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Functional Foods

Phytochemicals and Health Promoting Potential

Edited by Muhammad Sajid Arshad and Muhammad Haseeb Ahmad

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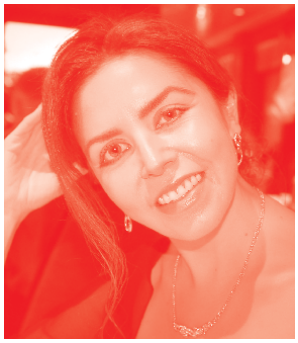

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Meet the editors

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Contents

Preface

Due to changing lifestyles and consumer awareness, there is a trend towards choosing healthy, nutritious, and safe food. Therefore, scientists and researchers are focusing on developing functional foods, an emerging field of food science that claims health-promoting benefits. Functional foods are just like traditional foods but with the potential to offer health benefits beyond proper nutrition. They may also be considered natural remedies against various diseases due to their bioactive components. Nutraceuticals can be used anonymously with functional foods. The bioactive components present in functional foods and nutraceuticals are sensitive to different processing technologies; therefore, researchers and scientists are continuously trying to adopt processing technologies like ultrasound, high-pressure processing, and irradiation, all of which minimally affect the bioactive components present in functional foods.

This book comprehensively discusses different phytochemicals, such as antioxidants, that have great potential for radical scavenging activity and play a role in the prevention of various types of disorders. Different products based on functional foods contain many therapeutic bioactive compounds that improve one's immunity to combat various types of health-related issues.

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Section 1

Overview and Importance of Functional Foods

Chapter 1

Functional Foods and Human Health: An Overview

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Abstract

Functional food is a whole ingredient or a part of food that used as food for specific therapeutic purposes. It is divided into two wide categories: Conventional and modified functional foods. Conventional functional Foods are composed of natural or whole-food ingredients that provide functional substances while modified functional is food or food products in which add additional ingredients for specific health purposes. Plant-based food such as fruits, vegetables, herbs, cereals, nuts and beans contain vitamins, minerals, fiber, omega-3 fatty acids, antioxidants and phenolic compounds that play a functional role in the human body against chronic diseases including cancer, cardiovascular and GIT-related disease. Some other foods or food products like juices, dairy products, fortified eggs and seafood are composed of functional components. Fish contain omega-3 fatty acids (EPA and DHA) that are played a functional role in heart health and brain development.

Keywords: functional food, conventional food, modified food, antioxidants, health

1. Introduction

Functional food is concerned with promoting health or protection from diseases [1]. The word functional food can also be used to describe traits that have been purposefully bred into established edible plants, such as purple or gold potatoes with lower anthocyanin or carotenoid content [2]. These foods are prepared for functional benefits and aid in reducing the risk of long term diseases beyond basic nutritional functions and can look like traditional food and be eaten as part of a daily diet [3].

The functional food sector, which includes the food, beverage, and supplement industries, is one of the many sectors of the food industry that has seen significant growth in recent years. The global demand for functional foods is expected to hit 176.7 billion dollars in 2013, with a compound annual growth rate (CAGR) of 7.4%. The functional food sector will grow at a 6.9% CAGR, while the supplement sector will grow at a 3.8 percent CAGR, and the functional beverage sector will grow at a

10.8 percent CAGR [4]. This type of expansion is fueled not only by technological advancements and the introduction of new goods to meet the needs of health-conscious customers but also by health claims that cover a broad range of ailments. Consumer skepticism remains, owing to the fact that the advantages of using the goods can be difficult to discern. Any businesses may be deterred from launching their products if any of the functional food statements are scrutinized closely [5].

Functional foods contain a rich source of bioactive components. When these components are used in reliable, non-toxic, and defined quantities, so these provide a scientifically validated and recorded health benefit for the prevention, control, or treatment of chronic diseases" [6]. However, establishing a formal concept for these foods would help in their commercialization. The inclusion of bioactive compounds, which are biochemical molecules that promote health by physiological processes, improves the idea of functional foods. Functional Food Center (FFC) has advanced the functional food concept to provide clarification and a more detailed understanding of its context [7].

Health benefits are commonly associated with functional foods. Physical performance, psychological activity, organ or system function, emotional, mental, and curing chronic illness are the physiological benefits of functional foods or bioactive compounds [8].

2. Definitions of functional food

In the early 1980s, a Japanese scholarly society proposed the idea of functional foods. The law for functional foods was first introduced in the 1980s as FOSHU,

Sources	Definition of functional food	References
Functional Food Center (FFC)	"Natural or processed foods containing known or unknown biologically active compounds that provide a scientifically supported and established health benefit for the prevention, management, or treatment of chronic diseases is prescribed, appropriate, and non-toxic amounts"	[11]
Food and Drug Administration (FDA)	"The word "functional foods," which is recently being used as a marketing idiom for the group, does not have a legal meaning"	[7]
Institute of Food Technologists (IFT)	"Foods and food ingredients that have health benefits in addition to basic nutrition." These products provide necessary nutrients in amounts that are often more than what is needed for regular operation, health, and production, as well as other pharmacologically active components that have a positive effect on health."	[12]
International Life Sciences Institute (ILSI)	"Foods that by virtue of the presence of physiologically active food components provide health benefits beyond basic nutrition"	[13]
European Food Safety Authority (EFSA)	"A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. A functional food can be a natural food or a food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet"	[6]

Table 1.
Different definitions of functional food.

which stands for "Foods for Specified Health Use." Functional foods were originally characterized as foods that can modulate body functions and thus help in disease prevention [9]. Functional foods are described by EFSA as "a food that, above sufficient dietary benefits, has a beneficial impact on the multiple body functions in a way that is related to either an enhanced health condition and well-being or a decreased risk of disease". The FSGs law encompasses a small number of well-defined food groups that are deemed important for human health and certain categories of, especially vulnerable individuals. Processed cereal-based diets and baby food, complete diet substitute for weight loss, infant formula and follow-up formula and food for special medical purposes [6]. A functional food should be a natural food or a portion of food to which an ingredient has been added or extracted using technical or biotechnological methods, and it must represent the effects in amounts that can be fairly believed to be consumed in the food" [10].

The Functional Food Center in the United States (FFC) has defined functional foods as "real or processed foods that contain known or unknown biologically active compounds that, efficient, in defined and non-toxic quantities, recorded health benefit or provide a scientifically validated using unique biomarkers for the prevention, treatment or control of chronic disease or its symbiotic diseases" [11]. There are different definitions of functional food are listed in **Table 1**.

3. Foods that contain functional components

Plant foods contain a variety of functional components such as vitamins (C, A and E) minerals, phenolic compounds, antioxidants and phytochemicals [14]. These Secondary metabolisms produce physiologically active molecules that are helpful for the human body. The various classes of health-promoting substances (phytosterols, carotenoids, phytosterols, phenolic compounds, nondigestible carbohydrates, tocotrienols and organosulfur compounds) are discussed together with the dietary origins as well as the biological and chemical properties that justify their mechanisms of action [15].

Plant-based functional foods (broccoli and other cruciferous vegetables, fruit, grapes, tomato, soybean, oat, oranges, flaxseed, garlic, wine, and tea) are played a functional role in the healthy body, as well as the phytochemicals concerned and benefits of health-promoting function. The study stresses the relevance of eating fruits and vegetables for people's overall wellbeing, as well as certain science and technological prospects in developing countries [16].

Phytochemicals found in whole grains, fruits, and vegetables are the most abundant sources of functional components. Conversely, animal products like milk, fermented milk products, and freshwater fish include antioxidants, covalently linked linolenic acid, long-chain omega-3, −6, and − 9 polyunsaturated fatty acids and organic acids [17]. **Table 2** lists several food ingredients, their biological roles and their typical origins.

4. Functional component

4.1 Fibers

Even though dietary fiber is not a "nutrient," it is an essential substance of our foods. Fiber is beneficial because it moves through the body without being digest. Dietary fiber is made up of polysaccharides, its present in soluble and insoluble form in different foods. Pectin, mucilages and beta-glucan soluble whereas lignin,

Component	Sources	Biological role	References
Flavones	Fruits and vegetables	Reducing cancer risk by neutralizing free radicals	[18]
Catechins	Tea	Tea Catechins improve diabetes, obesity and cardiovascular diseases	[19]
Anthocyanidins	Fruits	Due to antimicrobial and antioxidative ability anthocyanidins aid in neurological and non-communicable diseases.	[20]
Flavanones	Citrus fruit	It aids to prevent chronic diseases and Alzheimer's disease	[21, 22]
Lignans	vegetables, Flax seeds and rye	Cancer prevention and kidney cure	[23, 24]
Tannins	Fruits, vegetable, legume seeds, cereal grains and nuts	Reduce the risk of cardiovascular disease and improve urinary tract health	[25, 26]
Lutein	Green vegetable	Reduce the chances of developing muscle degeneration and improve visual problem	[27, 28]
Alpha-carotene and Beta-carotene	Fruits and vegetables	Free radicals can neutralize which can inflict cell damage	[29]
Lycopene	Tomato	Reduce the chances of developing prostate cancer	[30, 31]
Quercetin	Onion, red grapes, cherries and citrus fruits	Improve liver function and prevent obesity	[32]
Total phenolic content	Blueberries, and pomegranate	Reduce risk of cardiovascular diseases and improve neuron cells	[33, 34]
Carotenoid	Kiwifruit and kale	Aid in chronic deceases	[35]

Table 2.
Components, sources and biological role of functional food.

cellulose and hemicellulose insoluble are present in various plant foods [36]. These components are not digested or absorbed in the small intestine and passes into the large intestine in an intact form and is immune to enzymatic digestion. Fibers are graded as soluble or insoluble based on their solubility. Fiber fortification also helps dairy foods enhance their sensory qualities, shelf life, and structural properties [37].

The previous indications evaluated that fiber plays a functional role in the human body against various diseases. Because fiber is the non-digestible component that passes through the small intestine to the large intestine without absorbing. In the small intestine nutrients like fat and glucose are absorbed, however, fiber attracts extra fat and glucose, and pushes them to the large intestine, where stool is prepared. Moreover, extra fat and glucose are made part of the stool. However, in this way fiber prevents extra glucose and fat into the blood that's aids in inhibiting the chances of diabetes and CVD disease [37, 38].

4.2 Vitamins

Food fortification with essential micronutrients including vitamins A, D, and E improves human health by ensuring sufficient intake to avoid diseases (such as osteoporosis, osteoarthritis, a suppressed immune system, cancer, vision loss and

heart disease) and enhances life quality and survival. Malnutrition statistics in many populations, especially in developing countries, have increased the need for food fortification with lipophilic vitamins A, D and E. Foods usually contain these vitamins, but in inadequate quantities, and human body absorption is insufficient to achieve the optimal level of health benefits [39].

Some vitamins including A, C and E act as antioxidants. During processing, the oxidation process is started. These vitamins inhibit the oxidation process due to antioxidants activity. When these vitamins enrich foods are consumed then that's can help in different chronic diseases [40].

Around 2 billion people worldwide are believed to be deficient in one or more micronutrients, placing them at risk of death, illness, or disability. Food fortification and supplementation (i.e., the availability of nutrients in a form other than food) are both effective methods for ensuring adequate daily intakes of minerals and vitamins. A good vitamin or mineral compound for food fortification should have two main characteristics including high bioavailability and no reaction with the food matrix [41].

4.3 Minerals

The minerals have many roles and potentials in metabolism and homeostasis, mineral deficiency can cause a variety of common disorders and disease symptoms. Mineral absorption and bioavailability can be significantly improved by ensuring mineral content details in terms of safe food fortification and processing methods. The most popular minerals used to fortify various food preparations are iron, calcium, zinc, and iodine. The bioavailability of food minerals can be accurately measured using isotope ratio methods. Modern processing techniques have less detrimental effects on the consistency of micro-and macro minerals than conventional process parameters [42].

Food fortification and supplementation are the most cost-effective ways to combat global mineral malnutrition. Because of political, social, infrastructure-related, and technical constraints, most interventions to improve mineral nutrition have been less effective in most developing countries. The most potential solution has been salt iodization (iodine fortification), which has substantially decreased the incidence of goiter and other IDD symptoms in areas where it has been introduced [43].. Selenium (Se) is an effective nutritious antioxidant that exerts natural effects by incorporating it into selenoproteins. Since selenoproteins play a vital role in the regulation of reactive oxygen species (ROS) and redox states in practically all tissues. The selenium "improving" the immune system maintained by investigation on aging immunity [44]. Zinc is an important trace mineral and plays a vital role in many physiological functions. Zinc is essential for the growth and function of immune cells in the innate and adaptive immune system. Zinc homeostasis is well controlled in all cells, and any stress-free regulation will result in compromised normal function. In several disease models, such as infections, allergies, autoimmune diseases and cancer, the significances of homeostasis disorders can be detected [45].

4.4 Antioxidants

Antioxidants are substances that aid in prevent oxidation. Fruits, vegetables, nuts, cereals and beans contained different types of antioxidants. These substances are used as functional ingredients in many foods and food products [46]. As a functional ingredient, antioxidants help to protect from many diseases. In the human body, these functional ingredients played a vital role to protect from chronic diseases such as cancer and cardiovascular diseases [47].

5. Functional food categories

Consumer demands must be taken into account when designing functional foods, which can be found in nearly all food categories. Functional foods have been produced in almost every food category, but their distribution across consumer segments is not standardized, and product preferences different prebiotics, probiotics, symbiotic foods, isoflavones, phytosterols, anthocyanins, fat-reduced foods, sugar-reduced foods, and antioxidants are some of the functional food groups [48].

Functional foods have been introduced mainly in the dairy, confectionery, soft drinks, bakery, and baby-food industries, among other food markets. Vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron, and calcium are used to preparation of fortified food. Following that, the emphasis turned to foods fortified with various micronutrients, such as omega-3 fatty acids, phytosterol, and soluble fiber, to encourage good health and avoid diseases such as cancer [49]. Due to the wide range of health benefits, the producers of food have divided functional food into two groups including Conventional food and modified food that shown in **Figure 1**. Taken a stand proactive actions to produce foods that provide multiple health benefits in a single serving.

5.1 Conventional food

Foods that are composed of natural or whole-food ingredients that provide functional substances such as heart-healthy fatty acids, antioxidants, vitamins and minerals are called conventional functional food. A safe and organic diet includes more bioactive compounds and less harmful substances including persistent toxins, their metabolites, pesticides, and fertilizers. Organic food helps to maintain a balanced lifestyle and reduces the risk of developing. In the supply chain, the Purpose of these foods is aid to in ensuring the nutritional quality of particular foods or products [50].

Organic foods, rather than traditional foods, have become increasingly popular in recent years. Fruits (dry and fresh), vegetables (leafy green and non-leafy), nuts, seeds, legumes, herbs and spices are the conventional functional foods as show in **Figure 1**. Conventional functional foods contain bioactive substances that beneficial effects on health [51].

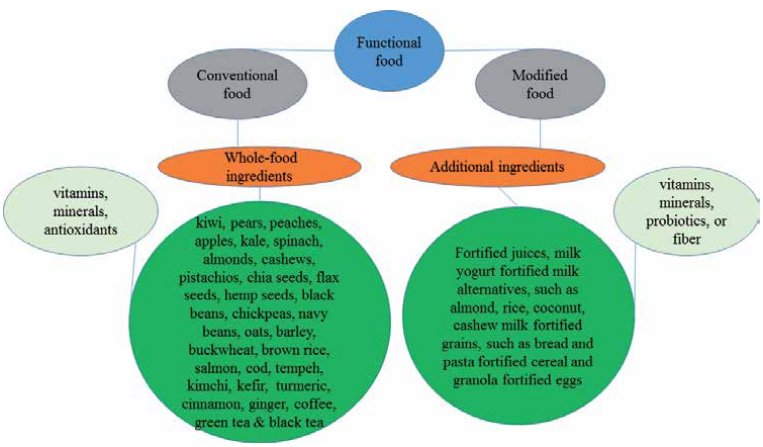

Figure 1.
Conventional food and modified food are forms of functional food. It is present in food products natural (whole-food ingredients) and additional (additional ingredients). The vitamins, minerals, fiber and antioxidants are functional components present in different functional foods and food products.

5.2 Modified food

Food or food products in which add additional ingredients (vitamins, minerals, probiotics and fiber) for specific health purposes are called modified foods. Fortified food products such as fortified juices, dairy products (milk and yogurt), fortified milk alternatives (almond, rice, coconut, and cashew milk), fortified grains (bread and pasta), fortified cereal and granola and fortified eggs are modified functional food products that show in **Figure 1**. The progress of modern biotechnological achievements has culminated in genetic modification or genetic engineering in the form of genetically modified plants and genetic modification, and eventually the outcomes in the form of genetically modified food. Which has the potential to end world hunger, poverty and malnutrition [52].

5.3 Fortified food

"Food fortification" refers to the addition of nutrients at levels greater than those found in the original food. Enrichment is associated with fortification. The term "restoration" refers to the addition of nutrients to food to compensate for the nutrients lost during processing. Food fortification is usually done at the industrial level, but it can also be done at the household or group level [53].

Nutritional fortification is one of three approaches used to resolve public health problems of micronutrient deficiency. When designing a micronutrient fortification program, a variety of factors must be taken into account. Trends in global micronutrient deficiency, dietary patterns, production and marketing of potential food vehicles, government policies, and regulations are all factors to consider. A five-step procedure, which can be visualized as a linked loop, is usually followed when constructing a fortification intervention (**Figure 2**). The measures can be performed in any order or even simultaneously. The cycle will begin at a different stage depending on what relevant information is already available in the region or country [54].

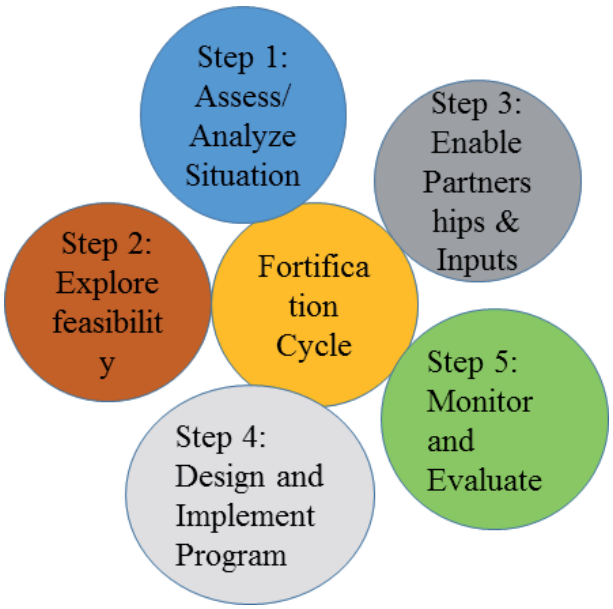

Figure 2.
Fortification cycle.

Although fortification has the potential to be a successful strategy, there is little evidence of its effectiveness in the developing world. Programs must monitor the direct impact of fortification on morbidity and mortality. According to the World Health Organization (WHO), more than 2 billion people are deficient in key vitamins and minerals, especially vitamin A, iodine, iron, and zinc. The majority of these individuals are from developing countries, where multiple micronutrient deficiencies (MMN) are normal [53].

6. Functional food development

From definition to effective consumer execution, functional food production includes several distinct phases. Consumers enthusiastically support food products with health claims attesting to a practical capacity to promote health beyond the provision of essential nutrients, which is likely to result in lower morbidity and mortality, as well as the improved overall quality of life in the general population [55].

The FDA's approval of qualifying health claims for many ingredients when used in specific quantities has aided the growth of the functional food industry and raised consumer awareness of nutraceutical ingredients like omega-3 fatty acids, dietary fiber, plant sterols, and soy protein. In the last decade, the number of functional food product releases with unique target health categories has increased. Gut health, cardiac health, immune function, bone health, and weight control have all been the subject of functional health statements [56].

For a successful product development process, a thorough understanding of the fundamental science of the heart, as well as a thorough understanding of the materials and processes available, is required. To create a cost-effective and tailor-made suitable for its intended use (final food product), the final product shape (liquid or dry) and demand (size and value) must be defined at the outset. These variables may have a huge impact on the products, formulations, and methods available. This is the time to verify physical performance and characteristics, core stability, and possible interactions with other ingredients during the formulation and operation [57].

Functional foods have been developed in almost every food group. From the standpoint of a commodity, the functional property can be used in a variety of ways. According to a different classification, certain functional products "bring good to your life," such as improving normal stomach and colon functions (pre-and probiotics), or "improve children's lives," such as promoting their cognitive ability and providing a conducive learning environment. On the other hand, finding good biomarkers for cognitive, behavioral, and psychological functions is challenging. Another form of functional food is designed to assist people in managing a pre-existing health condition, such as high cholesterol or high blood pressure. The third group includes items that "make your life easier" (for example, lactose-free and gluten-free products) [48].

The creation of indicators that could validate the effects of modern food ingredients and could be used during their protection evaluation is enabled by scientific knowledge of how particular Food ingredients have an influence on body processes that affect well-being and health [58]. The production of functional ingredients using cutting-edge food science and biochemical approaches will benefit consumers by improving their health and lowering their disease risk. These trials must be properly designed and carried out in order to provide empirical justification for the acceptance of health assurances and the effective modification of existing functional foods. The advancement of functional foods and their associated health

benefits will be accompanied by advancements in food security, which will ensure the legitimacy of the claims and also the food's protection. Technology cannot be governed in and of itself, and practical food science only serves as the systematic foundation for these legislation [59].

A food that is useful may be a normal food, one with which a diet element has been inserted or one through which a part has been omitted for particular health purposes, one in which a material has been changed by technical or biochemical processes to have a particular health benefit, one where the bioactivity of a product has been changed, or a mixture of all of these [60].

While the words "nutraceutical" and "functional food" are widely used across the globe, there is no general agreement about what they mean. The following concepts have been suggested by the Bureau of Nutritional Sciences of Health Canada's Food Directorate: A functional food resembles or is related to a traditional food, is eaten as part of a daily diet, and has been found to have metabolic advantages and/or lower the risk of developing chronic diseases beyond specific dietary functions. Nutraceuticals are foods that have been found to provide biochemical effects that offer protection against a chronic illness that are marketed in powders, tablets, and other medical products not typically associated with fruit. A nutraceutical, a pharma food, or a nutritional supplement are obviously not the same thing as a functional meal. It is a substance, not a drug, and it has health benefits that are typically disease prevention rather than medicinal [61].

