (Jenuwein and Allis, 2001).

1.3 Small Noncoding RNAs

Small noncoding RNAs family is a group of RNAs molecules, which act as negative regulators of the gene expression on posttranscriptional level. Based on the biogenesis process, three classes of small noncoding RNAs are known in the human genome: microRNA (miRNA), small interfering RNAs (siRNAs), and piwi interacting RNAs (piRNAs) (Kim, 2004). Among small noncoding RNAs, the best characterized and the most studied group is microRNAs, evolutionary conserved, small, ~22 nucleotides long noncoding RNAs. miRNAs posttranscriptionally inhibit gene expression by binding to its target mRNA, predominantly via the 3′ untranslated region (3′UTR). Complete base pairing leads to mRNA degradation, while partial complementarity results in translational inhibition of targeted mRNA. Over one-third of genes have been found to be regulated by miRNAs (Croce, 2009). miRNAs play an important role in a wide range of physiological processes, including cell differentiation, proliferation, apoptosis, and stress response, but also in the cancerogenesis (Croce, 2009). First report on deregulated miRNAs has appeared in 2002, when miR-15 and miR-16 were identified in a frequently deleted region at chromosome position 13q14.3 in chronic lymphocytic leukemia (Calin et al., 2002). The aberrant miRNA expression has been documented in almost all types of human cancers (Akcakaya et al., 2011, 2014; Caramuta et al., 2010; Ozata et al., 2011). miRNAs expression can be affected by the genetic changes, such as deletions, translocation, amplifications, single nucleotide polymorphisms, mutations, but also DNA methylation of their promoter regions (Croce, 2009; Dawson and Kouzarides, 2012). miRNAs could function as both oncogenes (oncomiRs) or tumor suppressor genes, depending on their target gene (Croce, 2009; Di Leva et al., 2014). The recent findings highlight importance of miRNAs deregulation in maintaining cancer stem cells (Garofalo and Croce, 2015). Its stability in circulation and transportation by exosomes, open various possibilities for clinical application of miRNAs as tumor biomarkers, and for the development of miRNA-based epigenetic therapy of cancer (Collins et al., 2014; Di Leva et al., 2014; Iorio and Croce, 2012) (Fig. 15.1).

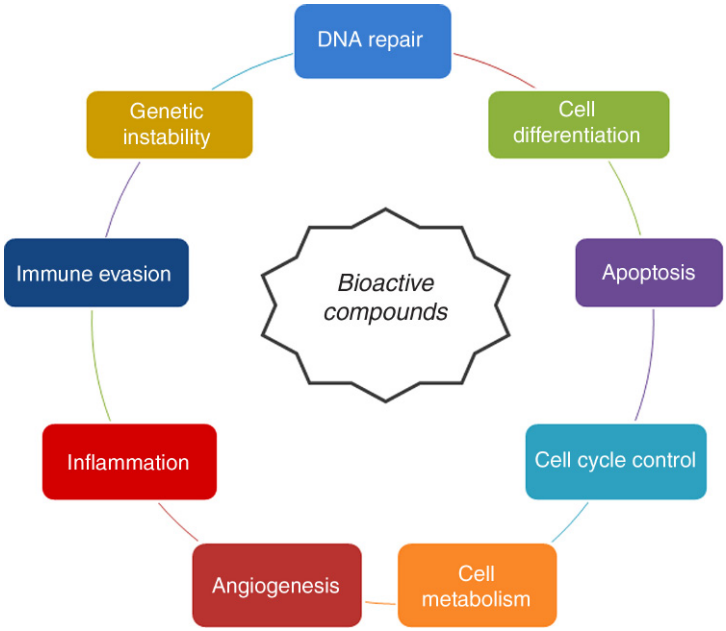


Figure 15.1: Bioactive Compounds in Cancer.
Dietary bioactive compounds in cancer modulate genetic and epigenetic changes in genes associated with the major hallmarks of cancer. Major target molecules of dietary bioactive compounds are enzymes DNMTs, HDACs, HMTs, as well as other chromatin structure modyfying enzymes and microRNAs. As a result of their changed activity, the pattern of DNA methylation and histone modifications is modified, thereby restoring the epigenetically silenced genes in cancer cells, which are involved in the major hallmarks of cancer. Dietary components and caloric intake can contribute to oncogenesis or play a role in the prevention of cancers by modulating the hallmarks of cancer, including apoptosis, cell cycle control, proliferation, invasion, angiogenesis, cellular energy metabolism, aerobic glycolysis, or pH gradient reversal.

2 Diet and Transgenerational Epigenetic Changes

Emerging evidence from experimental models and epidemiological studies on humans suggest that malnutrition and exposure to other adverse environmental conditions during the critical periods of development, prior to or during prenatal period or adolescence, result in epigenetic changes that persist later in life, and predispose individuals to complex diseases, such as diabetes, cardiovascular diseases, and cancer (Fig. 15.2).

Maternal dietary supplementation with methyl donor vitamins (folic acid, betaine, vitamin B12, and choline) in Avy/a mice, increase the level of DNA methylation of a transposon in the promoter of the agouti gene that change the phenotype of the offspring mice. These changes were indicated by an altered animal's coat color and the changes in the downstream metabolic and endocrine effects that ultimately affect obesity and life span of the offspring mice

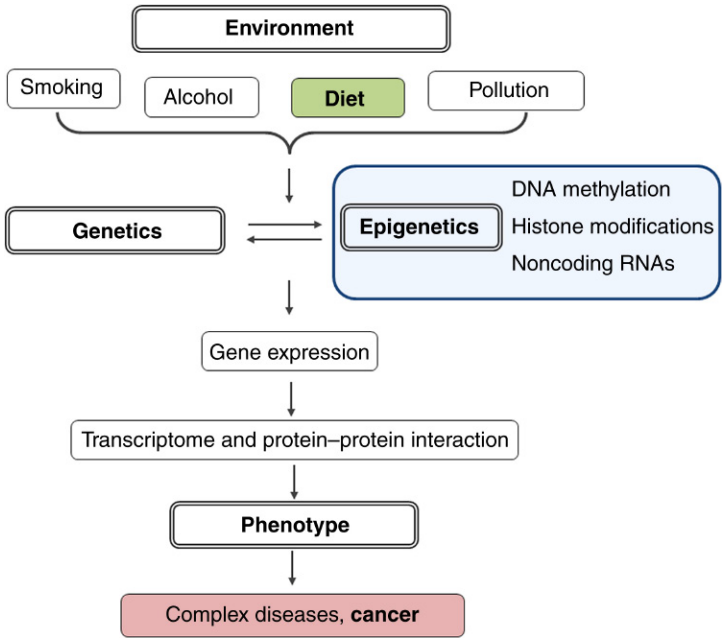


Figure 15.2: The Complex Interplay of Genetics, Epigenetics, and Environment That Affect the Phenotype.

Key epigenetic modifications in cancer involve DNA methylation, histone modifications, and noncoding RNAs. Unlike the genome, which is largely static, the epigenome is much more flexible, and its dynamic changes over time arise as an adaptive response to the different environmental conditions. Epigenetic mechanisms are highly coordinated, leading to transcriptional activation or repression. A number of recent studies indicate that the environmental factors, including diet and bioactive compounds, pollution, smoking, and alcohol, have an important role in establishing and maintaining epigenetic changes that can modulate the phenotype and the risk for complex diseases, such as cancer.

(Waterland and Jirtle, 2003; Wolff et al., 1998). This effect persisted in the F2 generation, suggesting possible transgenerational effect.

One of the earliest and most intriguing examples of diet impacting human health through epigenetic mechanisms came from several epidemiological studies performed on individuals and their descendants who experienced famine in the Netherlands during World War II. Individuals exposed to severe famine during gestation or as young adults during the "Dutch Hunger Winter" of 1944–45, had severe adverse health outcomes in adulthood, including psychiatric disorders, cardiovascular diseases, obesity, and colorectal cancer (Heijmans et al., 2007; Painter et al., 2006; Susser et al., 1996; Tobi et al., 2009). Individuals exposed to famine during childhood and adolescence had changes in the DNA methylation pattern of a panel of cancer-related genes (the CpG island methylator phenotype, CIMP) (Hughes et al., 2009), and a lower DNA methylation in the imprinted insulin-like growth factor 2

(IGF2) gene 6 decades later compared to their unexposed same-sex siblings (Heijmans et al., 2008). In addition, women exposed to famine in utero had children 20–30 years later with the same pathologies despite being conceived and born during a normal dietary state (Heijmans et al., 2008).

Another historical finding comes from the study of the descendants of individuals exposed to the poor food supply in Overkalix region in Sweden in the 19th century. The mortality rate of men born in the following years was associated with paternal grandfather's food supply in mid-childhood, whereas the mortality rate of the women was related to the paternal grandmother's food supply. In addition, the exposure to energy restrictions during a critical period of paternal grandmother's childhood to adolescence could result in a lower cardiovascular mortality and cancer and diabetes risk via epigenetic changes (Bygren et al., 2014; Tinghog et al., 2011). These findings indicate that environmental conditions in critical phases of development, such as gestation and adolescence, can cause epigenetic changes that persist throughout a lifetime, and indicate the transgenerational epigenetic changes in response to nutrition.