7. Conclusion

It is concluded that functional food prepared by a conventional or modified procedure, has a functional role in human health. Some natural foods like plant-based food including fruits, vegetable, nuts, herbs, cereals and beans, seafood for example fish, and dairy product like milk are rich sources of vitamins, minerals, antioxidants, fiber, phenolic compound and omega-3 fatty acid, these all components have kept the specific functional role in human health against diseases. Conclusively statement shows that functional foods are designed to improve health and chronic diseases.

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Chapter 2

Why Produce Food-Bioactive Compounds to Generate Functional Grade Foods?

Marina Marsanasco and Silvia del Valle Alonso

Abstract

Functional foods are those with health benefits but cannot incorporate and protect from oxidation or deterioration, maintaining the bioactive compounds (BC) activity. The liposomes have several advantages for BC encapsulation: ease of obtention, characterization, scaling-up, lipid protection for hydrophilic and lipophilic BC, and best, they are made with natural lipids of alimentary grade. In our studies, liposomes were made of soy phosphatidylcholine (SPC) with Stearic Acid or Calcium Stearate as membrane stabilizer. They encapsulated BC as vitamin E, vitamin C and folic acid (B9). The liposome's design strategy is that SPC lipid's components are BC like choline and essential fatty acids. These liposomes preserved and maintain the activity of the thermolabile vitamins C and B9. Like milk and fruit juice, in various food types can incorporate liposomes protecting BC. A series of laboratory studies will be performed to select the most stable liposomal formulations, like characterization, encapsulation efficiency, physicochemical, microbiological, thermal and sensory stability. Liposomes- BC design and development are discussed in the chapter. The food heat treatment and the conditions/storage time are also crucial and must be considered in these studies. Finally, incorporating the BC into a food production line is feasible with an excellent economic prospect until supermarket shelves are reached, like our food product proposal.

Keywords: bioactive compounds, liposomes, nutrition, stability, industry

1. Introduction

Functional foods are those with health benefits that must demonstrate that: a) they have a beneficial effect on one or more specific functions of the organism, beyond their usual nutritional effect, b) they improve the state of health and well-being, and c) they reduce disease risk [1]. Functional food must contain functional ingredients or bioactive compounds (BC), which are natural constituents that are generally found in small amounts in food. These compounds provide health benefits beyond the essential nutritional value of the product and for that reason, they are intensively studied to evaluate their effects on human health [2, 3].

BC include flavonoids, phytoestrogens, isoflavones, resveratrol, lycopene, organosulfur compounds, soluble dietary fibers, and isothiocyanates monoterpenes, plant sterols, olive oil. Among the BC of hydrophobic nature are carotenoids, tocopherols, flavonoids, polyphenols, and phytosterols stand out [3].

Regarding the importance of particular BC is the folic acid (FA) or vitamin B9, which belongs to the folates family and act as cofactors in carbon transfer reactions (formyl, hydroxymethyl, and methyl) in nucleotide biosynthesis (purine and pyrimidine bases), amino acid metabolism (methionine, histidine) and metabolism neurotransmitters (serine, choline) [4]. Animals and humans cannot synthesize folates. Thus, it is necessary to incorporate them into the diet from plant sources or food FA fortified [5].

The consumption of FA is critical since the deficiency of this vitamin is related to neural tube defects, coronary heart disease, and megaloblastic anemia, similar to that generated with the deficiency of vitamin B12, which occurs more frequently in pregnancy [5–7].

Scientific studies link low folate intake with neurocognitive dysfunctions. Folates play an essential role in developing the central nervous system and in the metabolism of some neurotransmitters; low folate concentrations may also be related to dementia and decreased cognitive function [6].

Various studies explain that FA is being used as a potential agent for preventing cancer and reducing the risk of heart disease [8, 9]. Epidemiological studies have shown that folate supplements can significantly reduce pancreatic cancer and breast cancer [10]. Besides, some studies link the intake of FA with a decrease in colon cancer and neurological diseases such as Alzheimer's [11]. The protective effect of FA on cardiovascular diseases, hematological and neurological diseases, and cancer has been associated with the antioxidant activity of this vitamin [12].

Another important BC is vitamin E (VE), also called α-tocopherol [13]. There are also foods such as eggs, seeds, nuts, and whole grains, which also contribute to VE's daily intake [5].

VE is the main fat-soluble antioxidant in the body. Its action is based on capturing peroxide radicals produced in cells by oxidative metabolism [7, 14]. Hence, it prevents the formation of hydroperoxides, delaying the initial phase of the oxidative process [13, 15]. This action protects cell membranes and other lipids from severe alterations produced by peroxidation [7, 14]. Various clinical studies also describe the beneficial effects of VE, alone or in combination with other vitamins, in some types of tumors such as prostate, gastric and lung. This fact is based on experimental studies that show the role of free radicals as a critical factor associated with the development of cancer. It is precisely the effectiveness of antioxidants from the diet such as tocopherols that have an essential role in the prevention of the development and progression of this disease [16].

Another group of BC are polyunsaturated fatty acids (PUFAs). There are two families of PUFAs: the n-6 family and the n-3 family. The PUFAs n-6 family is derived from linoleic acid, with two double bonds, and is characterized by having its first double bond at the carbon chain number 6, counting from the methyl at the end of the chain [17]. The n-3 PUFAs family derives from α-linolenic acid (with three double bonds), whose fatty acids have a first double bond at carbon number 3 in the chain. Besides being a source of energy, the PUFAs n-6 and n-3 families incorporate into cell membranes. They are precursors of eicosanoids like prostaglandins, prostacyclins, thromboxanes, and leukotrienes, that intervene in several physiological processes, like blood clotting or inflammatory and immune responses [17].

It is essential to highlight the contribution benefits of essential fatty acids. Man needs these fatty acids for normal functioning but must ingest them since body cannot produce them. That is why they are called essential [5]. Both linoleic and α-linolenic are essential fatty acids that cannot be synthesized by the body. Therefore, both fatty acids must be provided in the diet [17]. Research published has established that these fatty acids are very beneficial in the prevention of

cardiovascular diseases [18–20], schizophrenia [21], and cancer [22], among others. Also, they have vasodilator, antihypertensive, anti-inflammatory, and anti-atherothrombotic properties [23].

The problem is that many of these BC are not consumed in the necessary amounts for the body's normal functioning. According to the recent 2015–2020 Dietary Guidelines for Americans, VE and choline consumption is below the daily requirements, so it must be compensated with the intake of supplements in 50% of adults [24].

2. Developing formulations with bioactive compounds

The BC amount that must be added to food and its nutritional values detected are useful in preventing certain diseases. Then there is a need to study and bring to the industry the incorporation of matrices/carriers that permit BC in food. Various types of matrices in the food industry, like liposomes, nanoemulsions, microemulsions, solid lipid nanoparticles, and polymeric micro and nanoparticles have been studied for BC encapsulation [25]. A whole new market has even been generated related to nano-foods associated to nanotechnological techniques or tools, or to which manufactured nanomaterials should be added, either during their starting point or production processing or packaging [26].

Liposomes are considered within an emerging trend in the market called nano-feeding. They offer a series of competitive advantages compared to other matrices. For example, their production on a larger scale is of good feasibility. They are easy to obtain and scale-up, which allows the incorporation of BC compounds in this type of matrices in food production lines. Besides, their characterization and physicochemical, microbiological, and sensorial stability can be studied with different techniques. Also, liposomes components are low cost, and natural food can be easily obtained [25, 27].

Liposomes are microscopic spherical vesicles, formed by lipids that enclose liquid compartments in their structure [28], allowing the encapsulation of molecules, whether they are liposoluble or water-soluble BC [29, 30].

The liposomes can be formulated with phospholipids, which are polar lipids characterized by having hydrophilic and lipophilic groups on the same molecule [30]. These spherical vesicles are formed under certain conditions. After flash evaporating solvent, phospholipids are hydrated and organized into lipid bilayers. These lipid bilayers called lamellae, unite to form the phospholipid sphere that encloses the water [31]. Liposomes can have one or multiple concentric lamellae called a vesicle or multilamellar liposome [30].

These types of matrices have broad applications in the industry to transport BC or other types of compounds. For example, in accelerating cheese ripening, the vesicles offer a uniform distribution of hydrophilic enzymes [30]. In the encapsulation of flavorings, acidulants (citric acid, ascorbic acid, buffer, and alkalis), antioxidants, colorants, essential oils, vitamins, and minerals. Furthermore, these systems are used to encapsulate lactoferrin, a bacteriostatic glycoprotein, and nisin z, an antimicrobial polypeptide, to increase dairy products' shelf life. Liposomal systems are also used to trap Phosvitin (antioxidant), which inhibits lipid oxidation in various dairy products and ground pork. Besides, they are used to capture antioxidants like vitamin C, maintaining 50% of activity after 50 days in refrigerated storage and non-encapsulated vitamin C, which loses its activity after 19 days [32].

In this way, liposomes can encapsulate all kinds of BC. Besides, specific BC can be part of the carrier itself if a strategy is applied in these matrices' design.

To implement liposomes with BC in the food industry, the research and development of these matrices should be deepened to ensure the compounds' stability to be encapsulated and incorporated into food. It is essential to mention that for an industrial application, membrane stability and structure are important factors when designing liposomes [30], and must always ensure that they are food grade [33].

In our research line, we sought to incorporate saturated fatty acids that can act as membrane stabilizers and intervene against lipid oxidation processes [33]. Based on the research carried out by Hsieh and collaborators [28], stearic acid (SA) is an excellent alternative to prepare stable liposomes, structural benefits, and increase the efficiency of liposomal encapsulation, as well as oxidative stability. SA is an 18-carbon saturated fatty acid, insoluble in water, so it is located between the hydrophobic chains of the fatty acids in the bilayer. The authors carried out studies on liposomes formed with egg phosphatidylcholine (EPC) and SA in the molar ratio of 1: 0.25. The problem was that EPC has a much higher cost than soy phosphatidylcholine (SPC) in Argentina since EPC is a specific raw material obtained in the bench lab at a laboratory scale.

In comparison, SPC is a lipid product of the country's intense soy farming activity. It is a raw material that is easily obtained at a large scale and has a low cost. Therefore, SPC with SA was used in the molar ratio 1:0.25. A second strategy is developing liposomal formulations using the SPC base system and incorporating calcium stearate (CaS), with the double benefit that CaS can act as a possible stabilizer of the liposomal bilayer. CaS also incorporates a mineral such as calcium that increases the nutritional value [27, 29, 33, 34]. CaS is a salt composed of two 18-carbon saturated fatty acids linked to a calcium cation. The concentration used was the same as in SPC and SA. In this way, the possible effects of the stability provided to the liposomal bilayer incorporating saturated fatty acids are preserved, and at the same time, extra calcium is added to the formulation.

The formulations proposed to obtain liposomes that encapsulate and protect BC were the following [27, 29, 33, 34]:

- SPC.
- SPC:SA in a mol/mol ratio of 1: 0.25.
- SPC:CaS in a mol/mol ratio of 1: 0.25.

Besides, SPC is a natural lipid that generate the liposome's transporter, has essential fatty acids such as linoleic acid (omega-3) and linolenic acid (omega-6).

Table 1 shows the percentage composition of fatty acids in SPC and EPC considered essential fatty acids. Furthermore, SPC is also the source of choline, an essential nutrient needed to synthesize neurotransmitters (acetylcholine). It plays an essential role in the fetus's brain and memory development, and some researchers have indicated that choline and methionine intake may be necessary for reducing the risk of neural tube defects [35].

Multilamellar liposomes were prepared by the dehydration–rehydration method [36]. Briefly, 40 μmol of lipids were dissolved in 500 μL ethanol in a round bottom flask, and the solvent was dried in a rotary evaporator at 37 °C. Dry lipid film composed of SPC, SPC:SA (1:0.25, mol ratio), or SPC:CaS (1:0.25, mol ratio) was rehydrated with 2 mL distilled water to a final 50 mM lipid concentration.

To prepare liposomes with VE, a stock solution of this vitamin diluted in ethanol was prepared. Stock concentration was 22.4 mM. Then, 0.445 mL of this stock was mixed with a proper amount of lipids. The solvent was evaporated until the lipid film was obtained. Any liposoluble BC to be incorporated into the liposome

Fatty acids	% Fatty acids in SPC	% Fatty acids in EPC
Myristic acid 14:0	—	0.2
Palmitic acid 16:0	14.9	32.7
Sapienic acid 16:1	—	1.1
Stearic acid 18:0	3.7	12.3
Oleic acid 18:1	11.4	32.0
Linoleic acid 18:2*	63	17.1
Linolenic acid 18:3*	5.7	—
Eicosatetraenoic acid 20:4*	—	2.7
Docosahexaenoic acid 22:6*	—	0.4

Table 1.
*Composition of fatty acids in SPC and EPC (*essential fatty acids).*

must always be done in this step. Moreover, it is essential to use only ethanol to dissolve lipids since it is a solvent that is approved as an additive at a national and international level of the Food Committee with concentrations (possible trace) that do not exceed the maximum permitted [37–39]. When the film was rehydrated in 2 mL of distilled water, a final concentration of 5 mM was reached, and this is the step to incorporate the hydrosoluble BC's. In the case of FA, recently prepared solutions of this vitamin needed when the experiment is on the rehydration step. FA was weighed and diluted with distilled water to reach a 0.136 mM concentration.

Samples were prepared with the primary goal of fortifying food with the mentioned vitamins. According to Argentina regulations [40], the percentage of recommended daily intake (RDI) in a portion of fortified food must be between 20% and 50% for fat-soluble vitamins and between 20% and 100% for hydro-soluble vitamins. The RDI of VE is 10 mg and for FA is 400 μg. In order to fortify aqueous food like chocolate milk, regular milk, or juice, 2 mL of liposome suspension (50 mM) with vitamins was added to each serving of food (200 mL), which implies that it was fortified with 4.3 mg of VE (5 mM) equal to 43% of the RDI and 120 μg of FA (0.136 mM) equivalent to 30% of the RDI. Thus, 1 L of aqueous food will contain, for example, liposomes of SPC:CaS and BC in the proportion seen in **Table 2**.

Liposomal formulation SPC: CaS (mol / mol ratio of 1: 0.25) with 5 mM VE and 0.136 mM of FA	Mass of component
SPC	303.20 mg
Choline from SPC	41.66 mg
Linoleic acid from SPC	191.05 mg
Linolenic acid from SPC	17.30 mg
CaS	60.70 mg
Calcium from CaS	4.00 mg
VE	21.50 mg
FA	600 μg

Table 2.
Composition of SP:CaS liposomal formulation as carries of BC: VE, FA, choline and essential fatty acids in 1 L of product for example chocolate milk [27, 29, 33, 34].

The design, research, and development of carriers for BC, including all aspects, like the final quality of the food product and its feasibility, have to be considered until reaching the consumer's market. Thus, the liposomal formulation must be stable from a physicochemical, microbiological, and sensory perspective. The interaction of these matrices with BC must ensure their stability and protection until consumption. This data is not minor given that in the food industry, a series of treatments, usually thermal, must be applied to ensure the useful shelf life and safety of the product.

This fact presents a challenge in the development and research of foods with BC because many of these degrade or lose activity before reaching the industry's scale up regular treatments. VE is heat stable but oxidizes quickly in the air, with consequent loss of vitamin activity, especially in the presence of ferric ions and other metals [13]. Furthermore, VE is destroyed by exposure to UV light and to a great extent, during oil refining process [7]. Also, concerning food storage, during the storage of plant foods, VE has a weak antioxidant character, being much more active against animal fats, especially in the presence of synergistic substances [13]. On the other hand, FA is stable to alkalis under anaerobic conditions. However, under aerobic alkaline conditions, its hydrolysis occurs, separating the side chain and yielding glutamic acid, and pterin-6-carboxylic acid. Acid hydrolysis under aerobic conditions yields 6-methylpterin [41]. For this reason, to favor the stability of the FA, it must remain at a pH close to neutrality [42]. None of the degradation compounds mentioned shows biological activity; therefore, during the formulation of pharmaceuticals, nutraceuticals, or foods enriched with FA, it is necessary to protect this vitamin against environmental factors such as extreme light and pH [7, 43]. Specifically, in acidic media, it was shown that FA is unstable [44]. Furthermore, FA solutions decompose when exposed to light, forming glutamic acid, and pterin-6-carboxylic acid [41].

The milk pasteurization by the high temperature and short time method (2–3 seconds, 92 ºC) causes a loss of around 12% of total folates, and the loss caused by boiling the milk for 2–3 minutes is in the order of 17%. Sterilization of milk in bottles (13–15 minutes at 119–120 ºC) is the treatment that causes the most significant losses, about 39% [41].

Based on those aspects mentioned above of unfavorable conditions, if the objective is to add BC such as FA and VE, the liposomes must have oxidative stability not to affect the vitamin antioxidant activity, and they must protect the FA from the applied food heat treatments. Also, the liposomes can be food-incorporated at a neutral pH due to FA's stability.

The application of advanced liposomal formulation has shown that they can be applied in pH foods such as chocolate milk and orange juice. They demonstrate that liposomes can protect thermolabile vitamins such as vitamin C and FA [29, 33]). Other authors demonstrated that the capture of antioxidants like vitamin C in liposomes maintains 50% of activity after 50 days in refrigerated storage, and non-encapsulated vitamin C loses its activity after 19 days [32].

It should also be mentioned that the liposomal formulations of our research line presented values of oxidative stability under quality food parameters [45]. According to what is established by the authors [45], for a food to have a good quality, it must have an oxidative value below 0.2 mg of malondialdehyde (MDA) per Kg of food. In SPC, SPC:SA and SPC:CaS with VE-FA after pasteurization thiobarbituric acid reactive species (TBARS) value were 0.2380 ± 0.0248 μM, 0.2017 ± 0.0645 μM, 0.1816 ± 0.0581 μM, respectively. The results are shown as the mean ± SD of three independent assays; as published in Marsanasco and collaborators [29]. Taking as a reference the average value of SPC of 0.2380 μM that was the highest of the three formulations, if the transition from μM of TBAR to mg of

MDA/Kg of food is performed, it gives a value of 0.0166 mg per 1 Kg of chocolate milk (density was 1,033 Kg/L). Thus, is below 0.2 mg of MDA/Kg established by the authors, complying with the excellent quality parameter.

3. Incorporating bioactive compounds into different types of food

Liposomes have the particularity that they require an aqueous medium for their stability [30]. Based on the above, it is essential to consider that the choice of food to incorporate the liposomes with the BC must have a high water-activity (a_w) to maintain the liposomal formulations' stability. After considering this factor, the second thing is how the functional food to be produced will be positioned in the market. Alternatively, may be the product production has a social focus related to the country's nutritional deficiencies.

Our line research, based on the social requirements of our country and the market niche that we want to cover, the incorporation of liposomal formulations with BC in the following types of food was proposed:

- Milk/flavored milk.
- Fruit juices.

Thus, chocolate milk fortified with VE and FA was implemented with essential omega-3 and omega-6 FAs and choline (from the SPC-based liposome). Besides, when using the liposomal formulation SPC:CaS, calcium is added to the product. This type of food will provide energy and nutritional contribution to the consumer.

This research was complemented by studying the same liposomal formulations but with 5 mM of VE and 50 mM of vitamin C (VC).

In all cases, the focus of incorporating vitamins was towards the food fortification with the incorporation of these vitamins that are considered as BC.

The objective will be to offer a massive product such as milk, with an essential nutritional addition, in response to a growing demand for products with high nutritional value and nutritional deficiencies in the country's specific sectors.

Another type of food prepared with the addition of liposomal formulations-BC was orange juice. In this case, FA could not be incorporated; as explained in the previous section, considering it is not stable at extreme pHs. In this case, liposomal formulations were used with VE and VC in the concentrations already mentioned.

The liposomes in the food were incorporated in the volumetric ratio 1/100. Furthermore, they did not modify the base food's density, pH, or visual characteristics [27, 29, 33].

Once the essential foods have been established, the first phase of the study in the research and development of these functional foods is the characterization and physicochemical stability on a laboratory scale of the liposomal formulations with BC. At this stage, the following parameters should be studied: size, shape, electrical charge, encapsulation efficiency, oxidative stability, packing and lipid membrane stability, and rheological behavior of liposomal formulations with and without the addition of vitamins. Also, the heat treatment of the selected food must be considered, in our research line LTLT process was selected. LTLT is low temperature and longtime pasteurization process, that is 65 °C for 30 minutes applied to milk and juices.

There is a question of whether all liposomal studies with BC can successfully fortify the base food. Food has components that can interfere with analytical

determinations. It is for this reason that in this study stage, food simulants were used. Milk and flavored milk are found within foods with a pH > 5. Therefore, the food simulant distilled water was used instead of milk, while fruit juices are within pH <5, so the food simulant of 3% m/v acetic acid was employed [46].

Among the studies to be carried out at this stage, transmission electron microscopy is one of the most suitable methodologies to obtain information on the morphology of liposomes [47]. This technique must be complemented with optical microscopies that allow analyzing the shape and distribution of the liposomes, respectively. In the formulations developed, explicitly mentioning those with VE-FA, the liposomes presented a structure in which an outermost zone and a central nucleus were differentiated (**Figure 1**). They also presented a variety of sizes with spherical shapes and non-spherical related to the method of preparation and composition of liposomes. Moreover, isolated and aggregated liposomes were also observed [29, 33]. Data discussed coincide with those obtained by other authors [48], where the unilamellar liposomes of egg Lecithin at pH 7.2 showed liposomal aggregation. Nacka and collaborators [49] also demonstrated liposome aggregation of mainly phosphatidylcholine and phosphatidylethanolamine at various pH. In liposomal systems, aggregation is a physicochemical mechanism that can occur under certain conditions influenced by pH, heat treatment, external load, and cations' presence, among others [29, 33, 49].