3 Epigenetic Nutraceuticals in Cancer Treatment

A great number of bioactive compounds derived from food or herbal products, exert their anticancer effects through epigenetic mechanisms and thus have a promising potential to be used as cancer chemopreventive agents or nutraceuticals. Dietary bioactive components could change DNMT and/or HDAC expression and also modulate miRNAs expression (Supic et al., 2013). To date, the majority of studies reveal molecular mechanisms and epigenetic regulation of natural bioactive component in in vitro studies, but also in vivo experimental animal models and in humans. Thus, scientific interest in studying dietary bioactive components as epigenetic modulators in cancer has expanded during the last several years.

Natural compounds could be classified into several groups depending on their chemical nature into flavonoids (curcumin, quercetin, garcinol, apigenin), polyphenol catechins from tea and coffee [epigallocatechin gallate (EGCG), caffeic acid, and chlorogenic acid], stillbenoids (resveratrol, pterostilbene), isoflavonoids (genistein), isothyocyanates (sulforaphane, phenethyl-isothiocyanate), short-chain fatty acids (butyrate), and essential micronutrients (folate, vitamin D, selenium).

4 Essential Micronutrients–Folate, Selenium, Vitamin D

4.1 Folate

Folate is a water-soluble B vitamin that is naturally present in many foods, including green, leafy vegetables, that have a significant role in cancer development and progression (Kim, 2004; van den Donk et al., 2007). Folate and one-carbon metabolism have a strong

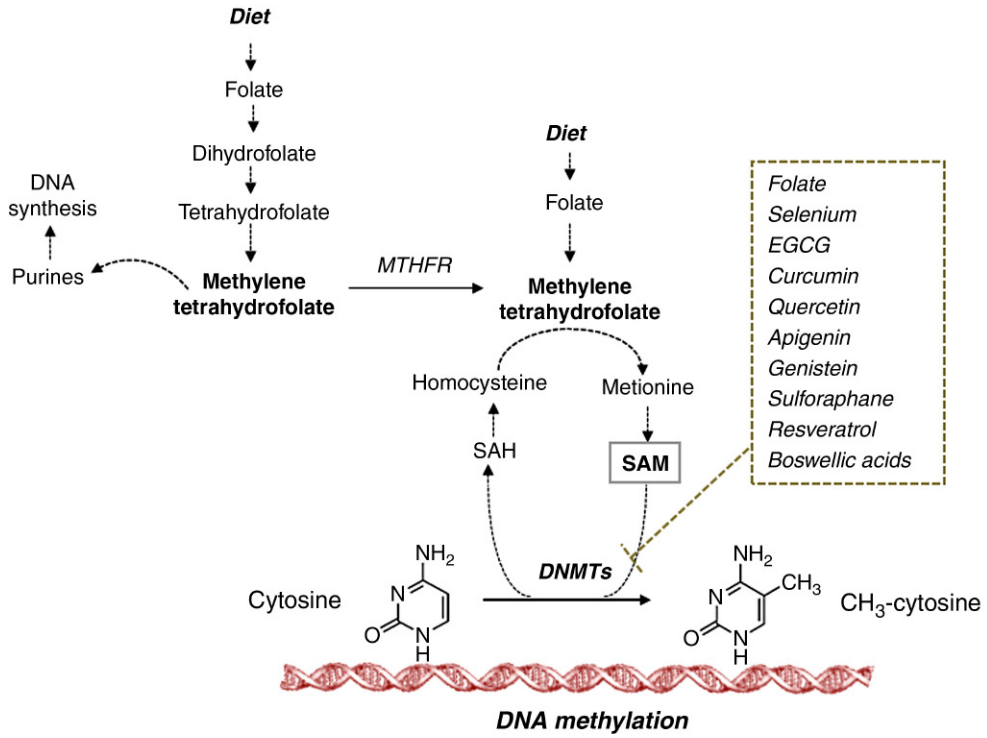


Figure 15.3: A Schematic Overview of the One-Carbon Metabolism, and DNA Methylation. Dietary folate conversion to methyl tetrahydrofolate is catalyzed by the enzyme methylene tetrahydrofolate reductase (MTHFR). Methionine is the precursor of S-adenosyl-methionine (SAM), the universal methyl donor for numerous biological reactions, including the methylation of DNA, histones, RNA, and proteins. DNA methylation of cytosine located 5′ to guanosine of CpG islands in promoters lead to transcriptional silencing and to the altered gene expression. Methyl groups from SAM are transferred to DNA by DNA methyltransferases (DNMTs). Dietary bioactive compounds, such as epigallocatechin-3-gallate (EGCG) from green tea, genistein from soybean curcumin, and sulforaphane, are bioactive food components with anticancer properties. The effects of dietary bioactive compounds on DNMTs are modulated through direct inhibition by interaction with the catalytic site of the DNMT enzymes, or affecting the SAM bioavailability. Thus, dietary polyphenols could affect the DNA promoter methylation status, thereby restoring the epigenetically silenced genes in cancer cells.

impact on biological methylation reactions, including DNA synthesis and DNA methylation (Fig. 15.3). Main mechanisms involved in the folate deficiency-mediated carcinogenesis are DNA damage (uracil misincorporation) (van den Donk et al., 2007), aberrant DNA methylation, and DNMT1 inhibition (Kim, 2004). Even though folate supplementation marginally reduces the risk of colorectal cancer (Sanjoaquin et al., 2005), supplementation of patients with a history of colorectal adenoma increased uracil misincorporation into DNA and is associated with a tendency toward promoter methylation (van den Donk et al., 2007).

Although questioned, one study indicated that supplementation with 5 mg of folate during pregnancy is associated with an increased maternal mortality from cancer (Charles et al., 2004).

DNA methylation, global and regional, depends on the availability of methyl-group donor compounds. Dietary methyl-group donors could influence the hypermethylation status of tumor suppressor genes. Dietary folate intake inversely correlated with RARB and BRCA1 promoter hypermethylation (Pirouzpanah et al., 2015). Folate, vitamin A, and vitamin B12 intake might have a role in tumor suppressors' demethylation and its reactivation in head and neck carcinomas (HNSCC) (Colacino et al., 2012). Strong evidence also indicates the age-dependent effects of methyl-group donor intake on the methylation status of genes (Pirouzpanah et al., 2015). Long-term supplementation with folic acid and vitamin B12 in elderly subjects resulted in DNA methylation of several genes implicated in development (Kok et al., 2015). High folate concentration enhanced cell growth in colorectal adenocarcinoma cells caco-2 and increased promoter hypermethylation of the estrogen receptor 1 (ESR1), p16, and p15 (Berner et al., 2010). However, another study showed that dietary folate and other methyl-group donor intake were not associated with that of E-cadherin, p16, and RAR-β hypermethylation in breast carcinomas (Tao et al., 2011). These findings suggest that dietary methyl-group donors and long-term supplementation with high concentrations of methyl-group donors could have different effects in enhancing carcinogenesis by inducing genetic and/or epigenetic changes. Additionally, methyl-group donor intake might modulate promoter methylation in normal or premalignant tissue. Recent studies of healthy women who underwent reduction mammoplasty revealed that alcohol consumption, breast tissue folate concentrations, and polymorphisms in one-carbon metabolism genes could be associated with p16 hypermethylation and decreased p16 protein expression (Llanos et al., 2015). These findings indicate that alcohol consumption can inhibit folate metabolism and alter DNA methylation in healthy tissues.

Several studies have demonstrated the association of cancer susceptibility and genes involved in one-carbon metabolism. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in folate metabolism, DNA synthesis, and DN methylation (Fig. 15.3). Single nucleotide polymorphism (SNP) C677T in MTHFR gene is associated with ras-association domain family protein 1A (RASSF1A) promoter hypermethylation in bladder and oral cancers (Cai et al., 2009; Supic et al., 2011a). In patients with colorectal, breast, or lung carcinomas, 677T allele carriers, characterized by reduced enzyme activity, show constitutive global hypomethylation, while patients with 2756GG genotype of the enzyme methionine synthase have hypermethylation in a large panel of tumor suppressor genes (Paz et al., 2002). These findings suggest that polymorphisms in one-carbon metabolism-related genes can modulate the epigenetic status of cancer-associated genes, but also the impact of environmental factors, such as nutrition and alcohol intake. Interaction between genetic polymorphism in enzymes involved in folate metabolism, folate intake, and alcohol

consumption might lead to aberrant DNA methylation. Higher incidence of promoter methylation in a number of tumor suppressor genes (APC, p14, p16, hMLH1, O^6-MGMT, and RASSF1A) was observed in colorectal cancer patients with low folate and high alcohol intake as opposed to patients with high folate and low alcohol intake (van Engeland et al., 2003). An interaction between heavy drinking and the MTHFR 667TT genotype was associated with multiple DNA methylations of tumor suppressor genes in oral cancer (Supic et al., 2011a). Possible mechanisms by which alcohol could cause aberrant DNA methylation include ethanol-associated folate deficiency, interaction with one-carbon metabolism, the impact on SAM synthesis, and an inhibition of DNMTs (Hamid et al., 2009). However, the interaction of polymorphisms in enzymes involved in folate metabolism, methyl-donors intake and alcohol, affecting the DNA methylation in cancer has not been fully elucidated and demands further investigations.

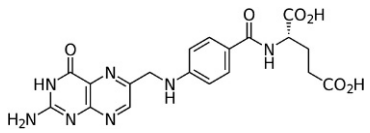
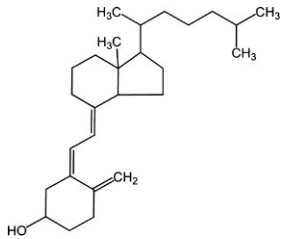
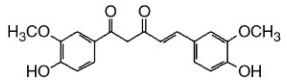
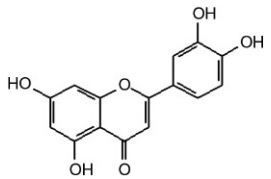
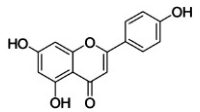
There are confirmations from clinical studies on folate and folic acid efficacy in cancer treatment. For instance, patients with colorectal adenoma were treated with daily 5 mg folic acid or placebo for 3 years. Due to the treatment, reduction of colorectal adenoma recurrence has been reported in folic acid treated group (Jaszewski et al., 2008).

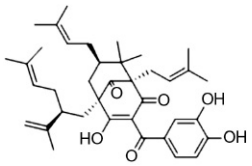
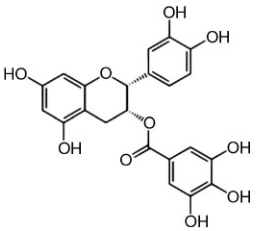
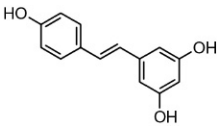
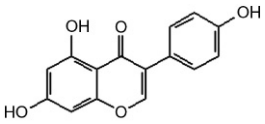
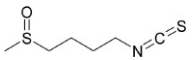
4.2 Selenium

Selenium, an essential microelement with antioxidant and proapoptotic effect (Davis and Uthus, 2002; Davis et al., 2000), was associated with the decreased proliferation, increased apoptosis, and epigenetic changes in a number of recent in vitro studies (Table 15.1). It has been demonstrated that selenium inhibits DNMT both directly and indirectly, by interacting with the enzyme and by influencing the plasma homocysteine level and the SAM:SAH ratio (Uthus and Ross, 2007). Selenium and green tea cotreatment exerted preventive effect in a rat model of colorectal cancer, through DNMT1 inhibition and H3 acetylation induction, followed by the reduced cell proliferation (Hu et al., 2013). Another study of colorectal cancer cells (caco-2, HCT116) showed that selenium alone or in combination with isothiocyanates sulforaphane and iberin, did not change the gene specific methylation of p16 and ESR1 genes, as well as global methylation (LINE-1) (Barrera et al., 2013).

Recent studies in esophageal cell carcinoma cells treated with methylseleninic indicated that selenium reduces HDAC expression, increased acetylation of histone H3K9, while inducing the expression of Krüppel-like factor 4 (Issa et al., 2015). Methylseleninic treatment of esophageal carcinoma cells downregulated Keap1 (kelch-like ECH-associated protein 1), through miR-200a induction (Liu et al., 2015a). Selenium-induced miR-34 expression, which led to the transcriptional activation of its target p53 and the induction of apoptosis in prostate cancer cells (LNCaP) (Sarveswaran et al., 2010). In diffuse large B-cell lymphoma, selenium (in the form of methylseleninic acid) inhibited HDAC activity, followed by the acetylation of histone H3 and α-tubulin, and downregulated HIF-1α expression and VEGF secretion

Table 15.1: Epigenetic dietary agents, structure, food sources, and epigenetic regulation.

Epigenetic Dietary Agents	Structure	Food Sources	Epigenetic Regulations	References
Folate		Green vegetables and dried beans	DNA damage (uracil misincorporation), DNA methylation, and DNMT1 inhibition	van den Donk et al. (2007); Pirouzpanah et al. (2015); Colacino et al. (2012); Berner et al. (2010)
Selenium	Se	Brazil nuts, seafood, and fish	HDAC inhibitor, miRNA modulation	Hu et al. (2013); Kassam et al. (2011); Liu et al. (2015a,b)
Vitamin D		Eggs, fish, mashrooms, and vitamin D fortified products	DNMT and HMT inhibitor, mirNA modulation	Lopes et al. (2012); Pereira et al. (2012); Ma et al. (2014); Padi et al. (2013)
Curcumin		*Curcuma longa* (turmeric) and curry	DNMT and HDAC inhibitor, miRNA modulation	Yu et al. (2013); Chen et al. (2013a,b); Hassan et al. (2015); Guo et al. (2015); Toden et al. (2015)
Quercetin		Fruits and vegetables	DNMT and HDAC inhibitor, miRNA modulation	Priyadarsini et al. (2011); Bishayee et al. (2015); Tao et al. (2015); Zhou et al. (2014)
Apigenin		Fruits and vegetables, celery, and chamomile	DNMT and HDAC inhibitor, miRNA modulation	Paredes-Gonzalez et al. (2014); Chakrabarti et al. (2013); Pandey et al. (2012)

Garcinol		*Garcinia indica* (mangosteen tree)	HDAC inhibitor, miRNA modulation	Huang et al. (2017); Balasubramanyam et al. (2004)
Epigallocatechin−3-gallate		Green tea	DNMT and HDAC inhibitor, miRNA modulation	Tao et al. (2015); Khan et al. (2015); Groh et al. (2013); Yu et al. (2016); Zhu and Wang (2016)
Resveratrol		Peanuts, cranberries, blueberries, and grapes	DNMT and HDAC inhibitor, miRNA modulation	Qin et al. (2014); Mirza et al. (2013); Zhang et al. (2016); Yang et al. (2015); Dhar et al. (2015)
Genistein		Soybeans	DNMT and HDAC inhibitor, miRNA modulation	Xie et al. (2014); Li et al. (2009); Li et al. (2012); Groh et al. (2013); Hirata et al. (2014); Chiyomaru et al. (2012)
Sulforaphane		Cruciferous vegetables (broccoli, cabbage, kale, etc.)	DNMT and HDAC inhibitor, miRNA modulation	Meeran et al. (2010); Clarke et al. (2011); Wong et al. (2014); Shan et al. (2013)

DNMT, DNA methyltransferase; HDAC, histone deacetyltransferase; HMT, histone methyltrasnferase; miRNA, microRNA.

(Kassam et al., 2011). Thus, selenium has been suggested as a potential nutriceutical agent for the cancer chemoprevention or treatment.

A phase III clinical study evaluated the effect of selenium supplementation of stage I nonsmall cell lung cancer patients (Karp et al., 2013) (Table 15.2). Even though selenium treatment (200 μM daily in duration of 4 years) has been safe, no promising effects have been reported compared to an untreated group in prevention of second primary tumor (Karp et al., 2013).

4.3 Vitamin D

Growing numbers of studies have indicated that vitamin D exerts anticancer effects (de la Parra et al., 2016; Deeb et al., 2007; Feldman et al., 2014). Vitamin D deficiency and vitamin D-related genes were associated with different cancer types, including breast, prostate, colorectal, oral, lung cancer, and melanoma (Feldman et al., 2014; Zeljic et al., 2012, 2014). The epidemiological data confirmed that the great majority of the world population is lacking vitamin D, and these findings could potentially indicate the association with an increasing incidence of various cancer types in those populations (Feldman et al., 2014). Vitamin D, in its metabolite active form calcidiol $1\alpha 25(OH)_2D_3$, binds to its receptor—vitamin D receptor (VDR)—after which retinoid acid receptor (RXR) is recruited (Christakos et al., 2016). Formed heterocomplex binds to vitamin D response elements (VDRE), located in the promoter region of a number of vitamin D responsive genes (Deeb et al., 2007).

Anticancer properties of vitamin D include antiproliferative, antiangiogenesis, antimetastatic and antiinvasive effects, induction of differentiation, and apoptosis (Deeb et al., 2007). Anticancer effects of vitamin D are mainly mediated by p21 and p27 induction, downregulation of MMP, cell cycle arrest and apoptosis, and in epithelial-mechenhymal transition (EMT) (Chen et al., 2015; Deeb et al., 2007; Upadhyay et al., 2013). Thus, there is an intense interest in studying vitamin D3 as a potential chemopreventive and chemotherapeutic agent.

The results of the recent studies indicated that the gene expression of VDR and CYP24A1, coding for enzyme 24-hydroxylase, involved in the vitamin D catabolism, as well as CYP27B1, coding for 1α-hydroxylase, involved in the vitamin D anabolism, are mediated by epigenetic mechanisms (Fetahu et al., 2014; Hobaus et al., 2013). In invasive breast carcinoma cells MDA-MB-231, vitamin D3 exposure led to the hypomethylation and induction of p21, PTEN, and RARβ2 (Stefanska et al., 2012a,b). In addition, vitamin D treatment induces promoter demethylation and the expression of cadherin 1 (CDH1) gene, and differentiation of triple-negative breast cancer cells, the most aggressive form of breast carcinoma (Lopes et al., 2012). In a large cohort of Canadian colorectal carcinoma patients, dietary vitamin D intake negatively associated with the promoter methylation of

Table 15.2: Clinical trials evaluating the key dietary epigenetic inhibitors in cancer.