It is important to mention the surface and viscosity behavior study of the matrices that will encapsulate the BC. The matrix that encapsulates the BC has a behavior like that of the product. It represents a great advantage to apply to the food industry, if visualizing an industrial-scale production. The size and shape of the liposomes with the BC are related to the final product's stability. But there is also another aspect that is related to the composition, shape, morphology distribution, tendency of aggregation, and membrane packing of the liposomal formulation with BC, and that is the rheological behavior and viscosity that they will contribute to the food product [29, 34]. Newtonian behavior occurs in almost all common liquids such as water, milk, orange juice, apple juice, and corn syrup. Furthermore, the pseudoplastic behavior is also present in common foods such as sauces and orange juice concentrate [50, 51].

For example, liposomal formulations SPC, SPC:SA, all with VE-FA and pasteurized, presented a behavior similar to that of a Newtonian fluid. While the SPC:CaS formulation with VE-FA pasteurized presented a behavior with a tendency towards a pseudoplastic fluid. This result is related to various factors as the membrane packing with the viscosity of formulation and rheology. According to the results obtained by other authors [52], a greater rigidity of the membrane of the phosphatidylcholine L-α-dipalmitoyl liposomes increased in viscosity. In the system with CaS, FA's addition would favor the association of two adjacent phosphatidylcholines with the calcium cation. It must be considered that FA (pka1 = 2.3) was shown to decrease the pH of distilled water from 6.0 to 3.88 by releasing the protons to the medium. Moreover, the

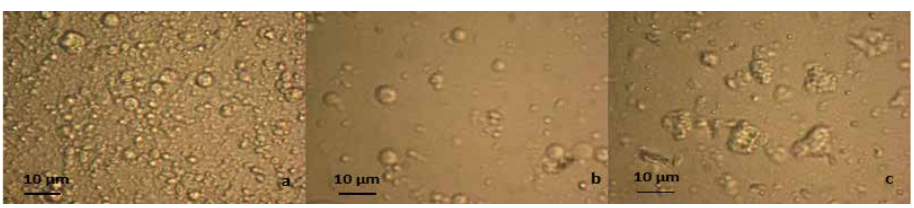

Figure 1.
Light micrographs of the liposomal system with 5 mM of VE and 0.136 mM of FA in distilled after pasteurization: a) SPC; b) SPC:SA, c) SPC:CaS.

low pH favors the dissociation of calcium from CaS, which generates bonds between calcium cations and two adjacent phosphatidylcholines [53, 54]. This greater stiffness of the membrane would also explain the pseudoplastic behavior of the SPC: CaS system [29]. Newtonian or pseudoplastic behavior in liposomal formulations with BC represents an advantage if, in the future, it is desired to apply liposomes on an industrial scale, especially the foods to be fortified, such as milk and orange juice.

Other studies carried out are the membrane packaging and its behavior, for this type of study; techniques are implemented that allow detecting the hydrophobicity factor (FH). This factor determines the degree of hydrophobic sites exposed at interface level and the possibility that the probe used, which is merocyanine MC540, is inserted into the bilayer. For this reason, the higher the value of this parameter, the higher the number of membrane surface defects [29].

In liposomes obtained, specifically those with VE and FA, considering that MC540 is slightly located above the domain of the main glycerol chain of phospholipids [55]. It can be inferred that the addition of vitamins favored membrane fluidity (specifically in the phospholipid polar zone) with the consequent greater probe entry and a greater number of defects in a said membrane appearance (**Table 3**).

Regarding VE, the result would be related to the effect of this vitamin that produces a general increase in the mobility of the headgroup in the lipid bilayer [56], which would favor the entry of the probe MC540 [57]. In the case of the SPC:SA (1: 0.25 molar ratio) or SPC: CaS (1: 0.25 molar ratio) system, the polar part of the fatty acids alternates with 21% SA or CaS, respectively. As the amount of saturated fatty acids increases, it is expected a membrane stiffness increment and fewer probes entering. But considering the membrane, the MC540 location; the addition of the SA or CaS could be favoring their income. In the case of SPC:SA, a possible explanation would be related to the fact that the polar heads of phospholipids such as phosphatidylcholine bind to water molecules through hydrogen bonds, and the polar heads of fatty acids such as SA also interacting with water molecules [58]. Although SA is an anion (as occurs in the pH of distilled water), it can also affect the hydrogen bonds with water [59]. The greater the hydration and formation of hydrogen bonds in the polar heads, the greater the water's penetration, which promotes the formation of more fluid domains [60]. In the case of the SPC:CaS system, the presence of FA (pka1 = 2.3) and the pH descent in distilled water with the consequence of the release of calcium ions from CaS and the association with close next phosphatidylcholines, increase the surface fluidity of the membrane and favors the entry of the probe [29].

Liposomal formulation	**HF**
SPC	2.02 ± 0.05
SPC VE-FA	2.61 ± 0.06***
SPC:SA	3.19 ± 0.02
SPC:SA VE-FA	5.310 ± 0.13***
SPC:CaS	5.12 ± 0.13
SPC:CaS VE-FA	6.16 ± 0.14***

*Values of the membrane packing of SPC, SPC:SA and SPC;CaS with 5 mM of VE and 0.136 mM of FA after pasteurization in distilled water. Results are shown as the mean ± SD of three independent tests for MC540 hydrophobicity factor (HF). Statistical comparison was made in each system with vitamin / s compared to the same system without vitamins (control), by means of the Dunnett's Test. The significant differences with respect to the control are shown as * p <0.5; ** p <0.01; *** p <0.001 [29].*

Table 3.
Values of the membrane packing of SPC, SPC:SA and SPC;CaS with 5 mM of VE and 0.136 mM of FA after pasteurization in distilled water.

In this case, the incorporation of SA or CaS increased the lipid membrane's fluidity and defects, but this does not imply ruling out the developed carrier. Because it can generate this effect but have important oxidative stability, protection, and encapsulation of BC, that is why all the characterization and stability studies must be carried out to have a precise knowledge of how the carrier interacts with the BC and, based on that, define which ones will continue in the second study stage.

Once the studies of physicochemical stability, encapsulation efficiency, and characterization of the BC liposomal formulations have been carried out, start the second study stage, the final product application. For that type of study, two points of great importance must be evaluated: the final functional food's microbiological and sensory stability. The purpose of microbiological tests is to analyze whether the composition, preparation, and incorporation of matrices with BC in food contributes to microbial load. It is crucial to ensure that certain factors such as manufacturing techniques, acceptable manufacturing practices and especially heat treatment contribute to the fact that liposomal formulation with BC does not provide bacterial load and is safe.

The other aspect of studying is the sensory evaluation of the product with the carrier and BC. Sensory evaluation is needed because changes in food can be physical or chemical and can affect the product's appearance, texture, taste, smell, aroma, taste, and safety.

In these studies, the product's heat treatment and shelf life must be considered, and in these cases, they must be carried out on the base food and not on a food simulant as applied in the previous stage of characterization.

The sensory analysis allows knowing the organoleptic properties of food because it is done through the senses. Sensory evaluation is innate in man since from the moment a product is tried, a judgment is made about it, whether it likes or dislikes, and describes and recognizes its characteristics of taste, smell, and texture. Discriminatory tests should be used when it is necessary to determine if two samples are significantly different. Within this type of test is the triangular one that consists of presenting to the evaluator three suitably coded samples, of which two are the same, and the third is different [61]. This test was used in our research line, considering that there were three liposomal formulations used with the vitamins: SPC, SPC:SA, and SPC:CaS. For that reason three triangles were used for each combination of vitamins, for example VE and FA. Each rater was presented with three coded samples in each triangular trial, two of which were identical, and one was different, all numbered with random three-digit numbers for identification. The same samples could be the product (e.g., chocolate milk) with or without liposomes-BC, and the difference could be the product (chocolate milk) without or with liposomes-BC, respectively [29, 34]). The results obtained concluded that the addition of the liposomal formulations with the BC produced significant sensory changes in some products, such as chocolate milk. For this reason, it was proposed to carry out global acceptability tests to analyze whether the differences found were positive or negative in the base food.

The global acceptability test is within the affective tests, which are those in which the evaluator expresses his subjective reaction to the product, indicating whether he likes or dislikes it, whether he accepts or rejects it, or whether he prefers it to another. The primary purpose of effective methods is to evaluate the response (reaction, preference, or acceptance) of a product's actual or potential consumers. Hedonic scales are used to carry out these tests. The word hedonic comes from Greek and means pleasure. In this sense, hedonic scales are instruments for measuring the pleasant or unpleasant sensations produced by a portion of food to those who taste it. Hedonic scales can be verbal and graphic, and the choice of the type of scale depends on the age of the evaluators and the number of samples to be

evaluated. These scales are those that present a verbal description of the sensation that the sample produces. They must always contain an odd number of points, and the central point, "I neither like nor dislike," must always be included [61, 62].

Objectivity is achieved in the evaluators' responses to the sensations caused by a food product using the hedonic scales [61]. The evaluators were summoned, and two samples were presented. One was the chocolate milk without liposomes-BC, and the other was the chocolate milk with the liposomes and BC. In this case, they did not know what they were evaluating. **Table 4** shows the global acceptability test results where the SPC and SPC:CaS liposomes in the chocolate milk do not modify its acceptability concerning the raw food. Only the SPC:SA formulation in chocolate milk generates a decrease in its acceptability, coinciding with the fact that it had shown significant differences in the triangular test.

This global acceptability test can be performed using the pairs of product samples with or without BC-liposomes but informing the evaluator who the functional food is and the base food—obtaining very positive results that demonstrate the excellent acceptability of potential consumers regarding wanting to consume a food with different nutritional properties [29].

Thus, the implementation of sensory tests that are already effective to evaluate matrices with BC in food allow inferring the impression that a consumer would have of this functional food, and the achievement is that it is positive, which implies a significant advantage at the moment for producing BC-fortified food on an industrial scale. Because if we consider the importance and above all the cost that would imply launching a product for sale, this task cannot be carried out without being sure that the incorporation of BC matrices does not modify the base foods' sensory aspect.

4. Elaborating carriers/bioactive compounds to a larger scale, at the level of a food production line

Once the research stage study, development and application of matrices with BC at laboratory scale is finished, the final stage begins. The valuable part of this procedure is that if its production feasibility is analyzed when determining liposomes' production or any other matrix on a laboratory scale, it is easy to bring it up to a higher volume. Once the laboratory stage has succeeded, it is possible to upgrade to a larger production scale.

Sample	Total assay acceptability
Chocolate milk with SPC-VE-FA	7.30 ± 1.24
Chocolate milk	7.10 ± 2.81
Chocolate milk with SPC:SA-VE-FA	6.18 ± 3.17
Chocolate milk	7.2 ± 1.76**
Chocolate milk with SPC:SA-VE-FA	6.83 ± 1.99
Chocolate milk	7.13 ± 1.70

*Total assay acceptability of liposomal formulations. Qualifications of 40 panelists in commercial chocolate milk with or without liposomes SPC, SPC:Sa or SPC:CaS or with 5 mM of VE and 0.136 mM of FA pasteurized. Statistics were performed using the test for paired samples between each chocolate milk, with and without liposomes-BC. The results with significant differences are shown as * p <0.5; ** p <0.01; *** p <0.001 [29].*

Table 4.
Total assay acceptability of liposomal formulations.

The larger-scale production phase comprises a series of studied, analyzed, and calculated stages in what is called project evaluation. The purpose of which is to verify whether the functional product can be produced and developed at an industrial level, which can also be positioned in a specific market niche according to consumers' needs and that it is economically profitable to produce and commercially market it. In this way, in our line of research, the evaluation of the project has been carried out, which analyzed the feasibility of installing a pilot plant that produces functional chocolate milk being fortified with VE and FA, and added with essential fatty acids omega-3 and omega-6, choline and calcium (from the liposomal formulation). This type of study should consider the project idea as a starting point, and that it is related to the product to be marketed, its definition and characteristics, the type of BC, the nutritional benefits, and the deficiencies and needs of the country population. Subsequently, a market study of the functional product must be carried out, analysis of supply and demand, and analyzing the future projection in the market.

In the second part of the project evaluation, technical analysis of the plant must be carried out, which includes determining the factory's location, the quantity, and characteristics of the industrial equipment necessary according to the process—also considering the capacity of the machines according to the volume of production, process, and space. The shifts and number of working days must be analyzed according to the production volume and established product demand. We are also carrying out an administrative study considering the various aspects such as organization chart, functions, and responsibilities of the plant's employees.

The last part of the project consists of carrying out an economic analysis considering the initial investment, total operating costs, financial and economic budgets, income statement, and breakeven point and culminating with the economic evaluation of investment and determination of the economic profitability of the project [63].

Regarding the type of plant to produce the functional food, in the beginning, a small-scale pilot plant can be established, which will grow as the functional foods demands increase. It is important to highlight that the capacity of the machinery and production volume are related to the supply and demand of functional foods, in which the area to be commercialized the functional product maters, as well as with the total and variable fixed costs of production and the price of sale that will have the functional food. This last aspect is not minor, given that a consistent and competitive sale price is needed, which allows the functional food to enter and position itself in the market. All these points mentioned will be considered in the equilibrium point calculation, which allows identifying the number of functional food units that must be marketed per month to cover the sum of the total fixed and variable costs of the enterprise and to be found in balance. The equilibrium point is a reference point that indicates the minimum level of sales that must be produced not to suffer losses [64]. If the functional product's sales exceed the breakeven point, the plant will make a profit. Nevertheless, to analyze the project's profits and profitability in-depth, a series of indices such as the net present value and the internal rate of return must be calculated [65].

Of all the detailed points of the evaluation of a project, the technical analysis of the plant has a close relationship with the production and incorporation of the matrices that the BC will carry in a food production line. A technical study analysis, studies and calculates how the production line will be assembled, its lay-out, machinery and capacity, and the production volume, among other aspects.

And it is at this stage that you should consider how to incorporate BC into food production line. In the technical study of the project evaluation in our line of chocolate milk with the incorporation of liposomal formulations with BC, and

considering that it is a pasteurized and homogenized chocolate milk, it was proposed that the liposomal formulations would be incorporated after the milk homogenization stage. This decision based preventing liposomes from damaging their structure and prior to pasteurization, to ensure food safety of the entire product.

In other words, the liposomes will be incorporated into the pasteurization tank with the chocolate milk so that the heat treatment is carried out on the functional food. The low temperature heat treatment will be applied for a long time, at a temperature of 65 ºC for 30 minutes, in order to eliminate pathogenic microorganisms and decrease the bacterial flora that cause the deterioration of the product. Subsequently, a cooling will be carried out in the same pasteurization tank in order to avoid intermediate temperatures that favors the growth of microorganisms and/or unwanted effects produced in the nutrients present in the milk resulting in a greater unnecessary exposure to heat. The milk will be cooled by circulating water in the same jacketed tank where the pasteurization was carried out.

A high-pressure homogenizer will be used for the formation and incorporation of the liposomes. It is the high-pressure homogenizer. The turbine's high rotation speed and the deflection of the materials through the plate create a continuous flow through the stator. The result is a smooth surface without lumps, a mixture of both emulsion and dispersion components. A microfluidizer will also be used to obtain a uniform distribution of particles with a built-in heat exchanger for product discharge at a constant temperature.

The necessary procedure will consist of adding 500 g SPC: CaS with VE to 9.5 liters of drinking water with the AF and dispersing the sample using the homogenizer at a temperature of 35 °C at 8000 rpm for 30 minutes. Next, the liposomal suspension obtained will be processed with the microfluidizer at a pressure of 100 MPa for 5 minutes [66]. The incorporation of the liposomes will be carried out from the microfluidizer, where they have been formed towards the batch pasteurization tank. The liposomal suspension will be added to the milk with the correct dilution since the concentration of the preparation is notably higher than that added to the milk.

It is essential to consider the implementation of equipment designed to work on a pilot scale, but with the possibility of expansion to production volumes. In this way, it will be possible to start with small production volumes but increase them as functional food is increasingly positioned in the food market.

5. Conclusion

It is evident that the knowledge and importance that BC are having in nutrition and especially in people's health is increasing. And as a result of the life system and / or socio-economic situations, it is of industrial relevance that a greater quantity of functional foods with specific BC needs to be incorporated into the market, due to the deficiencies and/or nutritional needs of the population. In this emerging production line, the need arises to investigate and develop matrices that allow the incorporation of BC in all types of products, but that also protect them and preserve their functional activity. And in addition, the laboratory scale that produces those matrices must be scalable to a greater volume like that of a production line.

This chapter has exposed all the various stages of research, development, production and incorporation into foods of liposomal formulations that allow encapsulation and contain BC. This allows us to conclude that when investigating and developing a functional food, not only the results obtained at the laboratory scale in relation to its stability and characterization are of utmost importance, but also whether it is feasible, profitable and marketable should be evaluated in industrial scale production.

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Conflict of interest

None of the authors have no competing interests to declare.

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Chapter 3

Sustainable and Healthy Food Ingredients: Characterization and Application in Functional Products

Țibulcă Dorin and Fogarasi Melinda

Abstract

Nowadays, and considering the increasing pieces of evidence of health-promoting abilities of numerous food classes, a pronounced market pressure has been observed both in agricultural and biotechnological industries. Thus, while the development of functional foods seems to be conceived as an interesting trend with large market potential, the increasing demand and interest of sustainable food ingredients seems also promissory. In order to contribute to this approach, the proposal chapter will provides a comprehensive overview of the healthy and sustainable ingredients as edible mushrooms, legumes and bison emphasizing the characterization and application of those as natural ingredients in functional food products.

Keywords: bison, functional and healthy foods, legumes, mushrooms, sustainable ingredients

1. Introduction

The growing demand for nutritious, healthy, sustainable but at the same time attractive food products drives the future of food processing to be multipurpose and more sophisticated. For this reason it became very challenging to develop novel food and/or functional food products, considering that it has to fulfill the consumer's expectations for products that are simultaneously palatable and healthy. Compared to conventional foods, the development of functional components requires technological solutions that can be demanding and expensive, and needs to establish a dynamic equilibrium between research and business. In addition it is important to consider the fact that functional food markets are continuously changing [1]. Food industry innovation is focused on scientific and technical approaches in food processing, along with the introduction of novel and functional foods. In order to promote and sustain healthy eating, food manufacturers need to offer suitable and attractive options on the market for consumers to choose from. There are two main factors that stimulate the interest among food manufacturers to apply these approaches. From one side there is a constant pressure from the public sector to produce "healthier" food products which is accompanied by increased consumer attention to healthy eating. According to the literature, the achievement of food

sustainability is not easy, even more if it is linked to an environmentally friendly diet which is a challenge for even the most dedicated eco-warriors. Concerning human nutrition, agriculture is one of the most important drivers of these changes, since innovation brought by the Green Revolution has completely modified process sustainability, leading to an irreversible tendency to adopt conventional and intensive practices [2].

Sustainable foods are defined as types of foods that are obtained in a manner that minimizes their negative impact on both the environment and the communities that produce them. It is mandatory that sustainable foods meet several criteria among which the most important inquire that there production be environmentally friendly that minimize greenhouse gas emissions, lowering the carbon footprint of the process, and use resources as sustainably as possible. According to the Food and Agriculture Organization of the United Nations (FAO) sustainable food production is the "method of production using processes and systems that are non-polluting, conserve non-renewable energy and natural resources, are economically efficient, are safe for workers, communities and consumers, and do not compromise the needs of future generations".

Besides environmental factors, sustainable eating also pays careful attention to the lifecycle of animals involving their raise and slaughter and how farmers are treated and paid. Therefore, foods must be sustainable considering the future trend of global population increase and limited amount of resources (land, water, and the food itself) that we have. In view of the above, the current section focuses on the presentation and discussion of important aspects related to the characterization of healthy and sustainable ingredients and the application of these natural ingredients in functional foods.

2. Foods with potential sustainability, functionality and healthy

Based on the literatures date, it is very clear to the scientific community together with food manufactures what is the main framework of a sustainable food and farming system even though, at the moment, there is no legal definition of 'sustainable food'. This is clearly reflected in many well known good accreditation schemes which are clearly defined examples, like those certifying 'organic' and 'Fairtrade' food.

Recently it can be noticed that there is keen interest for plant-based foods which more specifically by nutrition is playing an important factor in the definition of sustainable foods. These foods tend to have a greater emphasis on whole foods and fewer processed ingredients. Moreover, there is a growing affinity of the consumers to sustainable wild food products that are grown and produced in uncontaminated wild areas offering high quality food that is viewed in a completely different way as enriched ones with beneficial bioactive compounds [3, 4]. There are large numbers of wild edible food categories including annual and perennial herbs, forbs, ferns, as well as mushrooms, algae and lichens, vines, sedges and rushes, grasses, broad-leaved and needle-like or scale-like leaved shrubs, trees [5].

2.1 Mountain food products: wild edible mushrooms

Mountain Food Products have received an increasing interest in the last years due to the fact that these food products have numerous beneficial and unique qualities that are significantly more accentuated or it cannot be found in other products. It was also found that their quality is strongly influenced by the specific environmental and processing conditions of their mountainous regions of production

and transformation [3]. Mountain foods include a wide variety of products, such as dairy and meat products, fruits, olive oil, pastries, mineral waters, medicinal plants, mushrooms etc. In fact, increasing evidence have confirmed that mountain produced foods present a high level of health-promoting micronutrients, apart from having vestigial or even null amounts of toxins at same time that safe-guards environment.

Many studies reported in the literature pointed put the potential beneficial applications of mushrooms in human dietary, considering that they possess unique nutritional and chemical properties [6–9]. Researchers revealed that wild edible mushrooms provide an important amount of fiber and proteins together with other valuable components like essential amino acids but in comparison to other food products they have a low fat content and do not contain cholesterol [10–14]. In addition, wild edible mushrooms are recognized as a delicacy, due to their specific flavor and texture, especially in mountain areas where they are widely collected considering that many studies emphasized their important nutritional value and the fact that amino acids found in mushrooms are comparable with those of animal origin [15]. For this reasons, many studies have been performed in order to use wild edible mushrooms as raw materials for the production of functional foods considering the identified and extractable bioactive compounds, like terpenoids, unsaturated fatty acids and carotenoids, etc. Also, their exceptional chemical characteristics can be valorized in the fabrication of nutraceuticals or pharmaceutical products, exploring the synergies of the large group of bioactive compounds [5, 16–19]. **Figure 1** gives a summary of the range of beneficial properties of wild edible mushrooms, such as antioxidative, antibacterial, antiviral, anticancer, and anti-inflammatory properties, strengthening the immune system as well as the ability to improve the functioning of the cardiovascular system [14, 20, 21]. This explains why wild edible mushrooms are becoming more and more important in the definition of a balanced diet for humans all over the world, achieving exploitation of the health benefits and functioning mechanisms of mushrooms which give good results in the prevention of major diseases, such as cancer, heart and nervous problems [22, 23].

Mushrooms contain a number of chemical compounds of nutraceutical importance, such as terpenes, bioactive proteins and antioxidants, which make them a therapeutically stronger foodstuff in the battle against various degenerative diseases [7]. Mushrooms have a wide variety of compounds operating in their natural environment, but they can be used to ensure or promote human health in the form of nutraceuticals, additives, functional foods and others. Thus, the creation of a research-oriented field of study for the scientific and novel use of edible or medicinal

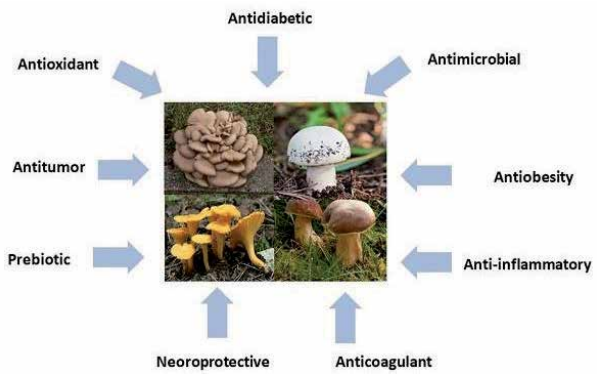

Figure 1.
Beneficial properties of wild edible mushrooms.

Aspect	Species	Habitat	Health benefits	References
	Cantharellus cibarius	Beech and Conifer forests	Excellent source of polysaccharides like chitin and chitosan, reduce inflammation and lower the risk of developing certain cancers	[24]
	Agaricus bisporus	Grassy areas following rain or forest	Hepatoprotective, immuno-stimulatory and antitumor activities Anti-aging activity, protect hepatic and nephric by improving serum, enzyme activities, biochemical levels, lipid contents and antioxidant status	[22, 25, 26]
	Lentinula edodes	Grows in groups on the decaying wood of deciduous trees, particularly shii and other chinquapins, chestnut, oak, maple, beech, sweetgum, poplar, hornbeam, ironwood, and mulberry.	Immuneregulator activity and anticancer potential Lung protection activity, regulate the antioxidant and inflammation status, Antitumor activity	[27]

Aspect	Species	Habitat	Health benefits	References
	Pleurotus ostreatus	On trunks of deciduous species	Cardiovascular, hypertensive, hypercholesterolemia antioxidant and antimicrobial activities, antidiabetic activity	[28–30]
	Boletus edulis	Conifer forests	Antiviral, antiinflammatory, antimicrobial, antioxidant	[31]
	Armillariella mellea	Grows solitary or in groups, on trunks of oak and beech but also on conifer trunks, roots, rotten logs	Meniere's Syndrome, vertigo, epilepsy, neurasthenia and hypertension, antioxidant, antimicrobial properties	[31]

Aspect	Species	Habitat	Health benefits	References
	Macrolepiota procera	Open woods and pastures as well as besides the paths in the forests (e.g. oak and beech or coniferous)	Antioxidant properties, Anti-tumor activity	[31, 32]
	Tuber indicum (truffle)	Conifer forests	Antitumor activity, inhibit the proliferation of hepatoma and human breast cancer cell lines	[22]
	Volvariella volvacea	Beech and Conifer forests	Reducing free radicals, Strengthening bones, Prevent Anemia	[33]

Aspect	Species	Habitat	Health benefits	References
	Flammulina velutipes	growing on dead or living wood or roots of deciduous trees, rarely on conifers	Regulation of the immune system, cancer immunotherapy, antioxidant Immunomodulating activity	[31, 34]

Table 1.
Health benefits of wild edible mushrooms.

mushrooms, the exploitation, and promotion of their full use is necessary. Some of the major properties of the mushroom are described and summarized in **Table 1**.

2.2 Legumes

Legumes and pulses have an important contribution to both human and animal dietary worldwide. The most significant ones are alfalfa, clover, beans, peas, chick-peas, lentils, soy and peanuts. These have long been a part of Western diets and agri-cultural management regimes, but they only recently gain the attention of researchers in the domain of in agri-food research. Promoted for their agronomic, nutritional and environmental benefits [35], legumes have been framed as plant-based solutions to an array of problems in the modern food system; becoming vegetable vessels that express the hopes and dreams of diverse researchers, marketeers and other food futurologists [36]. Moreover, research results concluded that legume consumption reduces the risk of numerous chronic diseases [37] but at the same time provide a range of essential macro, micronutrients and bioactive metabolites with synergic effect against inflammation, which plays a role in disease onset or progression [38]. The identification of resistant cultivars against abiotic and biotic stresses and development of sustainable field management practices, could address both nutri-tion and environmental concerns of modern society [2, 38]. These plants are viewed as a key component in many international research programs for the promotion of environmental sustainability development and the accomplishment of zero hunger. The concrete transition to a more sustainable diet rich in legumes requires a substan-tial change of the typical "western" dietary habits, and food choices (also supported by industrial stakeholders) as well as the suitable strategies to enhance legumes cultivation, distribution and consumption. It is well known that legumes show many environmental sustainability advantages such as the ones presented in **Figure 2**.

The healthy impact of legumes on human organism is based on the presence of The bioactive molecules [39] among which phenolic compounds, saponins, pep-tides and small proteins are the most significant [40]. Some of these are ubiquitous in the family, while others are typical of some genera or species and their synthesis

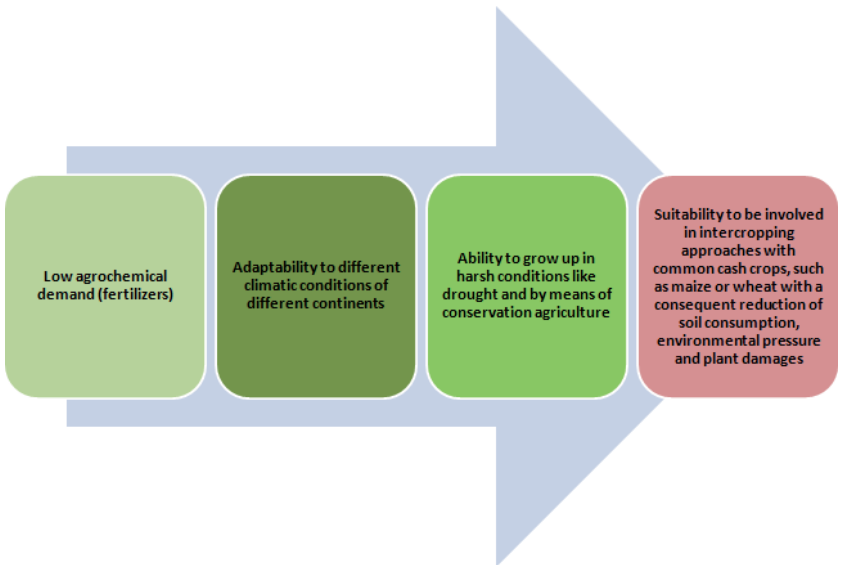

Figure 2.
Environmental sustainability advantages of legumes.

	Species	Health benefits	References
	*Phaseolus vulgaris* L.	Anti-diabetes, anti-hypertensive, antioxidant, prevention of cardiovascular disease, Hypocholesterolemic, prebiotic and fermentation modulator	[44–47]
	*Pisum sativum* L.	Protease inhibition, modulation of intestinal bacteria, glucose metabolism modulation, body weight regulation and anti-diabetes	[48, 49]
	Lupinus spp.	Anticancer, anti-inflammatory	[50]
	Arachis hypogea L.	Hypolipidemic, antioxidant and antimicrobial	[51, 52]
	*Cicer arietinum* L.	Antioxidant, hypoglycemic and anti-diabetes, antimicrobial, glucose metabolism modulation, body weight regulation and anti-diabetes	[53–55]
	*Lens culinaris* L.	Prebiotic, hypocholesterolemic, fecal bile acids and SCFAs enhancer, Anticancer potential	[56–58]

	Species	Health benefits	References
	*Glycine max* L.	Inhibition of proliferation, anti-inflammatory, osteoporosis prevention	[59]

Table 2.
The most common legume species and their health properties.

is highly dependent on plant growing conditions (e.g., development stage, amount of light, and water availability) [41]. Besides polyphenols, legumes proved to be an excellent source of peptides and small proteins that present many biological activities applicable as nutraceuticals and/or therapeutic agents [42, 43]. As a result, these small proteins have been subjected to many studies both in vitro and in vivo in order to quantify their beneficial properties and their potential role in prevention of chronic degenerative diseases. An important category of well-studied legume proteins are lectins which are adequate as carriers for target drug delivery considering their peculiar binding ability. Such an application is the use of lectins for the transportation and release of anticancer drugs during the different stages of tumor progression taking advantage of the well known fact that carbohydrates present on the cancer cell membrane are involved in recognition processes.

In order to highlight the health benefits of legumes, some of the most common legume species and their health impact are listed in **Table 2** based on the scientific literature. According to the data shown in **Table 2** it is obvious that these plants represent an important source for both diet and new nutraceuticals, considering the large number of bioactiv compounds and properties.

2.3 Bison

In the recent years, bison as an alternate meat variety is becoming more and more well-liked in North America [60]. One of the major advantages of bison meat is the fact that it is a sustainable and healthful alternative to cow beef and its production does not face so many environmental and ethical questions. In contrast to cattle which prefer to move around near water sources, bison cover more land leading to the preservation of the sensible ecosystems in the vicinity streams and ponds. Another important fact about bison is that they promote biodiversity considering that by going first for grasses and leaving patches where other plants can fill in. Their hoof prints leave depressions that collect water and their dung serves as a powerful fertilizer: both assist in seedling germination and establishment. Results reported in the literature indicate that in comparison to beef, bison meat presents a lowers energy and fat content and according to the feedback of consumers it seems that bison meat is also healthier than beef. However, the nutrient composition of bison and other meats are strongly influenced by a series of factors like age, type of feed, maturity, gender, type of cut, genetics and season [61]. It is also important to note that bison not only contains less fat than beef but in addition proved to offer an advantageable fatty acid profile, making bison meat a healthy red meat source [62, 63]. Many studies confirmed that bison meat has an hihg ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) [62–64], 3 to 4 times more anti-inflammatory omega-3 PUFA and

is particularly high in alpha linolenic acid [63]. In addition, ruminants such as bison are a major contributors of conjugated linoleic acid (CLA) to the human diet [65, 66], providing significantly more CLA than other non-ruminant meat sources such as pork, fish, chicken, and turkey. The dietary inclusion of a specifically rich source of CLA may be advantageous as CLA is believed to have anti-inflammatory properties [67] and may have an important role in the prevention of cardiovascular disease.

3. Application of sustainable healthy ingredients in functional foods

The recent tendency of functional food development had determined the orientation of the scientific community to attractive sustainable and healthy ingredients which can be used in the production. This demarche aims to valorize some of the

	Sustainable ingredients	Application	References
Mushrooms			
1	*Shiitake* powder	Frankfurter	[68]
2	Mixt of *Lentinula edodes*, *Pleurotus eryngii* and *Flammulina velutipes*	Yogurt	[69]
3	*Suillus luteus*	Cottage cheese	[69]
4	*Tremella fuciformis*	Pork patties	[70]
5	*Pleurotus eryngii*	Pork sausages	[71]
6	*Boletus edulis*	Frankfurter Bread	[72, 73]
7	*Cantharellus cibarius*	Frankfurter	[72]
8	*Agaricus bisporus*	Meat emulsion Beef patties Smoke sausages	[74–76]
9	*Flamulina velutipes*	Emulsiontype sausage Ham Chicken sausage	[77–79]
10	*Agaricus bisporus*	Snacks White bread Sponge cake	[80, 81]
11	*Pleurotus ostreatus*	Noodles	[82]
12	*Cordyceps militaris*	Extruded product	[83]
Legumes			
13	*Phaseolus vulgaris* L.	Tortilla Bakery product	[84, 85]
14	*Pisum sativum* L.	Cookies	[86]
15	Arachis hypogea L.	Bakery products	[86]
16	*Cicer arietinum* L.	Pasta, snacks	[87]
17	*Lens culinaris* L.	Bread, cake, crackers, pasta, snacks, dressings, soups, dairy and meat products	[88]
18	*Glycine max* L.	Noodle, meat product	[89]

Table 3.
Applications of different types of sustainable ingredients.

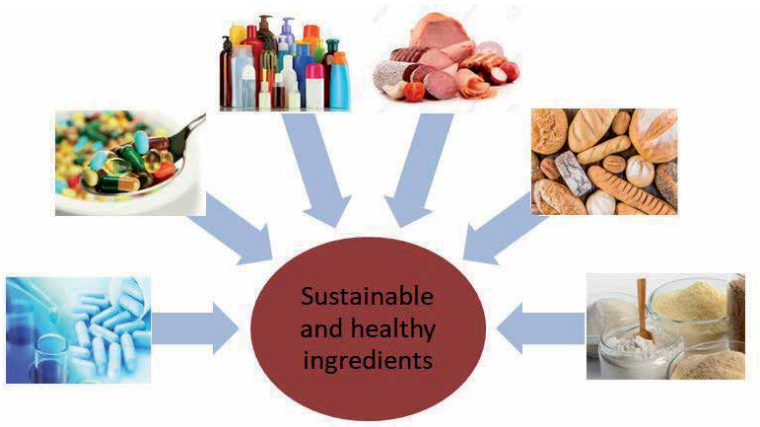

Figure 3.
Application of sustainable healthy ingredients in functional foods.

most important characteristics of sustainable and healthy ingredients such as easily digestible protein, healthy fat, and repertoire of various essential micronutrients for billions across the globe. In recent years, these ingredients were used in the different sector of the food industry being incorporated in various food products to obtain fortified functional foods (**Figure 3**) such as bakery and meat products, food supplements, pharmaceutical products, food additives, cosmetic products.

According to the data presented in **Table 3**, the benefits of sustainable and healthy ingredient are well known but their application in the elaboration of food products is quite recent mainly as meat, fat, phosphates, salt, flour, and nitrite replacer. As a result, many research teams are focusing on the assessment of efficient incorporation of the bioactivities of sustainable and healthy ingredients into newly developed food products. Despite the progress made there are several issues that need to be solved in the future such as the demonstration of the correlation between the functional activities and mechanisms, as well as their safety evaluation and safe range of intake.

4. Conclusions

Nowadays, sustainable ingredients gain more and more attention due to the beneficial and unique characteristics which make them an attractive source of high added-value compounds that could be utilized to fortify different products such as cosmetics and functional foods. Based on the result provided by several studies it can be concluded that healthy sustainable products provide protein, fat, minerals and vitamins in a very precise form and adequate content being recognized as a delicacy and therefore preferred by big part over the globe. It is important to note that literature data show an increasing demand to foods that has low calories, low fat and low cholesterol content and functional foods, which are defined as foods that have positive effects on human health. As an overall conclusion it can be stated that the studied and presented sustainable ingredients have great potential to be used as a natural source of bioactive compounds for the production of functional foods.

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Conflict of interest

"The authors declare no conflict of interest."

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Chapter 4

Eat Tasty and Healthy: Role of Polyphenols in Functional Foods

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Abstract

Adverse reactions to food such as allergies and celiac disease are increasingly recognized as a growing public health burden. There is currently no cure for these diseases so that there is an unmet need to evaluate different nutritional approaches aiming at improving the quality of life of affected patients and their families. In this context, healthy promising nature-derived compounds, most of which contained in fruits and vegetables, have been studied as an alternative to attenuate the epidemic. Indeed, phenolic compounds have become an emerging field of interest in nutrition in the last decades. A growing build of research suggests that phenolic compounds inhibit pro-inflammatory transcription factors by interacting with proteins involved in gene expression and cell signaling, leading to protective effects against many inflammation-mediated chronic diseases. However, the use of phenolic compounds as attenuating agents of immune reactions to food has to be aligned to the organoleptic characteristics of food, since many compounds present unpleasant taste properties, namely bitter taste and astringency. In this framework, tasty but healthy phenolic compounds arise as attractive ingredients in the design and formulation of functional foods. This book chapter is focused on revisiting the organoleptic properties of phenolic compounds while evaluating the role of these compounds in health promoting actions, namely the management of immune reactions to food such as Food Allergies and Celiac Disease.

Keywords: food allergies, celiac disease, functional foods, phenolic compounds, clean label

1. Introduction

"Healthy" and "natural" are two keywords appealing for consumers. Along with these, there is a growing demand for "functional" and "clean label" food products. The food system and consumers demand for new functional products that have potentially positive effects on health besides basic nutrition alongside to products made with few ingredients, preferably natural ones and assuring that these new ingredients are easy-to-recognize. In fact, "clean label" is a new "action" of food system to recover consumer trust, somehow lost in the past decades due to different issues, such as low transparency about ingredients. The term clean label is complex and multidimensional. While there is not a specific definition of the term, it is

while accepted that clean label refers to "natural" food, with simple, well-known and short ingredients list while avoiding the use of synthetic additives [1, 2]. In this context, the use of natural extracts rich in bioactive compounds able to reduce the use of synthetic additives arise as a new reality. Consumers' of nowadays are more informed and demanding. In addition, to healthy, functional and natural, consumers are also demanding for sustainable food products. Within this framework, plant-based ingredients and bioactive compounds arise as promising tools because they comprise all these topics. This chapter will focus on a class of plant-based bioactive compounds, the phenolic compounds, and their potential to be added to functional and clean labelfoods. This chapter will start by covering the structural classification and occurrence of phenolic compounds, their health bioactivities as well as their potential application for modulation of immune reactions to food (food allergies and celiac disease). While these compounds are undoubtedly healthy, their supplementation in food can affect the sensory properties leading to unpleasant effects, namely bitter taste and astringency perception. This will be also focused inside this chapter. The technological properties of phenolic compound as new ingredients will be also discussed considering the interactions within food matrices. Despite their importance, these interactions are usually overlooked in functional and clean label foods. At the end, this chapter aims to highlight that functional foods should constitute a part of a healthy but tasteful diet.

2. Nature and occurrence of phenolic compounds: from food to waste

Phenolic compounds (PC) are secondary metabolites with a high array of unique bioactive properties, which makes them vastly appreciated for their beneficial effects on human health and well-being. PC constitute a wide family of bioactive compounds comprising more than 8000 different structures already identified [3]. Besides the great structural heterogeneity, some common features allow classifying PC into two different families: flavonoids (flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins) and non-flavonoids (phenolic acids and derivatives, stilbenes and lignans) as summarized in **Figure 1**. In nature, these structures could also occur esterified, acylated and/or glycosylated. Indeed, the extreme diversity of these compounds joined with their extensively

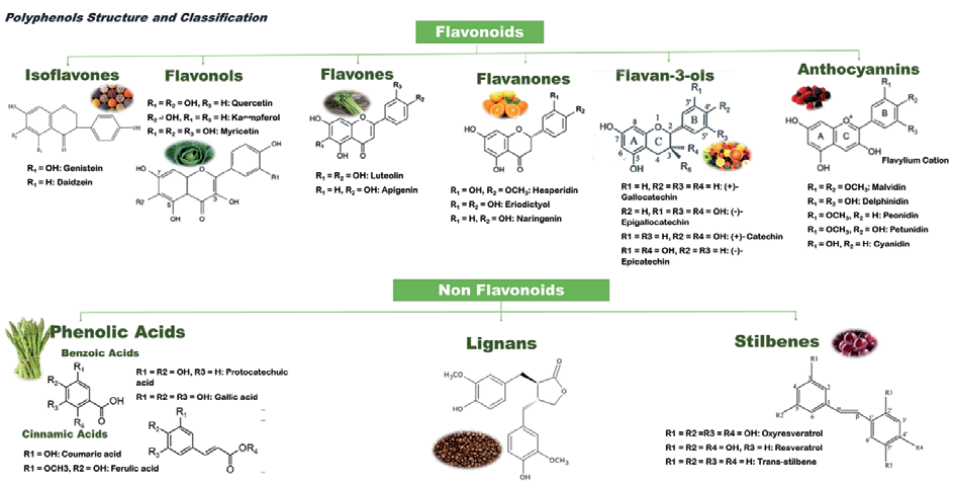

Figure 1.
Polyphenols classification and basic structures.

probed bioactivities makes them one of the most largely studied family of plant metabolites.

PC are found almost in all families of plants and are concentrated in leaf tissue, the epidermis, bark layers, flowers and fruits. The PC distribution could vary within the plant tissues. Indeed, differences in PC composition from seeds, pulp and peels have been extensively studied [4]. Overall, anthocyanin pigments are mainly accumulated in peels while flavan-3-ols appear in higher concentration in seeds [5]. Furthermore, PC occurrence in Plant Kingdom depend on biotic and abiotic factors such as genetic variations, environmental conditions and agronomic practices among others [6]. Besides these variations, the PC content could vary during processing from technological and industrial processes to homemade practices such as culinary treatments or vegetables storage [7, 8]. All these features must be considered before analyzing the real intake of PC and related health outcomes observed. Indeed, the amount of a bioactive compound required to deliver the health-related effects is the key to design a new functional food. Besides the great diversity of PC and the wide range of foods containing them, the major daily intake comes from cereal grain accounting for over 50% of their total intake. Among phytochemicals identified in cereal grain the most important are the phenolic acids and condensed tannins (flavan-3-ols polymers) [9]. In parallel, flavonoids are the main bioactive compounds found in fruits [10] and herbs and spices are rich in hidroxybenzoic acids [11].

PC are not only present in foods but also Agro-Food wastes such as fruit pomace, wood material or even waste water [12]. In this context, the valorization of Agro-Food by-products have emerged over the last years especially because of their easy obtention in large volumes with reduced costs [13]. Apart from their functionality as a source of energy, Agro-Food by-products should also be considered as value-added residues due to their chemical heterogeneity, structure and subsequent applications in the food sector as functional food ingredients or nutraceuticals contributing not only for a sustainable and circular economy but also for the implementation of zero waste politics [14].