Dietary Epigenetic Inhibitors	Cancer	Major Findings of the Study	References
Folate	Colorectum	Recurrence of colorectal adenoma was inhibited as a result of folate supplementation	Jaszewski et al. (2008)
Selenium	Lung	Treatment with selenium was safe but with no benefits in prevention of second primary cancer compared to placebo group	Karp et al. (2013)
Vitamin D	Breast	Treatment with high doses of vitamin D was safe, without toxicities and lead to normalization of vitamin D level	Jacot et al. (2016)
Curcumin	Prostate	Curcumin and soy isoflavones treatment decreased PSA serum level	Ide et al. (2010)
	Pancreatic	Curcumin and gemcitabine treatment was safe in phase I/II clinical trial	Kanai et al. (2011)
Quercetin	Prostate	Muscadine grape skin, which contains quercetin, was safe in patients treatmen in phase I	Paller et al. (2015)
Apigenin	Colorectum	No reccurence has been recorded in apigenin and EGCG treated patients	Hoensch et al. (2008)
Epigallocatechin-3-gallate	Chronic lymphocytic leukemia	Complete molecular remission of the disease has been reported in a patient treated only with EGCG	Lemanne et al. (2015)
	Breast	In phase I combined EGCG and radiotherapy treatment, EGCG showed good tolerability, safeness and efficacy	Zhao et al. (2016)
Resveratrol	Colon	Resveratrol treatment reduced tumor proliferation for 5%	Patel et al. (2010)
	Breast	Transresveratrol decreased RASSF1α methylation level, which was related to prostaglandin PGE2 change	Zhu et al. (2012)
Genistein	Breast	Treatment of soy protein supplementation upregulated genes related to cell cycle and proliferation	Shike et al. (2014)
	Prostate	Genistein redude PSA level and modulated expression of androgen and cell cycle related biomarkers associated with prostate cancer prediction and progression	Lazarevic et al. (2011); Lazarevic et al. (2012)
Sulforaphane	Prostate	Phase II brocolli sulforaphane-rich extract treatment did not reduce PSA level more than 50%	Alumkal et al. (2015)
		Sulforaphane effectively decreased PSA level in biochemical reccurence prostate cancer patients	Cipolla et al. (2015)

EGCG, Epigallocathechin-3-gallat; PSA, prostate specific antigen.

Wnt antagonist gene DKK1 (Rawson et al., 2012). However, the exposure of human blood mononuclear cells to calcitriol induced the expression of vitamin D responsive genes, without altering the genome global methylation (Chavez Valencia et al., 2014).

Vitamin-D3 also modulated the expression of several histone demethylases, Jumonji C and lysine-specific demethylase (LSD) families (Pereira et al., 2011, 2012). In the colon cancer SW480-ADH cells calcitriol increased the expression of the histone demethylase JMJD3, which upregulated SNAIL1 and ZEB1, the inducers of EMT, while downregulating the adhesion proteins E-cadherin, Claudin-1, and Claudin-7 (Pereira et al., 2011). In addition, JMJD3 knockdown decreases the expression of miR-200b and miR-200c, two microRNAs targeting ZEB1 RNA (Pereira et al., 2012).

Several studies suggested that the anticancer effects of vitamin D could be mediated through regulation of microRNAs expression (Ma et al., 2014). In colorectal cancer cells (HT-29) and in vivo xenograft mouse model, calcitriol treatment upregulated the expression of miR-627, which subsequently downregulated its target histone demethylase JMJD1A (Padi et al., 2013). Also, it has been reported that vitamin D induces miR-145 expression, which leads to downregulation of E2F3 and CDK6 in gastric cancer cells (Chang et al., 2015). These findings indicate that the antiproliferative and anticarcinogenic effects of vitamin D3, exerted through the modulation of a number of genes, including histone demethylases, cyclin-dependent kinase, transcriptional factors, could be mediated through microRNAs.

There are numerous clinical trials investigating vitamin D treatment in cancer patients. In a phase III randomized study, early breast cancer patients were supplemented for 6 months with high doses of vitamin D, which safely and with no toxicities normalized vitamin D level in patients compared with group treated with the conventional way (Jacot et al., 2016). Similar results have been achieved in phase II studies, where high vitamin D doses (500,000 IU) were administrated in melanoma patients with high recurrence risk. The treatment has been shown as safe and good tolerated (Saw et al., 2014).

4.3.1 Vitamin D cotreatment with other epigenetic nutraceuticals

Anticancer effects of vitamin D can be enhanced by cotreatment with other epigenetic nutraceuticals. In breast cancer cells (MCF-7), vitamin D and resveratrol treatment downregulated the expression of DNMT, induced demethylation and restored the activity of tumor suppressor phosphatase and tensin homolog (PTEN), and upregulated p21 (Stefanska et al., 2012b). Conversely, in invasive breast cancer cells (MDA-MB-231), PTEN demethylation was induced only by vitamin D treatment (Stefanska et al., 2012b). In vitro studies in the prostate cancer cells (LNCaP, PC-3, and DU-145) demonstrated that vitamin D3 exert synergistic the proapoptotic effect when applied with sodium butyrate, a natural short-chain fatty acid HDAC inhibitor, and trichostatin A, a synthetic HDAC inhibitor (Rashid et al., 2001).

5 Flavonoids

A great number of recent studies showed that high dietary intake of flavonoids, secondary plant metabolites, exert anticancer effects through epigenetic modifications (Busch et al., 2015). Flavonoids affect DNMT or HDAC gene expression and/or enzyme activities, suggesting potential clinical utilization of these phytochemicals in chemoprevention and/or chemotherapy of cancer. The most investigated flavonoids in cancer treatment, in in vitro and in vivo studies, are curcumin, quercetin, apigenin, and garcinol.

5.1 Curcumin

Curcumin (turmeric, diferuloylmethane) is a polyphenolic component of *Curcuma longa*, known for its antiinflammatory, antioxidative, and anticancer effects (Boyanapalli and Tony Kong, 2015) (Table 15.1). Curcumin treatment inhibits proliferation, induces apoptosis, and restores tumor suppressors, through modulation of various cancer related signaling pathways, via epigenetic mechanisms (Boyanapalli and Tony Kong, 2015). Both curcumin and demetohycurcumin, its more stable analog, induced cell cycle arrest in leukemia cells (Hassan et al., 2015). Cell cycle arrest induced upon a curcumin treatment was mediated through reduced cycline B1 expression, upregulation of proapoptotic Bcl-2, and decreased Bax and signaling pathway PI3K/AKT activity (Zhang et al., 2015b). Curcumin, as well as its analog demetohycurcumin, induced DNA damage and reduced expression of DNA repair proteins, including 14-3-3 protein sigma (14-3-3σ), O^6-methylguanine DNA methyltransferase (MGMT), breast cancer susceptibility gene 1 (BRCA1), and mediator of DNA damage checkpoint 1 (MDC1) (Ko et al., 2015; Ting et al., 2015). Suppression of tumor growth was also confirmed in xenograft lung cancer mouse model upon curcumin treatment (Jiang et al., 2015).

It has been demonstrated that curcumin induce epigenetic changes and reactivate tumor suppressor retinoid acid receptor β (RARβ) by decreasing promoter methylation, most probably through downregulation of DNMT3B (Jiang et al., 2015). In glioblastoma cells, curcumin increased mRNA and protein expression of receptor activator of NF-κB (RANK) by promoter demethylation (Wu et al., 2013). In acute myeloid leukemia, curcumin downregulates DNMT1, which leads to p15 promoter demethylation, cell cycle arrest, and induced apoptosis (Yu et al., 2013). In breast cancer cells, curcumin reactivated silenced RASSF1A by downregulation of DNMT1expression (Du et al., 2012). Other curcumin analogs, EF31 and UBS109, showed more potent activity than curcumin in suppressing proliferation and methylation in pancreatic cancer. Treatment with analogs reactivated p16, SPARC, and E-cadherin through NF-κB inhibition and downregulation of DNMT1 (Nagaraju et al., 2013).

Curcumin increases histone acetylation of suppressors of cytokine signaling SOCS1 and SOCS3 in myeloproliferative cell lines K562 and HEL. Also, curcumin has been confirmed

as an inhibitor of HDAC1, −3, and −8 (Chen et al., 2013b). In medulloblastoma, the most common brain tumor in children, curcumin treatment induces cell cycle arrest and decreases the HDAC4 expression and activity, as well as tumor growth (Lee et al., 2011b). In addition, global level of histone acetylation (H3K18 and H4K16) (Collins et al., 2013), and HDAC expression was increased upon curcumin treatment in breast cancer cells (Royt et al., 2011). Demetohycurcumin increased H3K36me3 near the promoter region of silenced genes (Hassan et al., 2015). Also, curcumin as demetohycurcumin treatment resulted in increased p53 and histone H2A.X phosphorilation (Ko et al., 2015; Ting et al., 2015).

It has been reported that growth and metastasis of lung cancer could be suppressed by curcumin inhibition of enhancer of zeste homolog 2 (EZH2) through modulation of let-7c and miR-101 and concomitant decreased expression of NOTCH1 (Wu et al., 2016). The results of the recent study showed that in prostate cancer cells, curcumin-induced CDKN1A at protein level through downregulation of miR-208 found to target CDKN1A and thus resulted in inhibited cancer growth (Guo et al., 2015). Curcumin attenuated colorectal cancer growth in in vitro and in vivo xenograft mouse model by miR-34 upregulation and miR-27a downregulation (Toden et al., 2015).