Agro-Food wastes have been traditionally used as organic fertilizer, livestock feeds, or as a source for biofuel production [13]. The evolution in Green-chemistry with cutting-edge technology to properly obtain bioactive compounds from Agro-Food wastes opens a new perspective to produce value-added products [15]. Much evidence has been highlighted to use by-products of fruit industry or wineries, among others. Edible parts of fruits and vegetables usually contain lower amounts of bioactive compounds than skin, twings or peels non-edible portions. Indeed, wastes obtained after pressing the juice are a valuable source of PC. In parallel, winery and overall beverages industry generates large volumes of pomace (a mixture of pulp, skin, seeds, and stem) with higher amounts of PC when compared with edible fruits and vegetables [16]. Among fruit-derived by-products or wastes, apple pomace contains high amounts of flavonols, flavanols, phenolic acids, dihydrochalcones and anthocyanins [17]. Furthermore, significant amounts of a well-known antidiabetic agent named phlorizin is widely found in apple-derived by-products [18]. Peels from fruit such as banana peel, rich in phenolic acids, flavonols, flavanols, and catecholamines accounting three times the edible part of the fruit, have been reported as providing strong antioxidant and anti-microbial activities, in addition to exhibit other health benefits like reducing cholesterol and blood sugar, neuroprotective effect and anti-angiogenic activity were also described [13]. Citrus peels have been also studied to recover high-value bioactive compounds like flavones (apigenin-glucoside and diosmetin-glucoside) and flavanones (eriocitrin and hesperidin) from lemon peel and phenolic acids (hydroxybenzoic and caffeic acids) and flavanones (hesperidin and narirutin) from orange peel and pulp [19]. Potato peel has been

reported as containing phenolic acids and beetroots peel and pulp contain flavonoids, phenolic acids and betalains, which exhibited good antioxidant activity and hepatoprotective effects [20]. One of the most recently studied food material was onion and garlic skin/peel, which generates huge volume of wastes especially rich in quercetin derivatives as well as other aglycone flavonoids exhibiting bioactivities like antioxidant, antimicrobial, antispasmodic, and antidiabetic activity [21]. Berries by-products such as branches obtained from elderberry processing are a valuable source of anthocyanins [22]. Furthermore, seeds/kernel which is a major waste after processing holds promise as a potential therapeutic source with numerous PC being isolated such as flavonols and gallotannins [23].

Some of the mentioned food by-products or Agro-Food wastes have been already proposed as additive in the formulation of bakery and dairy products to enhance their contents in bioactive compounds [24, 25]. In addition, the presence of natural compounds, pigments and volatile compounds can enhance the sensory properties and overall quality of the final product but some research is needed to deep in the technological effects of these new ingredients with highly probed bioactivities.

3. Phenolic compounds as modulators of immune reactions to food

Over the past several years, non-communicable diseases (NCDs) such as cancer, cardiometabolic, neurodegenerative and autoimmune disorders have become of important health concerns for consumers and a growing public health issue everywhere in the world [26]. They typically result from an imbalance between people and their environment and lifestyle patterns, including physical inactivity, tobacco usage, alcohol abuse and related metabolic risks [27]. Essential for life, diet provides a vast source of molecules that are largely harmless for the majority of the world population. Nonetheless, compelling observational and interventional evidence is now available on the implication of modern unbalanced dietary habits/diet - with its high saturated fat and sugar intake - on the incidence of low-grade, chronic, and systemic inflammation [28]. Furthermore, for some individuals, the intake of staple food like milk, eggs, nuts or bread can trigger a set of immune mechanisms that can lead to a severe allergic condition termed food allergy [29]. Besides food allergy there is a high number of immune reactions to food, some of them autoimmune disorders such as Celiac Disease. The rise of immunologic reactions to food are substantial and evolving public health issues, increasing over the last decade as epidemic [30]. Nevertheless, therapeutic options remain limited. One of the mechanisms leading to this subclinical, yet persistent adverse response to nonlife-threating situations, occurs through, for instance, food-induced structural and behavioral changes in gut microbiota [31]. The bacterial ecosystem living up in the gut play crucial roles in the induction of protective responses to pathogens, maintenance of body's homeostasis and tolerance to innocuous food antigens [32]. As a consequence, any environmental factor disturbing the richness and diversity of bacteria making up the gut microbiome could potentially affect host metabolism, impact intestinal barrier integrity and immune system functions [33]. A better understanding of the key nutritional mechanisms involved in such immune responses will likely be vital for disease prevention and development of new therapies. Indeed, consumption of antioxidants, mainly dietary phenolic compounds found in fruits and vegetables, has been related with low prevalence of immune reactions to food [34]. Used as nutraceuticals, PC are thought to dampen the onset of immune-related inflammation [35, 36]. Moreover, recent studies proved the ability of PC to bind food antigens [37, 38], which could modulate the disorders, but concerns still remain about their real function by the organism that assumes

PC through diet, because of their bioavailability, metabolism and pharmacokinetics. Scientific knowledge has to be improved to establish the basis for nutritional recommendations that help to prevent or minimize the prevalence and symptoms of immune reactions. A broad approach is herein explained to fully understand the immunomodulatory process of PC in food allergies and celiac disease from ingestion to immune systemic effects manifestation.

3.1 Food allergies

This hypersensitivity to particular proteins present in food, known as allergens, occurs when the immune system erroneously perceive foreign proteins as dangerous, initiating an allergic immune reaction [29].

The most common type of food allergy is mediated by immunoglobulin E (IgE), and is estimated to have an impact in the life of 5–8% of the children and up to 4% of the adults worldwide [39]. Food allergic reactions mediated by IgE comprise distinct phases; the allergic sensitization, where the food antigen is taken up, processed and displayed on the surface of antigen presenting cells (APCs); which, in the presence of interleukin-4 (IL-4) and/or IL-13, provide signals for the activation of the T helper 2 (TH2) subtype of T cells. Then, TH2 cells in conjugation with IL-4 and IL-13, will induce class switching in B cells, which differentiate into plasmocytes (antibody-producing cells) that secret allergen-specific IgE [40]. After the allergic sensitization, the subsequent re-exposure (elicitation phase) to the allergen, will now result in a more robust immune response. Here, the antigen-specific IgE binds to the surface receptor FcεRI expressed on mast cells and basophils. The cross-link of the FcεRI receptors with IgE will trigger mast cells and basophils degranulation, which leads to the secretion of inflammatory mediators e.g. β-hexosaminidase and histamine. In addition, allergen-induced cytokines (IL-4 and IL-13) are also released fostering the typical food allergic symptoms, which can range from mild to a life-threatening allergic reaction (anaphylaxis) [39, 40]. Given that, the incidence as well as the severity of food allergy, is gradually increasing, the search for novel therapeutics to mitigate this condition is in high demand [29].

As described earlier, there are various immune mechanisms implicated in food allergy that may, therefore, be targeted in prospective anti-allergic strategies. In this light, the extraordinary structural characteristics, wide distribution in fruits and vegetables, and the well-studied anti-inflammatory and anti-oxidant properties of PC, make these bioactive compounds fitting candidates for anti-allergic therapies [41]. In fact, various studies with PCs have suggested that some of these metabolites, especially phenolic acids and flavonoids, may exhibit certain anti-allergic benefits and although the exact mechanisms behind their action are not clear, data shows that PC can intervene at both the allergic sensitization and the elicitation phases [42, 43]. Moreover, PC can also modulate gut microbiota and potentially influence food allergy [44].

Several methodologies are currently in use to evaluate the capacity of distinct PC to interact with specific allergen proteins. Plundrich et al. performed in silico analyses to narrow down the search for PC present in cranberries/ and or lowbush blueberries (rich in anthocyanins), which could theoretically interact with Ara h 2, the most pro-allergenic protein in peanuts, specifically in the region that is thought to be the binding site for IgE [45]. This screening, in concert with further *in vitro* experiments revealed that procyanidin C1 and chlorogenic acid could potentially interact with Ara h 2 inducing conformational changes, which masked the IgE epitope [45]. Covalent interactions between chlorogenic acid and ovalbumin (OVA), the major allergen found in the egg white, also induced modifications in OVA conformation, resulting in the direct shielding of the linear IgE epitope, which

attenuated allergic mechanisms [46]. Accordingly, histamine release experiments, showed that the basophil degranulation was inferior in human basophils sensitized with the OVA conjugated with chlorogenic acid when compared to OVA unconjugated, implying a decrease in the crosslinking of the FcεRI receptors via IgE-allergen interaction [46]. Also, the ability of phenolic compounds to bind to dietary allergen is pointed out as having a beneficial effect due to precipitation events [47]. Accordingly, Yichen Li et al. observed that PC from pomegranate juice could form stable complexes with cashew nuts, thus reducing allergen recognition by antibodies, and consequently the immunoreactivity to cashew nuts [47].

Despite not completely mimicking the human pathophysiology of food allergy, animal models of food allergy are important pre-clinical research tools for the food industry. These models are elected due to their capacity to simulate the most common reactions observed after the exposure to specific allergens, namely IgE production, TH2 related cytokine expression and mast cells degranulation [48, 49]. In fact, various animal models are now used to study the effect of PC as modulators of allergy. For example, the metabolites derived from epicatechin found in the circulating plasma of a mouse model of OVA allergy fed with phenolic compounds extracts isolated from apple/ or purified epicatechin, were associated with the reduction of several clinical allergic symptoms [49]. Additionally, in the ileum, the mRNA levels of the TH2 – related cytokine IL-13 and the pro-inflammatory cytokine IL-12 were decreased as well, suggesting that epicatechin could be a possible modulator of allergic reactions [49]. Also, Abril-Gil et al., used Brown Norway rats to investigate the potential protective effect of cocoa diets, which contain high amounts of flavanols (e.g. epicatechin, catechin and procyanidins) in allergic immune reactions upon OVA re-exposure [50]. Strikingly, their findings showed that while in the group of rats deprived of cocoa high levels of serum specific anti-OVA IgE were observed, in the other groups where cocoa was offered, IgE was significantly lower [50]. Moreover, the *in vitro* analysis of spleen and mesenteric lymph node cells (MLN) cytokines secretion revealed that IL-5 and IL-13 were reduced in the MLN, and that in the spleen, IL-4 was also reduced in a specific cocoa diet. Interestingly, the cocoa diet was also important for attenuating degranulation events by reducing the FcεRI and mast cell mediators (proteases) gene expression and release [50].

More recently, a mouse model (C3H/HeJ mice) representative of peanut allergy was used to evaluate the capacity of PC rich-extracts obtained from blueberry and cranberry to minimize the allergenicity of peanut proteins [51]. Here, colloidal aggregates composed of PC extracts (with different percentages) and proteins derived from peanut were introduced in the diet of peanut sensitized mice for several weeks, before challenging the mice with a higher dose of peanut flour. At the end of the experiment, it was observed that mice pre-treated with the PC aggregates, showed reduced IgE and IgG levels; and lower expression of the allergen-induced basophil activation protein marker CD63 in spleen lysates, when compared to mice kept on a diet with non-complexed peanut proteins [51].

The manifestly promising results demonstrated by these and other *in vivo* experiments, suggest that in the presence of PCs, the re-exposure to allergens result in less exuberant allergic responses. In this way, the use of these bioactive compounds hold promise to surmount the immune responses triggered by the oral administration of food antigens, contributing therefore, to the oral tolerance to dietary proteins [52].

In summary, the urgent need for effective therapeutics for food allergy and given the complex mechanisms involved in this escalating pathology, diverse anti-allergic strategies are being explored. In this perspective, the natural ability of PC to interact with food allergens, interfere with IgE interaction or production, reduce

the secretion of allergic mediators and modulate the expression of allergy related cytokines make PCs attractive agents for mitigating food allergy.

3.2 Celiac disease

Celiac Disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten proteins by genetically susceptible individuals [53]. Although there is, so far, no clear explanation for the burst of gluten-related disorders in recent years, it has been speculated that this may have occurred due to an increase in the consumption of gluten-containing foods, some based on novel wheat stains produced for technological rather than nutritional reasons, and the humans' over usage of antibiotics and pesticides, both of which rendered people more sensitive to allergenic plant proteins [54].

Mechanistically, CD is characterized by an aberrant T-cell response towards gluten peptides that, because of their high proline content, are left undigested at the gut lumen [55]. According to recent findings, some of these gluten peptides have the ability to bind to the chemokine receptor CXCR3 on the surface of intestinal epithelial cells, leading to a PKC-α dependent tight junctions disassembly and increased intestinal permeability [56]. In the lamina propria, gluten peptides are selectively deamidated by the enzyme tissue transglutaminase 2 (TG2) and later presented to gluten-specific HLA-DQ2 or HLA-DQ8-restricted CD4+ T cells by antigen-presenting cells [57]. Once activated, and in response to tissue signals provided by stressed epithelial cells, gluten-specific CD4+ T cells support the activation and differentiation of both autoreactive TG2 and gluten-specific B cells into IgA and IgG-producing plasma cells while increasing the cytolytic properties of intraepithelial cytotoxic T lymphocytes which kill distressed intestinal epithelial cells based on the recognition of stress-induced ligands e.g. MHC-class I polypeptide-related (MICA/B) and HLA-E molecules [58].

The rapid increase in the global incidence of CD, together with the growing concern of CD patients regarding their quality of life when on a gluten-excluding diet, led researchers scrambling for alternative (or complementary) ways to tackle the celiac gut's response to gluten and potentially restore tolerance [59]. Among the candidates, recent evidence brough PC into the spotlight as promising agents to be used in CD management due to their wide range of beneficial properties and positive impact on human health. Nevertheless, and despite the advances made in the past few years, there are still many unresolved questions in this area, due to the multitude of action mechanisms underlying the response to PCs intake and large interindividual variability [60].

In a CD context, PC could act at several levels: they could impair gluten digestion and peptide availability at the intestinal lumen, reduce inflammation, enhance intestinal barrier integrity and function and have a prebiotic effect through inhibition of certain pathogenic groups and stimulation of beneficial bacterial growth [61]. As shown by Dias et al. green tea PC and grape seed procyanidins can readily interact with one of the most immunoreactive gluten peptides - the 33/32-mer - primarily through an unspecific, entropy-driven, hydrophobic effect [62]. In general, these interactions were found to be similar to the interactions between polyphenols and proline-rich salivary proteins in that they are the result of cooperative binding mechanisms involving both enthalpic and entropic effects. Staggeringly, the primarily PC-binding sites within the 32-mer peptide sequence were also unveiled: they correspond to leucine, tyrosine and phenylalanine-containing domains, located in four well-defined and almost indistinguishable hydrophobic clusters, equally spaced by non-polar proline residues [63]. Subsequent transepithelial transport studies on Caco-2 cell monolayers highlighted the ability of dietary doses of

EGCG to scavenge and reduce the apical-to-basolateral translocation of the 32-mer peptide in vitro to nearly undetectable levels [62]. Still, it remains unclear whether this attenuation will have any implication in the activation and triggering of a gluten-specific T-cell mediated immune response, though the structural changes induced on the peptide upon binding provides foundational support for functional changes in its immunostimulatory action [64].

On another recent breakthrough, green tea catechins were found to prevent gluten digestion through physical interactions with gluten proteins and prevention of hydrolysis by digestive enzymes [65]. According to this study, the presence of green tea catechins resulted in a decreased formation of low molecular weight gluten peptides, decreased intestinal permeability and reduced inflammation [66]. A similar finding was also made available by Kramer and coworkers which shown a significant inhibition of IFN-γ- or gluten peptide p31–43-induced increases in CD inflammatory biomarkers including TG2, COX-2, IL-15, IL-1β, IL-6, and IL-8 by procyanidin B2-rich cocoa extracts [67].

Regarding the effect produced by PC-rich dietary patterns on gut and blood microbiomics in CD patients, data are still scarce. Nevertheless, there is now several pieces of evidence suggesting that PCs may represent a relevant factor in shaping the intestinal microbial ecosystem (i.e. the microbiota and derived metabolic products) and modifying the relative abundance of specific bacterial taxa in dysbiotic CD subjects [68]. By modulating the concentration of health-affecting microbial metabolites in the gut e.g. butyrate, polyphenols are likely to regulate a plethora of biological responses at the intestinal level that control, for instance, tight junction integrity, anti-inflammatory signaling, immune cell migration, adhesion, and cellular functions such as proliferation and apoptosis [69]. Accordingly, it has been found that treatment of CD-derived organoids with microbiota-derived bioproducts, including butyrate, improved epithelial barrier functionality and reduced gliadin-induced IFN-γ and IL-15 secretion [70]. Of note, both butyrate and lactate have been shown to exert a relevant role in regulating FoxP3 isoform expression in T cells and consequent activation of a Th17-driven immune response in CD subjects [71]. But, as well-controlled intervention studies are still lacking, future studies should be focusing on providing a proof of concept of the reliability of a PC-based dietary intervention in the context of microbiota-intestinal permeability and CD health outcomes.

4. Use of phenolic compounds as alternative to synthetic additives in functional foods

In the last 50 years, food systems have dramatically changed where the access to foods, with high levels of salt, sugars and saturated fats have become cheaper and more widely available than micronutrient rich foods—such as fresh fruits and vegetables. Consequently, the incidence of NCDs greatly increase such as obesity, type 2 diabetes as well as immune reactions to food, claiming for action. Food industry faces important challenges regarding the increase in these NCDs. In addition, besides the health challenge, other key points demand advances for the production of food and significant changes in food systems, namely population growth, rural development, globalization and climate changes.

Diverse personal, cultural or economic factors influence consumers' dietary behavior. Furthermore, political issues as well as the food labeling, marketing, information about food and policies could impact on price affecting consumer demand. Under this framework, strategies to protect vulnerable populations can be addressed to achieve a global access to healthy and sustainable diets. Based on

Figure 2.
Use of polyphenols as ingredients in functional foods.

aforementioned, PC extracts arise as ingredients able to promote health benefits while reducing the use of synthetic additives. This section summarizes the interest in using PC as new ingredients in food formulations including the effects on technological processes (**Figure 2**).

4.1 Reduction of sugar

As already mentioned, the Food Industry needs to diminish the sugar in food and beverages to minimize the impact of sugar consumption in the prevalence of metabolic disorders. Furthermore, public health policy strategies including sugar taxes for food industry accelerate the process. Zero-calorie high-potency sweeteners with improved taste perception are needed as new ingredients. To date, the scientific community has made some efforts to obtain natural sweeteners or designing synthetic compounds with high sweetening power. The most widely artificial sweeteners used in the Food Industry are aspartame, sucralose, saccharine, neotame, advantame and acesulfame potassium-k but whether the use of these sweetener affect our health is still not well understood [72]. Natural alternatives have been explored such as molasses, honey, coconut sugar, date sugar, maple syrup, agave nectar, and xylitol [73]. Most of them are carbohydrates obtained from vegetables, trees, seeds, roots, and nuts. Moreover, dihydrochalcone sweeteners are PC-derived compounds with proved beneficial health effects arising as a good alternative for sugar reduction [74]. However, difficulties associated with browning, replacement of the bulking and other properties that sucrose, glucose and fructose provide in many solid food products must be carefully analyzed.

4.2 Reduction of salt

Reducing the consumption of salt in general population has been identified as a priority intervention to reduce NCDs. Indeed, the World Health Organization has

agreed to diminish 30% of salt intake by 2025. The use of vegetable extracts able to enhance flavor while improving health benefits arises as a promising alternative. Under this framework, extracts rich in PC have been already used in food industry. Indeed, green tea extracts were used to enhance the flavor of fish flesh [75], soybean isoflavones enhance the flavor quality in the muscle of grass carp while contribute to health benefits [76]. Furthermore, PC-rich extracts containing aromatic compounds such as onion, garlic, celery spices and herbs could also be a nutraceutical alternative to reduce salt.

4.3 Preservative agents

Spoilage are one of the main causes of economic loses in food industry [77]. Traditionally, the use of artificial preservative technologies such as drying, freezing, thermal treatments and more recently modified atmosphere packaging and non-thermal physical treatments (pulsed electric fields and high hydrostatic pressure) have been employed to extend shelf life of food [78]. Synthetic chemical preservatives such as, tartaric or citric acids; sulphites, sorbate, propionate and benzoate; or nitrites and nitrates, have been extensively used but in recent times the use of natural products as preservative agents acquire relevance [79]. The increase in the consumption of minimally processed foods joined with the clean label requirements boost the trend to explore the use of natural antimicrobials for food preservation. PC have been widely reported as antimicrobial agents [80]. Indeed antimicrobial extracts containing PCs have been already designed for this purposes, such as an extract of moso bamboo (Takeguard™) launched by Takex Labo (Osaka, Japan) or a mixture of different natural antimicrobial extracts (Biovia™ YM10) including green tea extract launched by Danisco DuPont [81]. **Table 1** summarizes the already tested PC from food byproducts with antimicrobial properties.

4.4 Colorant agents

Some PC are natural pigments with high potential to be incorporated into food systems as colorant agents. However, the great reactivity and lack of chemical stability make necessary to deliver these compounds in encapsulated forms. Among PC sources, flowers such as *Clitoria ternatea* petals are commonly used in health drinks and natural food colorants [95]. Moreover, Brazilian fruit peel - jabuticaba (*Plinia cauliflora* (Mart.) Kausel) and propolis from Tubuna (*Scaptotrigona bipunctata*) encapsulated in alginate beds have been tested as a new ingredient with colorant properties and health outcomes [96]. Betacyanins (red-violet) and betaxanthins (yellow-orange), from beets are also powerful antioxidants, which can be used as natural colorants in the food industry [97]. Furthermore, pecan nut shell has been already studied as a food colorant for active packaging for color stabilization [98].

4.5 Emulsifier agents

New alternatives to reduce the content of saturated fats while maintaining the emulsifying properties of sauces must be evaluated. To date, some amphiphilic plant proteins such as wheat gliadins and maize zeins have rheological properties suitable to fabricate colloidal particles for stabilizing foams and emulsions. However, in recent years the use of novel emulsifiers to obtain nutraceutical emulsions are being studied. In this context, the ability of PC to bind to proteins have been described as able to improve the chemical and physical stability of emulsions, arising as a good source of nutraceuticals while emulsifier agents. The emulsifying

Food Byproducts	Polyphenol	Target organisms	References
Coffee extract	Flavan-3-ols, hydroxycinnamic acids, flavonols, and anthocyanidins	*Pseudomonas fluorescens*, *Staphylococcus aureus*, Aspergillus flavus, *Listeria monocytogenes*, Bacillus subtilis, *Candida albicans*	[82]
Green tea waste	Flavan-3-ols (Tannins)	Staphylococcus aureus, *Escherichia coli*, Listeria monocytogenes, *Bacillus coagulans*, Shigella flexneri	[83]
Green, white and black tea extracts	Flavan-3-ols (Tannins)	Salmonella typhimurium, Listeria monocytogenes	[84]
Olive pomace	Phenolic acids (oleocanthal, deoxyloganic acid)	Escherichia coli O157:H7, Salmonella enteritidis, Listeria monocytogenes, and Staphylococcus aureus	[85]
Olive leaf extract	Phenolic acids and flavonoids	Listeria monocytogenes, Escherichia coli O157:H7, Salmonella enteritidis, Candida albicans	[86]
Pomegranate fruit peel extract	Phenolic acids and flavonoids	Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, and Pseudomonas fluorescens, *Pseudomonas stutzeri*, Gram-negative bacteria, Gram-positive bacteria, and fungi	[87]
Winery products	Phenolic acids, flavonoids, stilbenes	*Bacillus cereus*, Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, *Salmonella enterica*, Staphylococcus aureus, Yersinia enterocolitica	[88]
Grape pomace	Phenolic acids, flavonoids, stilbenes	Staphylococcus aureus, Salmonella, Enterococci, total aerobic mesophilic and psychrotrophic bacteria	[89]
Grape fruit seed extract	Flavonols, phenolic acids, catechins, proanthocyanidins and anthocyanins	Pseudomonas spp.	[90]
Myrtle berries seeds extract	Phenolic acids and flavonoids	Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus	[91]
Date extract	Phenolic acids and flavonoids	Escherichia coli, Bacillus subtilis, *Enterococcus faecalis*, and Salmonella spp.	[92]
Buckwheat hull extract	Flavonols (quercetin derivatives)	Gram-positive (Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis) and Gram-negative bacteria (Salmonella choleraesuis, Escherichia coli, and *Proteus mirabilis*)	[93]
Pumelo peel extract	Flavonoids	Escherichia coli, *Pseudomonas aeruginosa*, Bacillus subtilis, Staphylococcus aureus, Chromobacterium violaceum, and Vibrio anguillarum	[94]

Table 1.
Polyphenols as food preservatives. Adapted from [81].

properties of proteins have also been modified by introducing polysaccharides; however, little to nothing is known about how ternary interactions could affect the physical stability of emulsions. Ternary conjugates were fabricated by covalently bonding polyphenol, protein, and polysaccharide together. The protein was used to provide surface activity, the polysaccharide to provide strong steric repulsion, and the PC to provide functional properties [99]. But, some PC have poor interfacial activity, like green tea PCs [100]. However, the interaction between green tea PC and the protein β-lactoglobulin (β-lg) (spontaneous nanocomplexes formation) was sucesfully used as an emulsifier agent in fish oil [101]. Colloidal complexes were also prepared from pea protein and grape seed proanthocyanidin and the ability of these complexes to form and stabilize oil-in-water emulsions were verified [102]. Overall, covalent and noncovalent interactions between proteins and PC have provide novel insights into the interfacial behaviors of novel emulsifiers [103].

4.6 Matrix effect

There are several factors which could influence the PC delivery to bloodstream, to their target tissues and biological activities. Disruption of the natural matrix or the microstructure created during processing may influence the release, transformation, and further absorption of some nutrients as well as functional ingredients such as PC in the digestive tract. Some *in vitro* studies verified the effect of milk proteins in PC bioaccesibility and bioactivities after consuming oat based breakfast cereals with blueberry fruit [104]. The absorption of flavanols, such as green tea catechins, is influenced by epimerization reactions, which usually occur during technological processing as well as the presence of lipids and carbohydrates. Moreover is enhanced by the presence of piperine and tartaric acid [105]. Phenolic acids and Flavanones such as hesperidin are affected by the attached sugar, which can covalently link these compounds to the cereal bran matrix [106, 107]. There are only a few examples reported on PC release from the food matrix, but existing information established a direct relationship between the absorption and dose but is sometimes linear and sometimes saturated [108]. The lack of systematic information on the effects of other components on the bioavailability of PCs needs to be performed. This information must be completed by human studies to further establish general principles affecting absorption in vivo. Information derived from such experiments could be useful for the optimal design of future bioefficacy studies for functional foods production.

5. When sensory properties can compromise the intake of potential healthy phenolic compounds

It is undeniable that PC are a key target for the Food Industry on the design of new healthy products, as widely documented in the previous sections. Nevertheless, PC account for the main organoleptic properties of food products, mainly color and taste. Anthocyanins account for the (red) color of fruits and derived products, and flavanols and tannins are linked to unpleasant taste properties, namely astringency and bitterness. So, while PC have high expectations towards the development of health-orientated and functional products, a central challenge could be aligning their applications with consumer acceptability.

5.1 PC sensory properties

Astringency is a trigeminal sensation described as a mouthfeel of dryness, roughness and puckering sensations. It is induced by different classes of compounds

such as tannins, acids and alumns. In food, tannins are the main contributors to astringency perception. Several mechanisms are currently proposed to explain astringency molecular onset. The most studied one relies on the interaction and precipitation of particular families of salivary proteins, namely proline-rich proteins. The involvement of oral epithelial cells has been also reported and more recently the activation of G-protein coupled receptors was also reported. The involvement of oral mechanoreceptors is also a hypothesis despite no tangible data has been reported so far.

On the other hand, the perception of bitter taste is well characterized. Bitterness is perceived by activation of specific receptors, the bitter taste receptors (TAS2Rs). Humans express 25 TAS2Rs to perceive hundreds of bitter tasting molecules, with very wide structural features. These receptors occur at the cell-membrane of gustative cells within taste buds of human tongue. Among the wide diversity of TAS2Rs agonists reported there are several PC. In fact, some of these compounds are highly efficient agonists activating TAS2Rs at very low concentrations.

The importance of these taste properties is that they have elicited negative consumer reactions when present at high intensity in some products [109], decreasing the overall acceptability. This can compromise the intended intake of these new and functional food products.

5.2 Phenolic compounds as ingredients in functional foods

In the last years, the application of PC in functional and/or fortified foods has been widely reported. This has been mainly achieved by using either food industry by-products or wastes. Most studies use by-products from fruits (43%), followed by the application of winery (19%) and vegetable (13%) by-products [110]. Among fruit and vegetable by-products, citrus, tomato, grape, and apple by-products have been used in a wide range of food products (**Table 2**). One of these by-products is pomace (e.g. derived from apple or, grape), the main solid waste generated in juice or winemaking factories. It contains plenty of different varieties of nutritionally important compounds, such as dietary fibers, carbohydrates, PC, and minerals presenting a huge potential as a source of bioactive compounds. In addition to by-products or wastes, also some other high-PC content sources have been used to supplement food such as green tea.

Most of the reported studies use different fruit cultivars, obtained from different industry practices but without a deep characterization of the PC content. So, global relationships between PC composition-rheological-sensory properties-health inputs are not easily attained.

A cross-cut problem in most studies associated with the incorporation of these compounds in food matrices is that they can modify sensory properties leading to astringency and bitterness.

5.2.1 Addition of PC to bakery products

A significant amount of grape PC (near 70%) remains in pomace after wine making, the most important being tannins, anthocyanins, and phenolic acids, the quantities depending on the grape variety and winemaking practices. This by-product has been applied in bread, pancakes, pasta, biscuits, and other derived products. Some studies are summarized in **Table 1**.

White grape pomace has been also used to supplement biscuits [111]. In this case, the profile of PC was dominated by gallic acid, tyrosol and γ-resorcylic acid, which contributed nearly 87% of total PC. In this case, at sensory level, the replacement by grape pomace affected mainly on two attributes: fruity-acidic

PC matrix	Goal	Food product	Sensory properties				Ref.
			Physical	Color	Taste	Overall acceptability	
Wine grape pomace	Antioxidant activity	Biscuits	Reduced thickness, increased hardness	Lower lightness, higher red color, lower yellow		10% of grape pomace	[111]
	Functional food, wastes valuation, antioxidant properties	Wheat bread		Decreased brightness, increased red color	Increased bitterness, aftertaste	Zenel grape pomace was suggested to be used	[112]
Grape pomace	Healthier and high dietary fiber content	Muffins		Decreased lightness		High acceptability using at 10%	[113]
		Biscuits	No changes in thickness	Darkness	Increased bitterness	5% improved their acceptability	[114]
Grape seed extract	Healthier, extended shelf-life products	Yogurt	Increased hardness and consistency	—	Decreased taste rating	Decreased overall rating	[115]
Grape pomace	Higher antioxidant activity	Yogurt	General loss of textural quality	—	Unpleasant flavors, not enough sweet	Decreased overall rating	[116]
(Water-treated) Coffee sylverskin	Alternative fiber for oil replacement	Cake	Increased hardness, similar springiness and cohesiveness	Darker and more yellow color	Increased bitterness especially for non-treated coffee sylverskin	Water-treated coffee silverskin (up to 30%) induces no significant alterations on cake characteristics.	[117]
Tomato pomace	Alternative fiber and protein sources; increase antioxidant activity	Beef frankfurter, beef ham and meat-free sausages	Increased hardness and chewiness	Increased yellowness and lightness (meat-free sausages); highest redness	No significant changes in taste	Overall acceptance equal or higher than control	[118]
	Alternative fiber, byproduct valorization, increased health properties	Chicken sausages	Decreased chewiness and guminess, no changes in hardness	No significant changes	No significant changes	Overall acceptability (3%) equal to control	[119]

PC matrix	Goal	Food product	Sensory properties				Ref.
			Physical	Color	Taste	Overall acceptability	
Tomato powder	Replace synthetic colorants and reducing the nitrite level	Frankfurters	Decreased tenderness	Increased yellowness	Better flavor	Overall acceptability increased	[120]
Plant extracts (lemon balm, mint, lavender, rosemary and sage)	Functional (healthier and tastier) goat-milk new beverage	Milk-derived beverage	No significant changes	No significant changes	Some extracts (e.g. lavender) induced bitterness and astringency	Overall acceptability was found to be mint>rosemary>sage>lemon balm>lavender	[121]
Apple pomace		Cookies	Reduced thickness but no other significant changes	Decreased lightness and more brown color	Improved taste (higher fruity flavor)	Overall acceptability equal to control	[122]
	Fortification of bakery and meat products	Meat products (chicken patties and beef)	Lower hardness, springiness, cohesiveness, and chewiness	Darker and higher redness	—	—	[123]
	Increased antioxidant activity	Cider	—	Increased yellow color, higher luminosity	Higher bitterness and astringency	Overall acceptability higher than control	[124]
Rheology (dough) and texture properties							
Wine grape pomace	Antioxidant activity	Biscuits	Decreased water absorption	Reduced stability	Development time not modified		[111]
	Functional food, wastes valuation, antioxidant properties	Wheat bread	Decreased water absorption (Merlot dough); no changes to control (Zelen dough)	Increased stability	Higher development time (Merlot dough); no changes to control (Zelen dough)	Changes in all texture properties (stickier crumb, decreased crust toughness, increased sand feeling)	[112]

PC matrix	Goal	Food product	Sensory properties				Ref.
			Physical	Color	Taste	Overall acceptability	
Grape pomace	Healthier and high dietary fiber content	Muffins				hardness and chewiness increased; springiness, cohesiveness, resilience decreased	[113]
Grape peels	Supplementation of dietary fibers	Dough	Not modified	Reduced stability	Development time not modified	Higher hardness, lower adhesiveness, lower cohesiveness	[125]

Table 2.
Effect of fortification with PC in sensory properties of functional foods.

flavor notes and color. These authors studied the stability of PC during the baking process. Within the PC, the most stable ones were as follows: γ-resorcylic acid (loss of 11%) < gallic acid (loss of 18%) < tyrosol (loss of 21%) < catechin (loss of 31%) < isovanilic acid. Moreover, procyanidins B1 and B2, which were identified in the pomace were not retained after the baking process. At the end, there was an almost tenfold increase in bioactive compounds in the biscuits enriched with 30% of pomace, from 0.11 $mg.g^{-1}$ (control) to 1.07 $mg.g^{-1}$.

The stability of PC at the end of the baking process was also assessed upon supplementation of biscuits and bread with green tea extracts to increase the antioxidant ability of the final product [126]. While it was found ca. 30 and 21% of retention of epicatechin gallate and epigallocatechin gallate, respectively, for biscuits supplemented with 300 mg green tea extract per 100 g flour [127], the retention in freshly baked bread were ca. 83 and 91%, respectively [105]. At the end, it was determined that one piece of bread (53 g) containing 150 mg of GTE/100 g of flour will provide 28 mg of tea catechins, which is ~35% of those infused from one green tea bag (2 g). However, none of these studies inferred about the sensory profile of these food products.

Ross and colleagues studied the consumer acceptance of grape-seed (GS) flour-containing food products, namely pancakes and noodles [128]. The GS flour was obtained from winemaking by-products from different grapes (Merlot and Cabernet Sauvignon). Despite the PC content of the GS flour was not characterized, this organic material is well-known to have a high content in procyanidins. It was observed that the supplementation with GS flour led to a decrease on consumer acceptability of pancakes and noodles, especially for taste (bitterness), mouthfeel (astringency) and texture. This is not surprising since astringency and bitterness are the two main descriptors of procyanidins.

Coffee sylverskin, a byproduct of coffee industry highly rich in PC, has been also evaluated to supplement baking products such as cookies [129] or cakes [117]. The supplementation with this byproduct had improved the functional quality of cookies by increasing their PC contents, antioxidant capacities and *in vitro* bioaccessibilities. However, all supplementation concentrations had lowered the consumers' flavor-taste scores and overall acceptability, which was attributed to the bitter taste of this food matrix.

The use of apple pomace has functional ingredient has been recently reviewed [130] and has been applied with success in several bakery products, namely bread, scones, cakes, and muffins. The addition of 5% of the apple pomace was found to not significantly impact the sensory properties of cookies [131].

5.2.2 Addition of PC to coffee

GS pomace (derived from Chardonnay winemaking), was evaluated to be used as a functional ingredient in brewed coffee [132]. This GS pomace could be added at 6.25% replacement without significantly affecting the overall consumer acceptance of coffee compared to the control (0%). These authors chose the GS pomace from a white grape variety because it has lower levels of tannins and no anthocyanins compared to red varieties, and so it can be expected a lower contribution to bitterness and astringency. In fact, not only astringency and bitterness did not increase upon replacement as it was observed their significant reduction for all replacement percentages in comparison to the control coffee. Although the authors do not discuss this result, one hypothesis is that the white grape varieties are also well-known for a higher content in sugar. Mouthf eel and texture, pancakes made with Cabernet Sauvignon (25% replacement) showed the lowest acceptance, significantly different from 30% replacement with a higher acceptance. This result suggested that the

impact of GSF concentration was more apparent for in-mouth attributes mouthf eel and texture, pancakes made with Cabernet Sauvignon (25% replacement) showed the lowest acceptance, significantly different from 30% replacement with a higher acceptance. This result suggested that the impact of GSF concentration was more apparent for in-mouth attributes.

5.2.3 Addition of PC to pasta

Gaita and colleagues supplemented pasta with grape pomace [133]. These authors quantified the PC in control pasta and in the fortified one and showed an effective enhancement of PC levels. These increases were dependent on the pomace grape variety, but in general were effective for gallic, caffeic, ferulic and coumaric acids, rutin, and resveratrol. Moreover, the pasta samples with addition of pomace to a level of 3 and 6% showed improved sensory characteristics versus the control sample while the kneading and dough processing operations have not been affected.

5.2.4 Addition of PC to dairy products

Grape pomace of Chardonnay, Moscato and Pinot noir varieties has been used to supplement yogurt [116]. A total of nine PC were characterized in fortified yogurt depending on the origin of the grape pomace: phenolic acids (gallic acid, protocatechuic acid and vanillic acid); flavan-3-ols (procyanidin B1, catechin and epicatechin) and flavonols (rutin and quercetin). For most of these PC, they were stable at least for 21 days. Independently of the origin of grape pomace, all enriched yogurts add a lower liking score, especially regarding the organoleptic properties (flavor and taste). The Moscato yogurt was less accepted, with a very low mean liking score, particularly for taste and flavor. In contrast, Chardonnay was the sample with the highest mean scores for appearance, flavor and overall liking. Several informal attributes were reported by tasters such as "not enough sweet," with "unpleasant flavors", "not homogeneous" and "grainy/sandy." However, no correlation was found with the quantified PC.

Komes and colleagues used plant extracts (lemon balm, mint, lavender, rosemary and sage) for the development of functional and nutritively valuable goat's milk-based beverages [121]. The concentration of bioactive PC (rosmarinic acid, hydroxycinnamic acid derivatives and luteolin derivatives) were significantly increased in goat's milk in dependence of the added plant extract. While the extracts alone were found to be bitter and astringent, when added to the milk, some of the final beverages had acceptable levels of these two taste properties. However, the beverage enriched with lavender extract was characterized by the highest intensity of bitterness and astringency and thus consequently low overall acceptability. On the other hand, the beverage with mint extract was one of the preferred ones. Interestingly, this beverage was the one with the highest concentration of total PC, total flavonoids and antioxidant ability while was one of the less bitter and astringent beverages. This suggests that the profile of the PC present (not the concentration) should be the key to the perceived taste properties. At the end, apart from the accomplished bioactive enrichment and stability, the new functional beverages exhibited significantly enhanced sensorial properties when compared to plain goat's milk, with the highest overall satisfactoriness determined for samples fortified with mint and rosemary extracts.

5.2.5 Addition of PC to meat products

Tomato and derived-processed products generates considerable amount of by-products in the form of pomace, peel and seeds. They are rich sources of dietary

fiber and bioactive compounds. In addition to carotenes, tomato by-products are rich in vitamins as well as PC, namely phenolic acids and flavonoids. Due to their color properties, these by-products have major applications in meat and meat-derived food products, as reviewed recently [134]. The higher antioxidant activity observed for tomato waste (composed by skin and seeds) has been related with the fact that this product had the highest phenolic and flavonoid amounts, in particular rutin, quercitrin and naringenin may be more efficient as antioxidant than carotenoids with respect to preventing lipid oxidation in pressurized chicken meat. Different researches have observed some general trends in the application of tomato by-products, namely improved nutritional quality, reduced lipid oxidation and increased stability during the shelf-life period of meat products, while maintaining or increasing sensory properties and general satisfactoriness.

Green tea extracts have been also used in meat products (e.g. raw beef and chicken patties). In raw beef and chicken patties, the tea catechins treatment resulted in no significant differences in the sensory flavor, taste, and tenderness [135]. Moreover, even only a marinade with green tea instead of enrichment of meat was found to reduce the formation of heterocyclic aromatic amines while bitterness and astringency perception was neglectable [136].

At the end, surprisingly, the sensory profile reported for green tea extract-supplemented meat products (turkey burgers [126], raw beef and chicken patties [135], pan-fried beef [136] as well as other food products (biscuits, cake [137]) is usually equal or superior to the control conditions (usually containing synthetic antioxidants). Since green tea extract is well-known for its bitter and astringent taste properties this could be probably due to interactions with the food matrix compounds, as discussed ahead.

5.3 Interactions of phenolic compounds with food macronutrients

In the human diet, PC are generally consumed in foods along with macronutrients (e.g. proteins, lipids and carbohydrates). The effect of interactions between PC and food constituents is a very important topic since they can have several implications on their sensory properties and lastly on their biological effects.

5.3.1 Interaction with proteins

In food matrices, PC interaction with proteins may affect their physicochemical properties, and consequently, their sensory characteristics. The sensory implications of PC interaction with proteins are not just centered on taste. Indeed, these interactions can also influence the appearance (e.g. haze, color), aroma and texture of food products.

One of the most known effect of this interaction is haze formation in some plant-based beverages like beer, wine and fruit juice [138]. Consumers expect that these beverages are clear (free of turbidity) and to remain so during the shelf life of the product. The development of haze in beverages results in the formation of insoluble particles of colloidal or larger size that can be detected visually. This is often noted as a negative attribute affecting their acceptance and the likelihood of this product to be purchased again. Astringency and bitterness are also affected by the development of haze. Indeed, red wine astringency can be reduced by the addition of some fining agents (ovalbumin, gluten proteins or yeast protein extract) which remove reactive compounds capable of haze formation [139]. Also, in beers, the interaction between PC and malt proteins causes haze and flocculation which can be modulated by adding some fining agents that will help in the process of clarification [140]. However, fining agents should be used appropriately as they could also compromise

the flavor and the overall quality of the final product. Moreover, the use of fining agents can also remove a considerable amount of PC compromising their potential health benefits. Another example of PC interaction with proteins in beverages is the case of tea. In fact, tea astringency can be rectified by the addition of milk in which PC (flavan-3-ols) interact with milk proteins (casein and whey protein) [141].

Grace and colleagues [142] studied the effect of the fortification of soy protein isolate with concentrated PC-rich fruits and vegetables (muscadine grape and kale) by sensory analysis. These authors observed that the appearance of the incorporations had resulted in different colors, a purple-red powder for the incorporation with muscadine due to the presence of anthocyanins, and a mid-intensity green with kale caused by chlorophyll incorporation into the matrix. Also, panel evaluators indicated that unfortified protein formed clumps in the mouth, while the fortified muscadine and kale matrices presented a creamy consistency in the mouth. Furthermore, the panel evaluators mentioned that muscadine-protein matrix presented a pleasant flavor with delicate notes of grape aroma, slight astringency, no bitterness, and low sourness in comparison with unfortified soy protein. On the other hand, soy protein fortified with kale showed a reminiscent flavor of cooked beans, moderate sweetness, low sourness, and no bitterness.

In all these examples above mentioned, PC interact with proteins in food matrices, contributing to a lower amount of PC available to interact with oral cavity constituents, including salivary proteins, resulting in a decrease of astringency perception [143] and also bitter taste.