In one of the clinical trials (Table 15.2), combined curcumin and soy isoflavones treatment of prostate cancer patients synergistically decreased the level of prostate specific antigen (PSA) (Ide et al., 2010). Also, there are clinical confirmations that curcumin might be used in combination with regular chemotherapy. In phase I/II clinical study of pancreatic patients resistant to gemcitabine chemotherapy, daily treatment with 8 g of curcumin and gemcitabine was reported as safe with no toxicities (Kanai et al., 2011).

5.1.1 Curcumin cotreatment with other epigenetic nutraceuticals

Combined treatment of curcumin and other natural epigenetic agents showed promising synergistic anticancer effects. Curcumin and ellagic acid reactivated p53, induced reactive oxygen species (ROS) formation and DNA damage (Kumar et al., 2016). Furthermore, combined treatment with these two agents had anti-HPV effects by decreasing E6 oncoprotein in HeLa cells (Kumar et al., 2016).

Curcumin anticancer effects can be augmented by other epigenetic nutraceuticals, such as resveratrol. In transformed breast epithelial cells MCF-10A-Tr (induced by cigarette-smoke condensate) and in xenograft mouse tumor model, cotreatment of curcumin and resveratrol synergistically induced apoptosis through increasing p21 and the inhibition of the Hedgehog-Gli signaling pathway (Mohapatra et al., 2015). Cotreatment of cervical cancer cells (HeLa, SiHa, Caski) with curcumin and resveratrol reactivated tumor suppressor PAX1 (paired box) gene, independently of promoter methylation, through ubiquitin-like downregulation (Parashar and Capalash, 2015). Chemopreventive effects of curcumin in colorectal cancer might be enhanced by cotreatment with 3-acetyl-11-keto-β-boswellic acid (Toden et al., 2015).

5.2 Quercetin

Quercetin is an antioxidant flavonoid in fruits and vegetables, while the highest levels are found in onions, apples, and green and black tea. However, quercetin exerts low bioavailiability and poor water solubility (Sak, 2014). Quercetin-induced apoptosis through modulation of caspase 8, Bid, Bax, and cytochrome c release in leukemia cells, HL-60 (Lee et al., 2011a). As shown in hamster buccal pouch tumors, quercetin exerts anticancer effects by cell cycle arrest, inhibited invasion, angiogenesis, and induced apoptosis, which were correlated with HDAC1 and DNMT1 inhibition (Priyadarsini et al., 2011).

It has been demonstrated that quercetin can reactivate silenced p16 by promoter demethylation (Tan et al., 2009). Due to quercetin treatment, acetylation of histone H3 was increased followed with FasL increased expression, thus inducing apoptosis (Lee et al., 2011a). In ovarian cancer cells (SKOV3), quercetin potentiates tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by ROS generation, increasing TRAIL death receptor 5 (DR5) expression through JNK activation and upregulation of transcription factor CHOP (CCAAT enhancer-binding protein homologous protein), which was also confirmed in xenograft mice models (Yi et al., 2014).

Gold nanoparticles with quercetin were used in the treatment of hepatocarcinoma cells (HepG2). It has been shown that nanoparticles interact with DNA and reduce HDAC activity, especially HDAC1 and HDAC2 (Bishayee et al., 2015). Furthermore, nanoparticles with quercetin induce apoptosis by caspase 3 activation and ROS production, cell cycle arrest by increasing p21 while decreasing cdk1 and cyclin D1, and inhibit proliferation by downregulating pAKT expression (Bishayee et al., 2015).

A phase I study, conducted in recurrent prostate cancer patients, showed that muscadine grape skin, which contains quercetin, is safe and well tolerated, thus further confirmations in phase II are warranted (Paller et al., 2015).

5.2.1 Quercetin cotreatment with other epigenetic nutraceuticals or chemotherapeutics

Anticancer effects of quercetin could be enhanced in combination with other bioactive natural compounds. Quercetin augmented antiproliferative effects of green tea polyphenol EGCG in prostate cancer cells, but also in animal models (Wang et al., 2012, 2014). Quercetin and green tea cotreatment of prostate cancer xenograft model inhibited proliferation, androgen receptor, PI3K/AKT signaling, and induced apoptosis, which lead to significant tumor growth inhibition (Wang et al., 2014). Combined treatment of quercetin and curcumin was more effective than each agent alone in reactivation of androgen receptor via DNMT inhibition and global hypomethylation in androgen receptor negative prostate cancer cells (Sharma et al., 2016).

5.3 Apigenin

Apigenin (4′,5,7-trihydroxyflavone) is another flavonoid commonly found in vegetables and fruits. The anticancer potential of apigenin has been demonstrated in various cancer types, including breast, colorectal, head and neck, skin, prostate cancer, etc. (Shukla and Gupta, 2010) (Table 15.1).

Apigenin treatment causes cell cycle arrest and apoptosis, while inhibiting cell proliferation, migration, and invasion through an inhibition of Wnt/β catenin signaling pathway (Liu et al., 2015b; Xu et al., 2016), as well as PI3K/AKT and ERK1/2 MAPK signaling pathways (Lim et al., 2016; Lin et al., 2015). Also, apigenin induces caspase 3 and −8 and poly ADP-ribose polymerase (PARP) cleavage dependant apoptosis, and inhibits STAT3 signaling pathway (Seo et al., 2015a,b). Apigenin treatment of bladder cancer cells suppressed proliferation through regulation of cell cycle progression (increased p53 and p21, decreased cyclins, and cyclin dependant kinases), apoptosis, and ROS accumulation (Shi et al., 2015; Zhang et al., 2015a). Apigenin also exerts antiproliferative and apoptotic effects by triggering the oxidative stress and DNA damage (Banerjee and Mandal, 2015). The inducement of skin cancer by ultraviolet (UVB) radiation in mouse model was successfully inhibited by apigenin, through targeting mTOR signaling, independently of the AKT pathway (Bridgeman et al., 2016). In a mouse model of nonmelanoma skin cancer induced by chemicals, attenuated cancer proliferation has been noticed upon apigenin treatment (Kiraly et al., 2016).

The potential for using apigenine as an anticancer epigenetic agent has been demonstrated in a number of recent studies. Expression of DNMT1, DNMT3A, DNMT3B, and HDACs was reduced upon apigenin treatment of mouse skin epidermal JB6P+ cells, and reactivated silenced NF-E2-related factor 2 (Nrf2) (Paredes-Gonzalez et al., 2014). In breast cancer cells, as well as in tumor xenograft model, apigenin inhibited HDAC activity and increased histone H3 acetylation of the p21 promoter, which consequently increased p21 expression (Tseng et al., 2016). In prostate cancer cells (PC-3, 22Rv1) and xenograft model, apigenin inhibits HDACI class (HDAC1 and HDAC3) on mRNA and protein level, which leads to increased global histone H3 and H4 acetylation (Pandey et al., 2012). In addition, promoter histone H3 of p21 gene has been found hyperacetylated (Pandey et al., 2012). Also, due to apigenin treatment Bcl-2 expression was decreased, while Bax increased (Pandey et al., 2012). Proapoptotic effects of apigenin are enhanced in neuroblastoma cells with overexpressed miR-138 (Chakrabarti et al., 2013).

Limited number of clinical studies investigated the anticancer effect of apigenine in human subjects (Table 15.2). In one prospective study, cotreatment with 20 mg apigenin and 20 mg EGCG prevent recurrence in patients with resected colorectal cancer (Hoensch et al., 2008).

5.3.1 Apigenin cotreatment with other agents and epigenetic nutraceuticals

Synergistic effects have been shown for apigenin and other natural compounds in cancer treatment in vitro. Apigenin and curcumin synergisticaly induce cell cycle arrest, and apoptosis by targeting tubulin, which consequently causes microtubules depolymerisation in lung cancer cells (A549) (Choudhury et al., 2013). It has been reported that apigenin induces apoptosis through upregulation of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) signaling in HepG2 cells (Kim et al., 2013). Anaplastic thyroid carcinoma cells (8505C, CAL62 ATC) cotreated with apigenin and TRAIL induced cytotoxic effects, increased cleaved PARP and phospho-ERK1/2 through AKT pathway suppression and Bcl-2 regulation (Seo et al., 2015a). Combined treatment of gemcitabine and apigenin potentiate apoptosis and growth suppression of prostate cancer cells (MiaPaca-2, AsPC-1) via NF-κB downregulation and AKT inhibition (Lee et al., 2008).

5.4 Garcinol

Anticancer effects of garcinol, a polyisoprenylated benzophenone from the plant Garcinia indica, have been confirmed in a number of cancer types. Garcinol exerts proapoptotic, and antiproliferative (Ye et al., 2014), as well as antiangiogenic effects, through NF-κB, COX-2, MMP-9, and VEGF inhibition (Aggarwal and Das, 2016; Ahmad et al., 2010; Parasramka and Gupta, 2011). In addition, garcinol treatment induces apoptosis and autophagy by activating mTOR and PI3K/AKT pathway, as shown in prostate cancer cells (Wang et al., 2015a). It has been reported that garcinol induced reactive oxygen species (ROS) accumulation, induced growth arrest and expression of DNA damage-inducible gene 153, Bax Bcl2, Bid and caspase 8, thus inducing apoptosis independently of p53 status (Cheng et al., 2010; Yu et al., 2014). Also, garcinol inhibited invasion and induce apoptosis by ERK inhibition (Liao et al., 2005), and decreased the expression of CDK2, CDK4, and cyclin D1 and D3, while upregulating p21 and p27 (Yu et al., 2014). In breast, prostate, pancreatic, and hepatocellular cell lines garcinol inhibited STAT3 (signal transducer and activator of transcription 3) signaling (Ahmad et al., 2012a; Sethi et al., 2014). Antimetastatic and antiangiogenic effects of garcinol were exerted via MMP-9, VEGF, IL-8, and PGE2 modulation (Parasramka and Gupta, 2011).