5.3.2 Interaction with lipids

Contrary to PC-protein interactions that have been widely studied, interactions with other food constituents such as lipids are lacking a deeper and comprehensive research. The main references to the interaction between PC and food lipids concern on plant oils, especially olive oil. Bitterness is a key sensory attribute in olive oil determining its acceptability. However, the lipid matrix composition seems to be a determinant factor on the perception of bitter taste. García-Mesa and colleagues [144] demonstrated that two virgin oil matrices spiked with the same level of PC were able to produce different effects on bitterness, depending on the degree of unsaturation of the olive oil matrix. The most unsaturated matrices resulted in softer sensations and reduced bitterness in comparison with the less unsaturated ones.

The interest on using PC as food additives in food lipid matrices has also been growing. Indeed, lipid oxidation is the main source for food quality deterioration and generation of undesirable odors and flavors, compromising shelf-life, changing texture and color and reducing the nutritional value of food [4]. The use of green tea catechins as food additives with antioxidant properties is a good tool to increase the shelf life and to decrease the susceptibility of oxidative damage of food products. Furthermore, as previously referred, tea PC are able to interact with milk proteins suggesting a good retention in the cheese matrix [145]. Giroux and colleagues [145] evaluated the effect of green tea extract enrichment on the texture and organoleptic properties of Cheddar cheese during storage. The main effects observed were a decrease in the typical cheddar flavor, an increase in the global flavor intensity and astringency, color changes and increase in hardness. Nevertheless, the impact of green tea enrichment was dependent on the concentration used.

5.3.3 Interaction with carbohydrates

The first evidence of the interaction between PC and carbohydrates can be observed in fruits in which they interact in plant cell wall. Several classes of PC

have already been described to interact with carbohydrates such as anthocyanins, phenolic acids and procyanidins [143].

In the case of red wine, PC are the main contributors to color, astringency and bitterness. Several authors have reported that yeast mannoproteins interaction with PC have numerous effects on wine sensory properties, namely on color stabilization [146], reduction of astringency [147] and increased body and mouthfeel [148]. In fact, the formation of PC-carbohydrate complexes influences their association with salivary proteins leading to a decrease on astringency perception. The same reduction trend on astringency was observed for other matrices, in which soluble pectins were added to persimmon fresh juice, resulting in the complexation with soluble tannins [149]. The interaction between PC and carbohydrates depends on their structure and physicochemical properties (e.g., ionic character and viscosity). Indeed, carbohydrates which present higher viscosity can greatly affect sensorial properties. Peleg and coworkers [150] observed that the increase of viscosity of a PC-rich cranberry juice by the addition of carboxymethyl cellulose lowered the perceived astringency at 25 °C.

In conclusion, interactions between PC and macronutrients can occur in food items and impact their sensory properties. The design of new foods with high nutrient content, tasty and affordable could be a good tool to increase the consumption of these bioactive compounds. However, the creation of these foods without comprising quality, sensory properties and functionality remains a big challenge.

At the end, most of the studies based on supplementation of food products with extracts, or with food industry by-products rich in PC are somehow empiric approaches. They find an optimal dose of an extract, by-product or waste or able to have a high expected (functional/biological) activity while the negative side-effects (e.g. low loaf volume, undesirable taste properties and textural characteristics) are minimized. While this trial-error has led to some successful examples, the use of this knowledge by the food industry depends on a more systematic approach. A deep and extensive characterization of the PC profile of the extracts, by-products and wastes should be a critical point in these studies. Furthermore, consistent data regarding the binding of the PC with food matrix components, the effect of cooking practices as well as the final bioactivities are lacking. These topics will be a valuable tool to align tastiness to healthiness in a systematic and reliable way to aid food industry towards the development of functional and clean label food.

6. Conclusions

The consumption of plant-based foods, including fruits, seeds, cereals, vegetables, and derived foodstuffs, such as beverages, has been nowadays claimed to be beneficial for human health. This awareness has been shared not only by the scientific community but also by the general public. The increase in the prevalence of non-communicable diseases and particularly the immune reactions to food, prompted the establishment of nutritional recommendations to design functional and sustainable foods. In this framework, PC have a significant potential! However, attention should be paid to PC organoleptic properties, which can compromise the final consumer acceptance.

The potential use of PC on modulation of immune reactions to food has been recently explored. In this context, PC have a double potential, before and after the food intake. From one side, the recently discovered ability of PC to bind to food immunologic proteins, and general food macromolecules such as lipids, proteins or carbohydrates could influence their bioactivities opening a new way to explore the relationship between dietary PC and health outcomes. On the other side, the

modulator effects of PC on enzymatic activity, cellular redox potential, cell signaling transduction pathways or cell proliferation, as well as the ability to bind to cell receptors, that have been demonstrated sets them as potent anti-inflammatory and antioxidant compounds.

Although significant progress has been made to deepen these interactions with food macromolecules and bioactivities, considerable attention should be paid to the astringency and bitter taste elicited by PC. At the end, no matter how healthy a food is, if it does not appeal to its consumer, it is unlikely to succeed.

Going beyond, constructing food systems to all and designing foods suitable for people with specific nutritional requirements must be a priority. Under this framework, the design of clean label functional foods containing PCs as modulators of immune reactions to food while impact positively the organoleptic and technological properties of food emerge as a new reality.

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Chapter 5

The Impact of Dietary Compounds in Functional Foods on MicroRNAs Expression

Wittaya Chaiwangyen

Abstract

MicroRNAs (miRNAs) are a class of non-coding endogenous RNA molecules that are involved in post-transcriptional gene silencing via binding to their target messenger RNA, leading to mRNA degradation or translational repression. MicroRNAs can be modulated by several factors including hormones, transcription factors, and dietary compounds. These biologically active compounds have positive impact on the progression of human pathology including non-communicable diseases, which indicating that administration of diet may have potential as therapeutic agents in modulating the risk of chronic diseases. Interestingly, evidence emerging in recent years suggests that dietary miRNAs can be absorbed in human circulation, modulated human gene expression and biological functions. The exploitation of the miRNA functioning within different origins, cellular miRNAs and dietary miRNAs will help us to understand the molecular machinery as well as the regulatory mechanisms involved in fundamentally important biological processes. Therefore, this knowledge may be applied of natural bioactive compounds in preventive or therapeutic approaches.

Keywords: functional foods, microRNAs, dietary microRNAs, chronic diseases, non-communicable diseases

1. Introduction - Origin, biogenesis and functions of microRNAs

MicroRNAs or miRNAs are a class of small non-coding RNA approximately 21–25 nucleotides that modulate on gene expression post-transcriptionally via binding to the 3′ untranslated region (3′-UTR) of the target messenger RNA (mRNA), resulting in mRNA degradation or translational repression. The first miRNA, lin-4, was discovered by Ambro and his research group in 1993 and it was found to be related with larva development in *Caenorhabditis elegans* [1]. Up to date, almost 2000 miRNAs have been identified in humans (http://www.miRbase.org – 7.3.2019) [1]. It has been estimated that 1–4% of human genes expression can be regulated by miRNAs, which is the largest of genomic regulator [2]. In mammals, miRNAs have been associated with various cellular pathways with the regulation of cell differentiation, cell cycle, proliferation, apoptosis, hematopoiesis, and other cellular functions. Recent studies have highlighted the importance of mRNA regulation mechanism by validation and differential miRNA expression in a variety of human pathological conditions, including chronic diseases.

miRNAs are normally transcribed by RNA polymerase II from miRNA genes. This transcription leads to generate a primary miRNA transcript (pri-miRNA). Then, pri-miRNA is further cleaved by a microprocessor complex, which consists of Drosha, the double-stranded RNase III enzyme and DiGeorge syndrome critical region 8 (DGCR8), important cofactor, into a hairpin structure precursor miRNA (pre-miRNA) in the nucleus (**Figure 1**). The double strand pre-miRNAs with 70 nucleotides are then exported to the cytoplasm by the process of nuclear export factor exportin-5. The pre-miRNA is then processed by RNase III, Dicer, thereby generating a mature miRNA:miRNA duplex approximately 22 nucleotides in length and without a hairpin structure. The helicase enzyme cleaves miRNA duplexes into single-stranded miRNAs and incorporated into the Argonaute (AGO), TRBP and PACT proteins to form the RNA-induced silencing complex (RISC). Usually, other single strand called passenger strand or the star (*) strand will be degraded, while single strand mature miRNA is able to bind with its target mRNA and mediating translational inhibition or mRNA degradation, along with their sequence complementarity to the target [1, 3]. In plants, target mRNA will be degraded if miRNA has perfect or near-perfect complementarity to its target. In contrast to mammal, miRNAs bind to partially complementary sites in the 3′-UTRs of target mRNA, which leading to translational repression [4]. the target mRNA is either blocked (imperfect complementary) or degraded (perfect complementary) of the ribosomal translation, which sequentially impacts the cellular functions.

Phytochemicals are major plant-derived compounds that naturally found in vegetables, fruits, medicinal plants or other plants with medicinal properties including antioxidant, anti-diabetic, anti-inflammatory, antimicrobial, antidepressant, anticancer and prevention in other chronic non-communicable diseases [5–7]. Phenolic and flavonoid compounds are the most important group of bioactive compounds and second metabolites in plants which comprise of essential molecules

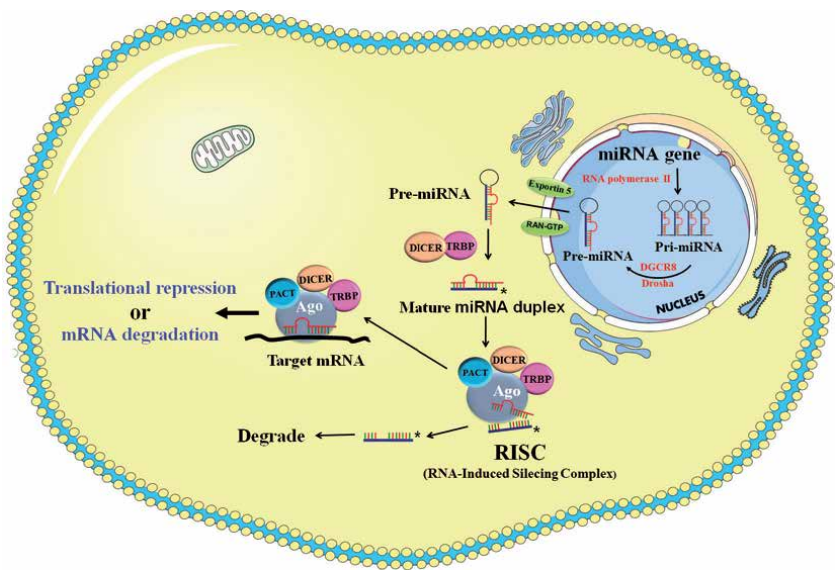

Figure 1.
miRNA biogenesis. miRNA gene is transcribed by RNA polymerase II and then forming the primary miRNA transcript (pri-miRNA), which is further cleaved by the Drosha/DGCR8 complex to generate the precursor miRNA (pre-miRNA). Pre-miRNA is then exported into the cytoplasm by exportin 5/RAN-GTP and further processed by dicer to create the mature miRNA, which is loaded into RISC, which contains AGO, PACT and TRBP proteins. Mature miRNA that binding to its target mRNA by perfect complementary binding and resulting in gene suppression by mRNA degradation. The partially complementary binding of miRNA and its target mRNA, which in turn inhibit the protein translation.

of human diet [6, 8]. It has been shown that bioactive compounds can modulate the endogenous miRNAs expression [1, 9–12]. Recently, some studies have revealed that plant-derived miRNAs (dietary miRNAs) as new bioactive compounds in plants can affect the synthesis of endogenous miRNAs [13–15]. Strikingly, miRNAs do not function only their origins but they are able to regulate the gene expression in cross-kingdom. Therefore, bioactive compounds present in functional foods are potentially regulate endogenous miRNAs expression.

2. Dietary compounds and endogenous miRNAs

Extensive studies have been performed to understand the molecular mechanism of bioactive compounds with a positive effect on chronic diseases or non-communicable diseases such as arthritis, cancer, cardiovascular diseases, diabetes and obesity [1, 16]. Emerging evidences confirm that alteration of endogenous miRNAs expression can be influenced by bioactive compounds in functional foods [16, 17] (**Figure 2** and **Table 1**).

2.1 Acetyl-11-keto-β-boswellic acid

3-acetyl-11-keto- β -boswellic acid (AKBA) is pentacyclic triterpene acids that mainly found in *Boswellia serrata* and it has been shown in medicinal properties for chronic diseases including anti-tumor, anti-inflammation, antioxidant, asthma, diabetes, atherosclerosis and analgesic [18–20]. AKBA showed the reduction of

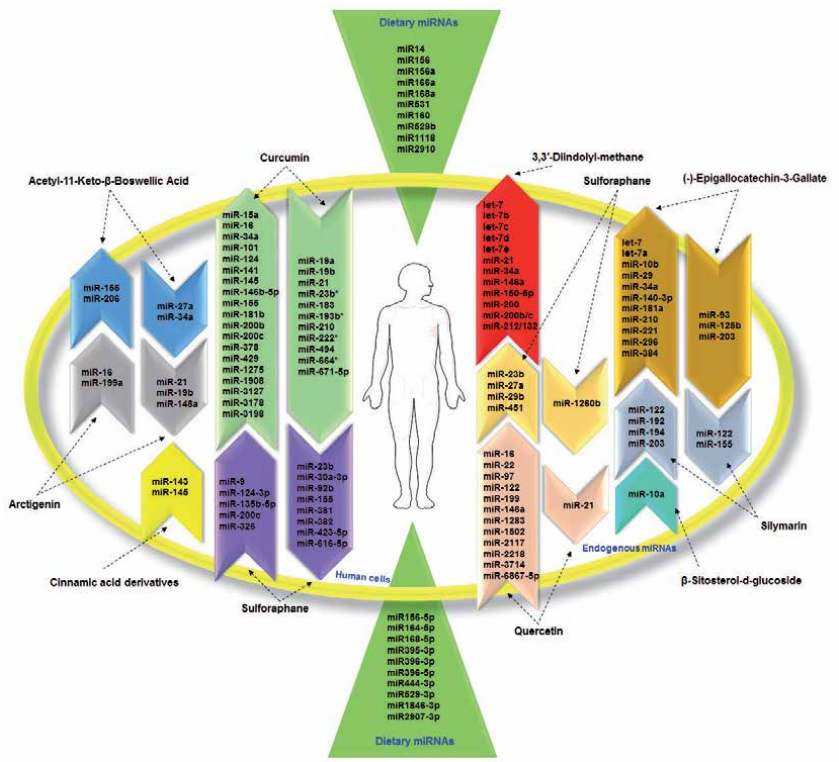

Figure 2.
Influences of bioactive compounds and dietary miRNAs on human non-communicable diseases. Ascending arrows represent up-regulated miRNAs and descending arrows represent down-regulated miRNAs by bioactive compounds. The green triangles show the positive impact of dietary miRNAs on human health.

Dietary compound	miRNA expression		Target of miRNA	Diseases	References
	Up-regulation	Down-regulation			
Acetyl-11-Keto-β-Boswellic Acid		miR-27a miR-34a	Unknown	Colorectal cancer	[23]
	miR-155		SOCS-1	Neuroinflammation	[21]
	miR-206		ER-α	Breast cancer	[22]
Arctigenin	miR-16 miR-199a		Unknown	Neuroinflammation	[28]
		miR-21 miR-19b miR-148a	Unknown	Prostate cancer	[29]
Cinnamic acid derivatives	miR-143		MAPK/ Erk5	Colon cancer	[31]
	miR-145		Unknown	Gastric cancer	[33]
Curcumin	miR-15a, miR-16, miR-34a, miR-146b-5p miR-181b	miR-19a miR-19b	Unknown	Breast cancer	[38]
	miR-101, miR-200b, miR-200c, miR-141 miR-429	miR-21	Unknown	Colorectal cancer	[39, 40]
		miR-21		Gastric cancer	[41]
	miR-145 miR-1275 miR-1908 miR-3127 miR-3178 miR-3198	miR-23b*, miR-183 miR-193b* miR-210 miR-222* miR-494 miR-664* miR-671-5p	Oct4	Prostate cancer	[42]
	miR-181b		CXCL1 CXCL2	Breast cancer	[43]
	miR-378		p38	glioblastoma	[44]
	miR-124 miR-155		Unknown	Neurodegenerative disorder	[45]
3,3′-Diindolyl-methane	let-7 miR-34a miR-150-5p		EZH2, Notch1 AR Ahr	Prostate cancer	[46]
	miR-200		FoxM1	Breast cancer	[47]
	miR-212/132 cluster miR-21		Sox4 Cdc25A	Breast cancer	[48, 49]
	let-7b, let-7c, let-7d, let-7e, and miR-200b/c		ZEB-1, E-cadherin	Pancreatic cancer	[50]
	miR-146a		Unknown	Pancreatic cancer	[51]

Dietary compound	miRNA expression		Target of miRNA	Diseases	References
	Up-regulation	Down-regulation			
(−)-Epigallocatechin-3-Gallate	miR-296		STAT3	Nasopharyngeal carcinoma	[57]
	let-7a miR34a		c-Myc	Hepatocellular carcinoma	[58]
	miR-34a	miR-93	Unknown	Prostate cancer	[59]
	miR-29 miR-210	miR-125b miR-203	Unknown	Cervical cancer	[60]
	let-7		HMGA2	Melanoma cell	[61]
	miR-384		Beclin-1	Myocardial ischemia/ reperfusion	[62]
	miR-140-3p		Unknown	Osteoarthritis	[63]
	miR-10b miR-181a miR-221		Unknown	Liver fibrosis	[64]
Genistein	miR-23b		Unknown	Breast cancer	[66]
		miR-1260b	sRRP1 Smad4	Prostate cancer	[67]
		miR-1260b	sFRP1, Dkk2, Smad4	Renal cancer	[68]
	miR-27a		Unknown	Lung cancer	[69]
	miR-29b		Unknown	Lung cancer	[70]
	miR-451		Unknown	Chronic liver disease	[72]
Quercetin	miR-200b-3p		Notch1	Pancreatic cancer	[75]
	miR-146a		EGFR	Breast cancer	[76]
	miR-16		HOXA10	Oral cancer	[77]
	miR-22		WNT1/β-catenin	Oral cancer	[78]
	miR-97 miR-298 miR-2218 miR-1502 miR-2117		Unknown	Oxidative stress in pheochromocytoma	[79]
	miR-503-5p miR-1283, miR-3714 miR-6867-5p		CCND1	Endometriosis	[80]
	miR-122	miR-21	Unknown	Liver fibrosis	[81]
	miR-199		Sert1	Hypoxia	[82]
Silymarin	miR-203		class 1 HDAC proteins and ZEB1	Lung cancer	[84]
		miR-155	Unknown	Rheumatoid arthritis	[85]
		miR-122	Unknown	Liver damage	[86]
	miR-122 miR-192 miR-194		Unknown	Liver damage	[87]
β-Sitosterol-d-glucoside	miR-10a		Unknown	Breast cancer	[89]

Dietary compound	miRNA expression		Target of miRNA	Diseases	References
	Up-regulation	Down-regulation			
Sulforaphane		miR-23b miR-92b miR-381 miR-382	Unknown	Breast cancer	[92]
		miR-616-5p	GSK3β/β-catenin	Lung cancer	[93]
	miR-135b-5p	miR-30a-3p	RASAL2 Cx43	Pancreatic cancer	[94]
	miR-200c		Unknown	Oral cancer	[96]
	miR-9 miR-326		Unknown	Gastric cancer	[97]
	miR-124-3p		STAT3	Nasopharyngeal cancer	[98]
		miR-423-5p	Unknown	Liver fibrosis	[99]
		miR-155	Unknown	Neuroinflammation	[100]

Table 1.
Summary of miRNAs bioactive compounds and miRNAs expression in human pathology.

inflammatory miRNA expression, miR-155 and increased the expression of miR-155 target gene, suppressor of cytokine signaling-1 (SOCS-1) in neuroinflammatory mice model [21]. Therefore, AKBA might be used for treatment of neuroinflammatory disorders. AKBA also induced breast cancer cell cycle arrest, apoptosis and decreased the expression of estrogen receptor alpha (ER-α) via the up-regulation of miR-206 [22]. In addition, combination of AKBA and curcumin suppressed colorectal cancer growth through the down-regulated miR-27a and miR-34a expression [23].

2.2 Arctigenin

Arctigenin (AR) is a phenylpropanoid dizbenzylbutyrolactone lignin and was first identified in *Arctium lappa* L. Several studies showed anti-inflammatory, anti-cancer, anti-viral, immune modulatory activities of AR [24–27]. The study demonstrated that AR upregulated miR-16 and miR-199a expression by decreasing upstream protein (IKKα and IKKβ) expression and inhibiting NF-κB signaling pathway activity, thereby reducing inflammatory cytokines production in neural cells [28]. The combination treatment of AR and quercetin significantly inhibited the oncogenic miRNAs expression including miR-19b, miR-21 and miR-148a in prostate cancer cells. AR and quercetin also showed anti-migration activity in prostate cancer cells [29].

2.3 Cinnamic acid derivatives

Cinnamic acid derivatives can occur naturally in plants and their structure composing of benzene ring and acrylic acid group. Several compounds of cinnamic acid derivatives have been identified including artepilin C, baccharin, drupanin, ferulic acid, curcumin, caffeic acid, p-hydroxycinnamic acid, coumaric and chlorogenic acids, etc. [30, 31]. Medicinal activities of cinnamic acid derivatives have been reported such as anti-inflammatory, anti-oxidant, anti-viral, anti-microbial, anti-diabetic, neuroprotective and anti-tumor activities [30–32]. Cinnamic acid derivatives

from propolis significantly induced colon cancer cell apoptosis through TRAIL/DR4/5 and/or FasL/Fas death-signaling pathways and via the upregulated miR-143 expression, resulting in decreased the target gene MAPK/Erk5 expression and its downstream target c-Myc [31]. Moreover, Li et al. demonstrated that cinnamic acid derivatives decreased gastric cancer cell proliferation through the up-regulation of miR-145 and down-regulation P13K/Akt signaling pathway [33]. Therefore, cinnamic acid derivatives have a potential as therapeutic agents for cancer.