Garcinol act as a HAT inhibitor, linked to hyperacetylation of H3 and nonhistone proteins (NF-κB/p65) in breast cancer cells MCF-7 (Ye et al., 2014). In triple negative breast cancer cells, garcinol upregulated E-cadherin and reversed the EMT, which was followed by miR-200s and let-7 upregulation and the inhibition of NF-κB and Wnt signaling pathway (Ahmad et al., 2012b). Furthermore, PANC-1 prostate cancer cells with stem-like properties and metastatic potential have been inhibited upon garcinol treatment by downregulation of Notch1 signaling and upregulation of miR-200c, found to directly target Notch1 (Huang et al., 2017). Gemcitabine treatment inhibited proliferation and induced apoptosis via modulation of

miR-21, miR-196a, miR-495, miR-605, miR-638, and miR-453 (Parasramka et al., 2013), demonstrating that the anticancer effects of garcinol could be modulated through microRNAs.

5.4.1 Garcinol cotreatment with other epigenetic nutraceuticals

Combined treatment of garcinol and other natural chemotherapeutics has been studied as a potential epigenetic anticancer agent. Garcinol and curcumin exerted synergistic antiproliferative and proapoptotic effects in prostate cancer (Parasramka and Gupta, 2012), and in human leukemia cell line HL-60, via caspase 2 and −3 activation, PARP cleavage and cytochrome c release (Pan et al., 2001).

6 Polyphenol Catechins

6.1 Epigallocatechin-3-gallate (EGCG)

A major polyphenol component of green tea, epigallocatechin-3-gallate (EGCG), shows the most promising results in cancer prevention and treatment (Table 15.1). EGCG suppressed proliferation and induced apoptosis through p53 inhibition (Irimie et al., 2015), and through Bcl-2 family modulation and activation of caspase-dependent pathway (Irimie et al., 2015; Wang et al., 2015b). Antiproliferative and antimigration effects of EGCG are achieved by binding to tubulin and inducing depolymerisation of microtubules (Chakrabarty et al., 2015), and by reducing the expression of MMP2 and MMP9 (Chen et al., 2016). EGCG treatment inhibited invasive and metastatic potential via targeting MEK/ERK pathway (mitogen-activated protein kinase/extracellular signal-regulated kinase) in osteosarcoma cells (Tang et al., 2015), and nasopharyngeal carcinoma cells (Fang et al., 2015).

EGCG is the potent inhibitor of DNMTs activity, which, in a dose-dependent manner, restores methylation-silenced genes in cancer cells (Fang et al., 2003). EGCG reactivated silenced tumor suppressor genes, retinoid acid receptor β (RARβ), CDH1, and death associated protein kinase 1 (DAPK1) through promoter demethylation (Khan et al., 2015). In colon cancer cell lines, EGCG restores RXRα activity by decreasing promoter methylation, without changing of global demethylation (Morris et al., 2016). EGCG treatments of cervical cancer cells decrease DNMT3B expression, but not HDAC1, even though reduced enzymatic activity has been confirmed for DNMT and HDAC (Khan et al., 2015). In colorectal cancer cell line (HT29) EGCG treatment reduced HDAC activity and HDAC1 protein level (Groh et al., 2013). EGCG inhibited SIRT3 expression on mRNA and protein level in oral squamous cell carcinoma (SCC-25) through regulating estrogen related receptor α (ERRα) localization (Tao et al., 2015).

EGCG anticancer activity has found to be mediated through microRNAs modulation of gene expression related to AKT, NF-κB, MAP kinase pathways in tobacco-induced lung cancer mouse model (Zhou et al., 2014). Treatment of oral squamous cell carcinoma with EGCG upregulate miR-204 followed by downregulation of its targets Slug and Sox4, thus inhibiting

proliferation, EMT, and metastasis in vitro and in vivo (Yu et al., 2016). EGCG treatment of osteosarcoma induced miR-1 overexpression, which was followed by the proliferation inhibition, cell cycle arrest and apoptosis induction (Zhu and Wang, 2016). Another study reported that EGCG induced apoptosis by miR-126 overexpression in osteosarcoma cells (U2OS) (Jiang et al., 2014).

Recent case reports reported that EGCG treatment, without standard medical therapy, resulted in complete molecular remission of the disease in a patient diagnosed with chronic lymphocytic leukemia (Lemanne et al., 2015) (Table 15.2). EGCG and black tea theaflavin synergistically reduced growth of prostate cancer in men treated with combination of EGCG and theaflavin prior to prostatectomy (Kobalka et al., 2015). In a phase I clinical study, patients with breast cancer were treated with EGCG and radiotherapy, EGCG showed good tolerability, safeness, and efficacy (Zhao et al., 2016). In a phase II trial, EGCG treatment of stage III lung cancer patients during and after radiotherapy, was shown to be is an effective and safe method to manage radiation-induced esophagitis and to reduce dysphagia and pain (Zhao et al., 2015).

6.1.1 Epigallocatechin-3-gallate cotreatment with other epigenetic nutraceuticals

Curcumin and EGCG cotreatment inhibited cancer stem phenotype of breast cancer cell lines (MDA-MB-231, MCF7) by downregulation of STAT3 and NF-κB signaling (Chung and Vadgama, 2015). Curcumin and EGCG synergistically induced cell cycle arrest through p21 upregulation in prostate cancer cells (PC3) (Eom et al., 2015).

Cotreatment of EGCG and low dose of 5-aza-2′-deoxycytidine, a synthetic DNMT inhibitor, showed better effects in inhibiting breast cancer cells proliferation and survival, while inducing the apoptosis-specific and tumor suppressor genes (RASSF1A) (Tyagi et al., 2015). DNMT1, DNMT3B, and MBD4 were maximally downregulated upon cotreatment on both mRNA and protein levels, and HDAC1 at protein level. Histone H3 demethylation (H3K17me3) and acetylation p-Ac-H3 (S11/K15) was higher after EGCG and 5-aza-2′-deoxycytidine joint treatment than in single agent treated cells (Tyagi et al., 2015).

6.2 Resveratrol

Resveratrol is phytoalexin polyphenol found in peanuts, cranberries, blueberries, grapes, and in red wine (Hardy and Tollefsbol, 2011). Antioxidative, antiinflammatory, and anticancer effects of resveratrol have been demonstrated in numerous in vitro and in vivo studies (Table 15.1).

Resveratrol decreases expression of DNMT1 and DNMT3B genes in breast cancer and reactivate RASSF-1α tumor suppressor by promoter demethylation (Qin et al., 2014). DNMT3B was decreased most probably through upregulation of miR-129, miR-204 and miR-489 upon resveratrol treatment (Qin et al., 2014). In women with increased

breast cancer risk, resveratrol also hypomethylated and reactivated RASSF-1α gene (Zhu et al., 2012). In breast cancer, resveratrol, as SITR1 antagonist, inhibits androgen receptor stimulated proliferation (Zhang et al., 2016). Natural phytochemicals, including resveratrol, decrease expression of DNMT1, -3A, and -3B, as well as protein level of DNMT1, HDAC1 and methyl-binding proteins in breast cancer patients (Mirza et al., 2013). It has been demonstrated that resveratrol exerts antiproliferative and antiinvasive effects in colorectal cancer by NF-κB suppression and targeting SIRT1 (Buhrmann et al., 2016).

Resveratrol induces cell cycle arrest, apoptosis, and nuclear translocation of SIRT1 and p53 in bladder cancer cell line and in vivo (Wu et al., 2014). In osteosarcoma, resveratrol suppressed matrix metalloproteinase 2 (MMP2) via miR-328 upregulation and inhibition of CREB-DNA binding activity, which reduced invasive and metastatic potential of osteosarcoma (Yang et al., 2015).

Resveratrol suppresses colorectal cancer cell proliferation, invasion and induces apoptosis by upregulating miR-34c and concomitant downregulation of Kit ligand precursor, and this effect is enhanced by p53 (Yang et al., 2015). In prostate cancer cells, resveratrol treatment leads to the acetylation and reactivation of PTEN, by inhibition of the metastasis associated protein 1 (MTA1)-HDAC complex and inhibition of Akt signaling (Dhar et al., 2015).

Using resveratrol as a chemopreventive agent is demonstrated in clinical trials (Table 15.2). For instance, administration of daily reseveratrol doses (0.5 or 1 g) for 8 days prior surgery in colorectal cancer patients, reduced tumor proliferation for 5% (Patel et al., 2010). Women with increased breast cancer risk were treated with 5 or 50 mg of transresveratrol twice daily during 12 weeks (Zhu et al., 2012). Due to the treatment, tumor suppressor RASSF1α methylation level was decreased and related to prostaglandin PGE2 change (Zhu et al., 2012).

6.3 Genistein

Chemopreventive effects of genistein, soybeans isoflavone, have been reported for various cancers types. According to the epidemiological data, an Asian diet rich with soybeans could be associated with decreased incidence and mortality of breast cancer (Li and Tollefsbol, 2010).

Silenced tumor suppressor genes, p16, RARβ, and MGMT, could be reactivated due to genistein treatment, as shown in esophageal carcinoma cell line (KYSE 510) (Fang et al., 2005). Genistein directly interact with the catalytic domain of the DNMT1 enzyme, decrease its expression and DNMT1 enzyme activity, and the global DNA methylation level (Xie et al., 2014). Moreover, genistein treatment of breast cancer cells (MCF-7, MDA-MB-231) reactivates silenced tumor suppressor genes ataxia telangiectasia mutated, adenomatous polyposis coli (APC), PTEN, and mammary serpin peptidase inhibitor (Xie et al., 2014). In neuroblastoma, genistein demethylated tumor suppressors p53 and CHD5 by

decreasing DNMT3B expression (Li et al., 2012). Genistein induced the expression of sFRP1 and Smad4 via DNA demethylation and histones modifications (H3K9-me2, H3K9-me3, and H3K27-me3) (Hirata et al., 2014).

Genistein inhibit hTERT activity through both genetic and epigenetic mechanisms. In prostate cancer cells (DU-145, LNCaP) genistein induced hTERT promoter demethylation, as well as cancer growth inhibition and apoptosis induction (Karsli-Ceppioglu et al., 2015). Furthermore, in breast cells (MCF10AT and MCF-7), genistein inhibited hTERT by decreasing DNMTs expression, and decreasing the histone methylation (H3K4me2) of the hTERT promoter (Li et al., 2009).

Genistein demethylated and increased acetylation of histones H3 and H4 in known breast cancer-associated genes, such as BRCA1, ERα, ERβ (Dagdemir et al., 2013). Upon genistein treatment of colorectal cells (HT29), HDAC activity was reduced and the HDAC1 protein level was decreased (Groh et al., 2013).

Genistein treatment of prostate cancer upregulated the expression of miR-574-3p, which subsequently induced apoptosis by Bcl-xL reduction and caspase 3 and −9 activation (Chiyomaru et al., 2013). Genistein inhibited growth of metastatic breast cancer cell lines (MDA-MB-435, Hs578t), through miR-155 downregulation, and induction of its targets FOXO3, PTEN, casein kinase and p27 genes (de la Parra et al., 2016). Genistein treatment also decreased miR-1260b, and upregulated its target genes in the Wnt pathway, sFRP1 and Smad4, in prostate cancer (Hirata et al., 2014), and renal carcinoma cells (Hirata et al., 2013). In prostate cancer cells PC3 and DU145, genistein inhibited tumor growth, migration and invasion by downregulation of miR-151 (Chiyomaru et al., 2012), and miR-221/222 (Chen et al., 2011a,b). Tumor growth was inhibited and apoptosis induced in pancreatic cancer cells after exposure to genistein through miR-27 downregulation (Xia et al., 2014). Also, downregulation of miR-27b upon genistein treatment was demonstranted in SKOV3 with concomitant Sprouty2 upregulation (Xu et al., 2013). The downregulation of Notch-1 signaling by genistein in pancreatic cancer cells was modulated through miR-34a (Xia et al., 2012), and miR-200b (Bao et al., 2011). However, genistein treatment shows inconsistent results on miR-23b expression, found to be upregulated in breast cells (MCF-7) (Avci et al., 2015), while downregulated in renal carcinoma cells (A498) (Zaman et al., 2012a,b), indicating possible tumor-type specificity of genistein effects.

Although a number of recent studies show the promising potential of genistein as an anticancer agent, this bioactive compound raised controversy in relation to breast cancer risk. In a clinical trial, early-stage breast cancer patients were treated with soy supplementation prior surgery and gene expression was evaluated in pre- and posttreatment tissue (Shike et al., 2014). It has been shown that in soy-treated breast cancer patients, expression of genes related to cell cycle and proliferation is induced (Shike et al., 2014). A randomized phase II trial has reported that after 6 months of soy supplementation in healthy breast cancer

women, breast epithelial proliferation was not reduced (Khan et al., 2012). Several meta-analyses have reported inverse associations between genistein/soy intake and breast cancer risk. However, these results show inconsistency among pre- and postmenopausal women (Dong and Qin, 2011; Wu et al., 2008). Also, there are data indicating that time of genistein exposure during the fetal development, childhood, adolescence, and adulthood (pre- or postmenopause), could be critical in modifying breast cancer risk (Anderson et al., 2013; Korde et al., 2009; Li and Tollefsbol, 2010; Warri et al., 2008; Wu et al., 2008). Therefore, there is justified doubt on using soy supplementation in breast cancer treatment and prevention. Due to its phytoestrogen properties, genistein could be contraindicative in women with increased breast cancer risk.

However, genistein treatment showed promising effects in treatment of prostate cancer. Prostate cancer patients were treated with 30 mg genistein prior to prostatectomy for 3–6 weeks, after which PSA level, androgen-related biomarkers and cell cycle related genes expression has been evaluated (Lazarevic et al., 2011, 2012). Due to the treatment, PSA level was reduced (Lazarevic et al., 2011) and expression of androgen and cell cycle related biomarkers were modulated, which was related to prostate cancer prediction and progression (Lazarevic et al., 2012).

6.3.1 Genistein cotreatment with other epigenetic agents

Cotreatment of genistein and other epigenetic agents, natural or synthetic, exert synergistic effects in modulating cell proliferation and apoptosis (Dhandayuthapani et al., 2013; Hsieh and Wu, 2009; Park et al., 2010; Shiau et al., 2010). Due to genistein treatment of easophageal carcinoma cells (KYSE510), activation of DNMT enzyme was decreased, which subsequently resulted in p16, RARβ, and MGMT promoter demethylation (Fang et al., 2005). In addition, genistein enhanced the effect of sulforaphane, trichostatin, or DNMT inhibitor 2′-deoxy-5-aza-cytidine (5-aza-dCyd) treatment in epigenetic reactivation of these genes and inhibition of cell growth (Fang et al., 2005).

6.4 Sulforaphane

Sulforaphane is isothiocyanat commonly found in cruciferous vegetables, such as broccoli and cauliflower. Numerous evidences confirmed sulforaphane anticancer and antioxidant effects (Tuorkey, 2015). Molecular mechanisms through which sulforaphane exerts anticancer effects are mainly demonstrated in in vitro studies on various cancer cell lines, but also in in vivo. Sulforaphane induced G2/M cell cycle arrest, caspase activation, PARP inactivation, upregulation of p21, and inhibition of mTOR/AKT signaling in vitro and in xenograft models (Suppipat et al., 2012). Prostate cancer cells invasion inhibition by sulforaphane treatment was mediated through an activation of ERK1/2, E-cadherin upregulation, and MMP2 downregulation (Peng et al., 2015).

It has been demonstrated that sulforaphane reactivates ERα expression in ER negative breast cancer cells (MDA-MB-231), which was correlated with ERα hypermethylation and hyperacetylation (Meeran et al., 2012). In prostate cancer cells, sulforaphane treatment reactivated previously silenced genes by decreasing DNMT1 and DNMT3B gene expression (Hsu et al., 2011; Wong et al., 2014). Furthermore, in prostate cancer TRAMP C1 cells, sulforaphane induced demethylation and expression of Nrf2 through DNMT1, DNMT3A, and HDACs downregulation (Zhang et al., 2013). However, recent studies indicated that colorectal cancer cells (caco-2, HCT116) treated with sulforaphane and/or selenium, do not show changes in ESR1 and p16 genes mehylation level, neither did the global LINE-1 methylation (Barrera et al., 2013), indicating that sulforaphane may exert tumor-type specific effects.

Breast cancer cells (MCF-7, MDA-MB-231), treated with sulforaphane had repressed hTERT via decreased DNMT1 and DNMT3A (Meeran et al., 2010). Expression was decreased in prostate cancer cells upon sulforaphane treatment, which correlated with chromatin changes (Abbas et al., 2016). Upon sulforaphane treatment, promoter of hTERT gene showed increased histone H3 and H4 acetylation, and decreased H3K9me3 and H3K27me3 methylation (Meeran et al., 2010), which highlighted sulforaphane as a potential epigenetic modifier of hTERT gene expression.

In prostate cancer cell lines and in xenograft mouse model, sulforaphane decreased activity of HDACs (Clarke et al., 2011; Myzak et al., 2006a,b, 2007; Zhang et al., 2013). Sulforaphane inhibited HDAC activity and increased histone acetylation, followed by the expression of proapoptotic genes in lung cancer cells (A549, H1299) (Jiang et al., 2016). It has been shown that sulforaphane treatment of bladder cancer cells upregulated miR-200c, which mediate E-cadherin and EMT inhibition via ZEB1 (Shan et al., 2013).

In one of the first clinical studies on human subjects, HDAC activity was inhibited in peripheral blood mononuclear cells after 3 or 6 h after broccoli consumption, and this effect was prolonged up to 24 h after the comsumption of singe portion (68 g) of broccoli sprouts (Myzak et al., 2007).

A recent phase II clinical study has evaluated effects of sulforaphane rich broccoli extracts in men with recurrent prostate cancer (Alumkal et al., 2015). Daily treatment with 200 μmoles of sulforaphane extract was not found as promising in reducing PSA level more than 50% (Alumkal et al., 2015). However, results of the other clinical study are confirming sulforaphane efficacy in reducing PSA level after treatment of cancer patients with higher doses. In patients with biochemical recurrence of prostate cancer after prostatectomy, administration of daily 60 mg sulforaphane for 6 months was followed with 2 months without treatment (Cipolla et al., 2015). It has been shown that the PSA level was significantly decreased compared with the placebo group during the treatment period with prominent results after 3 months. PSA levels remained low in the treated group for 2 months after sulforaphane treatment (Cipolla et al., 2015).

6.4.1 Sulforaphane cotreatment with other epigenetic nutraceuticals

Combination of sulforaphane and EGCG reactivated ERα in breast cancer cells (MDA-MB-231) (Meeran et al., 2012). Also, antiproliferative and apoptotic effects of tamoxifen were augmented by combination with sulforaphane and EGCG (Meeran et al., 2012). In colon cancer cells (HT-29 AP-1) joint treatment with sulforaphane and EGCG synergistically induced AP-1 activation (Nair et al., 2008).

Combinations of sulforaphane and EGCG showed promising effects in potentiating cisplatin-induced apoptosis by p21 upregulation and cycle arrest in ovarian cancer cells (A2780, A2780/CP20) (Chen et al., 2013a), indicating that cotreatment of these epigenetic nutraceuticals can be used in restoration of cisplatin sensitivity. Prostate cancer cells (MIA PaCa-2, Panc-1) were treated with a combination of low-dose aspirin, curcumin, and sulfaraphane, upon which apoptosis was induced by caspase 3 and PARP activation and NF-κB inhibition (Thakkar et al., 2013). Progression of the advanced pancreatic cancer was inhibited upon combined treatment with sulforaphane, quercetin and green tea catechins through miR-let-7a induction and K-ras inhibition (Appari et al., 2014).

7 Conclusions, Future Perspectives, and Challenges

Recent studies have provided convincing evidence that dietary components and phytochemicals can have an important role in cancer prevention, possibly through modulation of epigenetic processes. The growing interest in nutritional epigenetics of cancer in recent years is largely due to the reversible nature of epigenetic changes that may be targeted by dietary interventions or epigenetic therapy. Epigenetic changes could be influenced by environmental factors, diet, and the joint effects of gene-to-environment interactions, and be a link between cancer, susceptibility genes, and nutrition (Supic et al., 2013, 2016b). A number of bioactive phytochemicals have been identified as potential epigenetic modifiers that may affect DNA methylation, histone modifications, and miRNAs in cancer. Natural epigenetic modifiers could potentially exert anticancer properties without the undesired toxic side effects that occur during chemotherapy with synthetic epigenetic agents (Magic et al., 2009). Another key aspect of the chemopreventive action of polyphenols and other dietary factors is their potential ability to selectively target cancer cells, while maintaining negligible cytotoxicity to normal cells (Khan et al., 2012). However, the precise mechanisms whereby bioactive food components exert anticancer actions through epigenetic mechanisms in human cancers are still unknown and require further investigation.

The interaction of dietary components with one another, in addition to interactions with genetic, lifestyle, and environmental factors, makes it very difficult to determine key components, which might induce or prevent cancer development. There is limited information about tissue- and cell-type specificity, optimal doses, and temporal associations of dietary

treatments, as well as metabolism. It is necessary to identify the relevant bioactive dietary components and their metabolites to provide safe, but effective, dietary recommendations. Future animal, clinical, and epidemiologic studies are necessary to characterize the specificities of bioactive dietary compounds and their kinetics. Establishing the optimal doses and scheduling of bioactive dietary components are required to obtain desirable epigenetic changes.

One of the important challenges is the increasing of the bioavailability from oral delivery and plasma half-life of phytochemicals, which could be used for the treatment of various tumor types. Anticancer effects of one phytochemical compound could be enhanced when given in combination with other bioactive natural compounds. An important challenge in anticancer therapy by epigenetic nutraceuticals is the discovery of new strategies in increasing the delivery of bioactive compounds to the site of actions. It has been shown that liposomes and nanoparticles have promising results in more efficient delivery of bioactive compounds into cancer cells. Thus, synthesis of more stable analogs, with increased bioavailability and stability, as well as novel delivery systems, is warranted.

An emerging and intriguing question is whether bioactive food components and phytochemicals have only beneficial effects or if there are conditions, doses, or vulnerable developmental phases when they might exert harmful effects. Natural DNMT and HDAC inhibitors lack targeting in genetic levels or might impair global epigenetic patterns. Higher doses of natural DNMT inhibitors might cause global DNA hypomethylation of the genome, which could affect genome stability, and promote neoplastic transformation. Even nonselective local epigenetic effect on target genes involved in multiple signaling and cancer-related pathways could have a substantial global consequence. Also, high doses of DNMTs and HDAC inhibitors used for cancer treatment could exert substantial toxicity and drug resistance effect. Alternatively, low doses of nonselective epigenetic modifiers could be combined with more targeted therapy, immunotherapy, or chemotherapy. It has been shown that genistein and apigenin could have a stimulatory effect on the proliferation of ERα-positive breast cancer cells (Seo et al., 2006), demonstrating that in specific cancer types phytochemicals could exert detrimental effects. These findings indicate that comprehensive evaluations are warranted for the use of concentrated phytoestrogen supplements or for long-term exposure.

Additional research is needed to determine beneficial versus deleterious effects in healthy individuals, as well as individuals with different types of cancers, advanced stages, or different physiological conditions, such as aging. Changes in DNA methylation profile observed with aging (Fraga et al., 2005), indicate that epigenetic changes accumulate over time and that long life exposure to bioactive compounds at low concentrations could remodel the epigenome in a cumulative fashion (Szarc Vel Szic et al., 2011). Further studies are needed to determine the doses of bioactive food components effective and optimal for cancer prevention or for cancer treatment.

Another question arising is whether inadequate nutrition, or even transient exposure to phytochemicals, in critical times for development (preconception, gestation, lactation, neonatal life, adolescence) can affect the long-term epigenetic changes that occur later in life or in offspring. Evidence emerged that prenatal exposure to caloric restrictions or specific bioactive compounds can affect long-term DNA methylation changes in offspring that occur for decades later in life (Heijmans et al., 2008; Hughes et al., 2009). A more recent study showed that prenatal supplementation with dietary polyunsaturated fatty acid affected imprinting in infants (Lee et al., 2014).

The effects of dietary components and phytochemicals have been demonstrated in a number of in vitro experiments, but sometimes at concentrations that can hardly be achieved in vivo. In addition, results from animal models cannot be fully translated to prevent human malignancies in clinical settings due to species differences in epigenetic mechanisms. Therefore, animal models and human dietary intervention studies should be carefully designed to further elucidate the nutriepigenetics impact in the management of human cancer.

Limited numbers of clinical studies investigated the anticancer effect of bioactive compounds in human subjects. The translation of the promising in vitro data into clinical practice of cancer treatment is a relatively slow process, and further clinical studies are warranted. Although a few human dietary intervention studies have been conducted, observational studies, in particular studies involving individuals exposed to severe famine of the Dutch Hunger Winter and Overkalix cohorts, provided insights into interactions between epigenetics, diet, and health (Bygren et al., 2014; Heijmans et al., 2008). The first study in human subjects demonstrating that single ingestion of broccoli sprouts, containing sulphoraphane, inhibited HDAC activity in circulating peripheral blood mononuclear cells as early as 3 h after broccoli intake, which persisted for at least 48 h (Myzak et al. 2007). These findings indicate that HDAC activity in peripheral blood mononuclear cells could be used as a biomarker for the evaluation of novel dietary HDAC inhibitors in human subjects. Also, the question as to nonhereditary cancers could be prevented, postponed, or managed by interventional nutrition and controlled lifestyle now arises (Toden and Goel, 2014).

The complex interactions among environmental, genetic, and epigenetic factors in cancer development have not yet been fully elucidated. Identifying the mechanisms that underlie epigenetic modifications may serve as a tool to predict the individual's genetic susceptibility to cancer. Future research should further explore the ability of nutraceuticals to act as epigenetic modifiers, and to provide recommendations for chemoprevention, or therapeutic applications against cancer. The field of nutritional epigenetics seems to have a very promising future in cancer prevention, nutritional interventions, and therapeutic applications of natural compounds in the treatment of cancer.

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HANDBOOK OF FOOD BIOENGINEERING
VOLUME 8

THERAPEUTIC FOODS

Edited by
Alina Maria Holban and **Alexandru Mihai Grumezescu**

Therapeutic Foods, a volume in the *Handbook of Food Bioengineering* series, aims to bring together the most investigated and innovative approaches regarding the development of valuable and functional food products with improved quality, ensuring health promotion and environment protection in the context of a sustainable food industry. It covers many spices, plant extracts, essential oils, vegetal mixtures that have a great immune-stimulatory effect and can be efficiently utilized in the treatment of infections and other severe disorders. The book is a comprehensive resource for food scientists, engineers, biotechnologists, biochemists, and industrial companies.

Key Features

- Presents introductory chapters for background and practical examples of therapeutic foods, investigated for the therapy of different diseases
- Provides scientific proof regarding nutritional content and nutraceutical potential of some intensively debated natural foods and ingredients (chia, lipids, polysaccharides, mushrooms)
- Includes benefits of the applications of functional properties of food and food ingredients to benefit health and well-being

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