2.4 Curcumin

Curcumin[(1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione] is well known as natural polyphenol and derived from the rhizome of turmeric or *Curcuma longa* Linn [34, 35]. Curcumin has been shown to possess of several medicinal properties such as anti-inflammation, antioxidant, pro-apoptosis, chemoprevention, anti-proliferation, wound healing, anti-nociception, anti-parasite, anti-malaria, anti-diabetes, neuroprotection and anti-tumor [34, 36]. Numerous studies have been focused on curcumin as a novel anti-cancer drug due to the inhibition of NF-kB, Akt/PI3K, and MAPK pathways and enhancement of p53 by curcumin, thereby inhibited several cancer cells proliferation, migration, invasion and induced apoptosis [35, 37]. Emerging data suggest that curcumin dysregulate oncogenic miRNAs and tumor suppressor miRNAs expression in various type of cancers such as lung cancer, prostate cancer, breast cancer, colorectal cancer, nasopharyngeal carcinoma, pancreatic cancer, ovarian cancer and etc. [35]. Curcumin have been shown to up-regulation of miR-15a, miR-16, miR-34a, miR-146b-5p and miR-181b and down-regulation of miR-19a and miR-19b upon treatment of several breast cancer cell lines with curcumin [38]. Curcumin but not 5-fluorouracil, upregulated the expression of miR-101, miR-200b, miR-200c, miR-141 and miR-429 and down-regulated oncogenic miR-21 in colorectal cancer cells [39, 40]. In addition, miR-21 was down-regulated in gastric cancer with curcumin treatment, resulting in inhibition of cell migration and invasion by regulation of the PTEN/PI3K/AKT pathway [41]. Lui et al. showed that curcumin up-regulated 6 miRNAs (miR-145, miR-1275, miR-1908, miR-3127, miR-3178, and miR-3198), whereas 8 miRNAs (miR-23b*, miR-183, miR-193b* miR-210, miR-222*, miR-494, miR-664*, miR-671-5p) were down-regulated when treated with curcumin in human prostate cancer stem cells (HuPCaSCs) [42]. Experimental confirmed of miR-145 function in HuPCaSCs revealed that miR-145 inhibited cell proliferation by targeting transcription factors Oct4 [42]. Another study also reported that miR-181b was up-regulated by curcumin and inhibited breast cancer cell proliferation, invasion and induced cell apoptosis by targeting CXCL1 and CXCL2 [43]. Inhibitory effect of curcumin on glioblastoma cell growth was observed and curcumin also up-regulated miR-378 expression and p38 was the target of miR-378 [44]. Curcumin and Pioglitazone combination have a potential as therapeutic applications for neurodegenerative disorders by increasing of miR-124 and miR-155 expression, thereby inhibiting the inflammatory cytokines TNF-α, IL-1β and IL-6 production and inflammation-associated enzymes COX-2, iNOS through inhibition of NF-κB activity in animal model [45].

2.5 3,3′-Diindolylmethane

3,3′-diindolylmethane (DIM) is a naturally active compound found in stomach, which derived from indole-3-carbinol (I3C) that present in cruciferous vegetables. DIM has been reported to regulate several miRNAs expression in cancer. Tumor suppressor miRNAs was upregulated by DIM in prostate cancer cells including let-7, miR-34a and miR-150-5p by targeting EZH2, Notch1 and AR and Ahr, respectively [46].

DIM also upregulated tumor suppressor miR-200, which led to inhibit the expression of FoxM1 in breast cancer cells [47]. miR-212/132 cluster and miR-21 were upregulated by DIM, which downregulated the expression of Sox4 and Cdc25A, respectively in breast cancer [48, 49]. Moreover, DIM upregulated let-7b, let-7c, let-7d, let-7e, and miR-200b/c expression, which led to inhibit the expression of ZEB-1, E-cadherin in pancreatic cancer cells [50]. It has been reported miR-146a was upregulated upon treated with DIM and suppressed the expression of MTA2, NF-κB, IRAK1, EGFR in pancreatic cancer cells [51].

DIM showed the modulation of miRNAs expression in other inflammatory diseases. The expression of miR-106a, miR-20b, and miR-125b-5p were increased after treatment with DIM and suppressed the expression of IRAK4 and TNF-α to limit responses to TLRs activated by LPS in acute liver failure (ALF) animal model [52]. DIM significantly upregulated miR-200c, miR-146a, miR-16, miR-93, and miR-22 in brain CD4+ T cells and inhibited the expression of cyclin E1 and B-cell lymphoma-2 in experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis [53].

2.6 (−)-Epigallocatechin-3-gallate

(−)-Epigallocatechin-3-Gallate or EGCG is a major polyphenol compound in green tea (*Camellia sinensis*) and derivative of catechin [3, 16]. EGCG is a powerful antioxidant, anticancer and antiangiogenic properties, which has a potential role to influence human diseases [54–56]. EGCG suppressed nasopharyngeal carcinoma cell migration and invasion through a novel signaling axis of miR-296/STAT3 regulation [57]. Gold nanoparticles (AuNPs) have been used for drug delivery as their stability and increase drug bioavailability as well as accumulation of drug in cancer cells. EGCG-capped gold nanoparticles upregulated the tumor suppressor miRNAs, let-7a and miR34a, which consecutively their targeted gene, caspase-3 was upregulated, and c-Myc protein was decreased in hepatocellular carcinoma cells [58]. miR-34a is one of the tumor suppressor miRNAs that downregulated, whereas miR-93 is highly up-regulated in prostate cancer cells. Co-transfection of miR.34a mimic and miR.93 inhibitor along with EGCG significantly decreased androgen receptor (AR) and prostate-specific antigen (PSA) expression when compared to the co-transfection without EGCG [59]. In cervical carcinoma cells, Hela (HPV16/18+), EGCG inhibited cell growth and up-regulated miR-29 and miR-210 expression, while down-regulated the expression of miR-125b and miR-203 [60]. Up-regulation of let-7 was observed in EGCG treated melanoma cells, which led to inhibit the expression of high mobility group A2 (HMGA2) [61].

EGCG showed the protective effect against myocardial ischemia/reperfusion (I/R) injury through up-regulation of miR-384-mediated autophagy by targeting Beclin-1 via activating the PI3K/Akt signaling pathway [62]. EGCG also demonstrated the anti-arthritic effects by inhibited IL-1β-induced ADAMTS5 expression and up-regulated the expression of miR-140-3p in osteoarthritis chondrocytes [63]. EGCG treatment has potential role of preventing toxin-induced fibrosis by suppression of osteopontin expression and up-regulation of miR-10b, miR-181a and miR-221 in liver hepatocellular carcinoma cells [64].

2.7 Genistein

Genistein belongs to isoflavone family and presents in soybeans with antiangiogenic, anti-metastasis, anti-inflammatory, anti-oxidant, cell cycle arrest and induction of apoptosis effects [65]. Genistein can regulate the expression of miRNAs in several call types [65]. It has been reported that treatment of genistein up-regulated miR-23b and inhibited breast cancer cell growth [66]. Genistein also exhibited anti-tumor effect

by down-regulated miR-1260b and targeting sRRP1 and Smad4 through DNA methylation or histone modifications in prostate cancer cells [67]. The same research group reported that miR-1260b was highly expressed in renal cancer cells and miR-1260b was down-regulated in genistein treated renal cancer cells [68]. The treatment of miR-1260b inhibitor inhibited the expression of its target genes, sFRP1, Dkk2, Smad4 [68]. Treatment with genistein induced non-small lung cancer cell apoptosis, caspase-3/9 activation and inhibited cell proliferation via up-regulation of miR-27a -mediated MET signaling [69]. Co-encapsulate miR-29b with genistein in hybrid nanoparticles (GMLHN) has been studied to treat effectively in non-small lung cancer cell and GMLHN showed the anti-proliferative effect by down-regulation of phosphorylated AKT (pAKT) and phosphorylated phosphoinositide-3 kinase (p-PI3K) [70].

Genistein promoted myoblast proliferation and differentiation through down-regulated miR-222 expression, resulting in increased expression of its target genes, MyoG, MyoD, and ERα [71]. Interestingly, genistein up-regulated miR-451 expression and inhibited IL1β expression and inflammation in chronic liver disease nonalcoholic steatohepatitis (NASH) mice model [72].

2.8 Quercetin

Quercetin is bioactive flavonoids that can be found in fruits and vegetables including onion, kale, apple, many berries, citrus fruits and tea [73]. Anti-cancer, anti-inflammatory, antioxidant, anti-diabetes, anti-atherosclerosis and anti-viral effects have been reported in different in vitro studies for quercetin [74]. Several studies have focused on quercetin and miRNAs modulation for therapeutic approaches. miR-200b-3p was up-regulated in pancreatic cancer cells when treated with quercetin, resulting in inhibition of self-renewal and decrease of proliferation through Notch1 signaling pathway [75]. Quercetin significantly inhibited breast cancer cell proliferation and invasion via up-regulated miR-146a expression and targeting EGFR [76]. Quercetin inhibited cell viability, migration and invasion by up-regulated miR-16 and targeting HOXA10 in oral cancer cells [77]. In addition, quercetin decreased oral cancer cell viability and increased cell apoptosis via miR-22/WNT1/β-catenin pathway [78].

Recently, quercetin modulated 34 miRNAs expression (5 upregulated and 29 downregulated) and novel miR-97, miR-298, miR-2218, miR-1502, and miR-2117 were identified in pheochromocytoma of the rat adrenal medulla that responded for protective effect against oxidative stress through PI3K-AKT signaling pathway [79]. Treatment of quercetin inhibited proliferation of endometriosis through up-regulated miR-503-5p, miR-1283, miR-3714 and miR-6867-5p by targeting CCND1 [80]. TGFβ1 is a fibrosis inducer and quercetin significantly down-regulated miR-21 and TGFβ1 and up-regulated miR-122 in liver fibrosis [81]. Protection of cardiomyocyte against hypoxia caused insults of quercetin has been reported by up-regulation of miR-199 mediated sirt1 expression and AMPK phosphorylation [82].

2.9 Silymarin

Silymarin is a flavonolignans extracted from the milk thistle *Silybum marianum* (L.) gaernt and recent studies have demonstrated the anti-cancer, anti-inflammatory, vascularization inhibitory, antioxidant, hepatoprotective, cardioprotective and anti-metastasis activities of silymarin [83]. Several miRNAs have been implicated in the invasive potential of cancer cells. Tumor suppressor miRNA, miR-203, was up-regulated and class 1 HDAC proteins and ZEB1 were repressed with silymarin treatment, resulted in inhibition of non-small cell lung cancer migration [84]. Silibinin, the major active constituent of silymarin extract, induced apoptosis and ERβ expression, inhibited cell proliferation, and reduced pro-inflammatory cytokines expression including IL-17 and

TNF-α, through ERβ binding and down-regulated miR-155 in rheumatoid arthritis [85]. miR-122 is liver-specific miRNA and was down-regulation upon silymarin treatment in rat model for hepatoprotective and radio protective effects via increased superoxide dismutase (SOD), glutathione (GSH) and reduced lipid peroxidation (MDA) [86]. It has been reported the hepatoprotective activity of silymarin on thioacetamide-induced liver damage by restored miR-122, miR-192, and miR-194 expression levels [87].

2.10 β-Sitosterol-d-glucoside

β-Sitosterol-d-glucoside is bioactive compounds that has been isolated from *Agave angustifolia* and sweet potato [88, 89]. Pharmacological activity of β-Sitosterol-d-glucoside has been reported including immunomodulatory, anti-inflammatory, cytotoxic, and antiparasitic activities [88]. β-Sitosterol-d-glucoside exhibited cytotoxic effect in breast cancer cells by up-regulated miR-10a expression and decreased the PI3K/Akt signaling pathway [89]. Treatment of β-Sitosterol-d-glucoside can down-regulate miR-322-5p, miR-301a-3p, miR-129-5p, miR-322-3p, and miR-129-2-3p in neural stem cell and their targets are related to the regulation of proliferation [90]. Therefore, β-Sitosterol-d-glucoside could be developed for further therapeutic applications.

2.11 Sulforaphane

Sulforaphane is dietary compounds in broccoli (*Brassica oleracea*) and cruciferous plants. It has been demonstrated the capability of sulforaphane for anti-inflammatory, antiaging, antidiabetic, antioxidant, anti-tumor, hepatoprotective and cardioprotective effects [91]. Plant-derived phytochemicals including sulforaphane are potentially affected miRNAs expression. Sulforaphane inhibited breast cancer cell cycle arrest and senescence via down-regulation of miR-23b, miR-92b, miR-381 and miR-382 [92]. Anti-tumor effect of sulforaphane also reported in non-small cell lung cancer by down-regulation of miR-616-5p and targeting GSK3β/β-catenin signaling pathway [93]. Sulforaphane inhibited the progression of pancreatic cancer through down-regulated miR30a-3p with the increasing of its target, Cx43 expression and upregulated miR-135b-5p mediated RASAL2 expression [94, 95]. In addition, sulforaphane treatment significantly increased the expression of tumor suppressor miRNA, miR-200c, resulted in inhibited the cancer stemness and tumor-initiating properties in oral squamous cell carcinomas and cancer stem cells both in vitro and in vivo [96]. Anti-proliferative and apoptotic effects of sulforaphane have been reported in gastric cancer cells, which leading to alter the expression of miR-9 and miR-326 [97]. Up-regulation of miR-124-3p and inhibition of its target, STAT3 by sulforaphane treatment were observed and thereby induced apoptosis, inhibited proliferation and decreased the stemness of nasopharyngeal cancer cell [98].

Sulforaphane has potential to inhibit hepatic fibrosis by downregulating miR-423-5p in hepatic stellate cell [99]. Sulforaphane showed the protective effect in microglia-mediated neurotoxicity by inhibited LPS-induced expression of inflammatory miRNA, miR-155 [100].

3. Dietary miRNA and human gene regulation

Several evidences demonstrated the direct modulation of cellular signaling pathways by dietary compounds could decrease the risk of chronic diseases [101]. Interestingly, it has been reported that small non-coding RNA including miRNAs can be transferred across Kingdoms, for example dietary miRNAs have been found in human body fluids and these circulating miRNAs are likely to regulate human gene

expression [15, 102–107]. The uptake of plant derived miRNAs could be in the form of raw and cooked plants in capable of stability forms [107, 108]. Due to high temperature cooking process, low pH and enzymes in digestive tract as well as enzymes in blood circulation, miRNAs might be destroyed before their functions with target mRNAs [15]. Strikingly, GC base content, 2'-O-methylation on the 3′-terminal, unique nucleotide sequence of dietary miRNAs and extracellular vesicles (exosome and microvesicle) are preventive features of plant derived miRNAs in harmful conditions [109–114].

There are numerous studies to support the functional roles of dietary miRNAs in cross kingdom gene regulation. Rice miR156a and miR168a were detected in human serum and miR168a down-regulated low-density lipoprotein receptor adapter protein 1 (LDLRAP1) expression, resulted in an increase of plasma LDL cholesterol level, **Table 2** [105]. miR2910 from *Populus euphratica* was identified in human plasma and targeting Sprouty RTK Signaling Antagonist 4 (SPRY4) gene of the Janus kinase/signal transducers and activators of transcription (JAK–STAT) signaling pathway [115]. Based on the computationally predicted miRNAs from *Camptotheca acuminate,* 14 potential miRNAs were found to be regulated 152 target human genes such as miR4723–3p, miR5780d, and miR548d-3p targeting discs large MAGUK scaffoldprotein 2 (DLG2), NUMB endocytic adaptor protein (NUMB) and glycogen synthase kinase-3B (GSK3B) genes which were related to cancers such as breast cancer, lung cancer and leukemia [116]. *Ocimum basilicum* is a medicinal plant and its bioactive compounds have potential for therapeutic approaches. miRNA target prediction analysis revealed the target of *O. basilicum* miRNAs, miR156, miR531, miR160, miR529b, and miR1118 were 87 human target genes associated with the Ras-mitogen-activated protein kinase (Ras-MAPK) signaling pathway, Alzheimer disease, breast cancer, cardiomyopathy, HIV, lung cancer, and several neurological disorders [117].

Plants	Plant derived-miRNAs	Human target gene/ Disease	References
Oryza sativa	osa-miR156a osa-miR166a osa-miR168a	LDLRAP1	[105]
Populus euphratica	peu-miR2910	JAK–STAT pathway	[115]
Camptotheca acuminata	14 miRNAs	Cancer (breast, lung and leukemia)	[116]
Ocimum basilicum	miR156 miR531 miR160 miR529b miR1118	Ras-MAPK signaling pathway, Alzheimer disease, breast cancer, cardiomyopathy, HIV, lung cancer, several neurological disorders	[117]
Curcuma longa	miR14	Rheumatoid arthritis	[120]
cabbage, spinach and lettuce	miR156a	Cardiovascular disease	[118]
Oryza sativa	miR156-5p miR164-5p miR168-5p miR395-3p miR396-3p miR396-5p miR444-3p miR529-3p miR1846-3p miR2907-3p	Cancer, cardiovascular and neurodegenerative diseases	[119]

Table 2.
Dietary miRNAs and human gene regulation.

The abundantly expressed miRNA in dietary green vegetable, miR156a which was detected in human serum and targeted the junction adhesion molecule-A (JAM-A) [118]. The JAM-A was up-regulated in atherosclerotic lesions from cardiovascular disease patients and miR156a could suppressed inflammatory cytokine-induced monocytes adhesion by targeting JAM-A [118]. The very recently report using a computational approach to predict the potential target of rice miRNAs including miR156-5p, miR164-5p, miR168-5p, miR395-3p, miR396-3p, miR396-5p, miR444-3p, miR529-3p, miR1846-3p, miR2907-3p, which can bind to the human mRNA [119]. Most of these target genes were associated with cancer, cardiovascular and neurodegenerative diseases [119]. miR14 derived from *Curcuma longa* was detected and remarkably stable in human serum for 48 h. The potential targets of miR14 were associated with inflammation in rheumatoid arthritis such as Phosphotidylinositol-specific-phospholipase C (PLCXD3), Adenylate cyclase 9 (ADCY9), and 3′ (2′), 5′-bisphosphate nucleotidase (BPNT1) [120].

4. Conclusion

It has been widely known that functional foods and their bioactive compounds have the capacity for human health benefits. To date, miRNAs have been shown a significant effect on gene expression and modulate the cellular biological functions in physiological and pathological conditions. There is emerging evidence suggesting that dietary bioactive compounds can be effective in human diseases as a result of altering miRNAs expression levels, resulting in modulation of cellular signaling pathway. Additional research the possibility of bioactive compounds for developing as novel drugs with less side effects is required *in vitro* and *in vivo*. Recently, it has been revealed in several studies that dietary derived-miRNAs are bioavailable and alter human gene expression. The cross-kingdom gene regulations of dietary miRNAs from plants to human have raised our expectations for evaluating the active therapeutic potential and dietary supplements.

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Section 2

Antioxidants Based Functional Foods

Chapter 6

Functional-Antioxidant Food

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Abstract

Nowadays, people face many different dangers, such as stress, unsafety food, and environmental pollution, but not everyone suffers. Meanwhile, free radicals are the biggest threat for humans because they lead to over 80 different diseases composed of aging. Free radicals can only be eliminated or minimized with antioxidant foods or antioxidants. The chapter on the functional-antioxidant food presents the antioxidant functional food concept, the classification, the structure, and the extraction process of antioxidant ingredients. Various antioxidant substances such as protein (collagen), polysaccharides (fucoidans, alginates, glucosamines, inulins, laminarins, ulvans, and pectins), and secondary metabolites (polyphenols (phlorotannins, lignins, polyphenols), alkaloids, and flavonoids) also present. The production technology, the mechanism, the opportunity, and the challenge of antioxidants functional food also present in the current chapter. The current chapter also gives the production process of functional-antioxidant food composed of the capsule, the tablet, tube, the pills, the powder, and the effervescent tablet.

Keywords: antioxidant, functional food, proteins, secondary metabolites, polysaccharides

1. Introduction

Today, the greatest danger of humans is free radicals, the source of about 80 different human diseases including aging [1–14]. Free radicals are produced from the pollution of the climate environment, water, food, human life, and work, and also from the natural transformation of the earth [8, 15]. In order to reduce free radicals, nature itself also has complex metabolic processes to produce inactivates and convert free radicals to a more stable form in nature. Free radicals are known as products of antioxidants that occur naturally or are produced by biosynthesis [1, 7]. These antioxidants are mainly in the form of biopolymers such as proteins, polysaccharides, and secondary metabolites (polyphenols, alkaloids, flavonoids). Each group of antioxidants possesses different antioxidant properties for different applications. They are found in a loose or persistent bond in natural resources and are difficult to extract in varying concentrations into different species. Under the increasing pressure of society and nature, human aging and disease are increasing [4, 9]. To meet the social needs

and personal development. Antioxidant supplements are increasingly popular and welcomed by consumers, and are more interested in by regulators, manufacturers, and researchers.

Hence, the chapter focus on the functional-antioxidant food composing of the structure, the extraction process, and the production technology of antioxidants into functional food, the mechanism of functional-antioxidant food. Functional-antioxidant food will mainly contain protein, polysaccharides, and secondary metabolites, for example, polyphenols (phlorotannins, lignins, polyphenols), alkaloids, and flavonoids. Antioxidant polysaccharides focus on fucoidans, alginates, glucosamines, inulins, laminarins, ulvans, and pectins, while protein is collagen from marine resources. Antioxidant functional foods will exist in the capsule, the tablet, tube, and the effervescent tablet. The materials used for extracting bioactive substances are diverse from terrestrial flora and fauna to the sea.

2. Functional-antioxidant food - concept and classification

2.1 Concept

Functional-antioxidant food is food containing one or more antioxidant substances extracted from plants or animals or synthesized. Bioactive substances belong to the group of protein, carbohydrate, or secondary metabolites.

2.2 Classification

Functional-antioxidant food could be classified according to different criteria, for example, shape or bioactive ingredient style. Classification of functional-antioxidant food follows their shape are susceptible to drug classifications. Hence, functional-antioxidant food classification base on their bioactive ingredient style (**Figure 1**). Functional-antioxidant food is mainly three large groups (protein, carbohydrates, and secondary metabolites. The current chapter presents antioxidant substances commonly used in the manufacture of functional-antioxidant food such as such as collagen, pectin, alginate, fucoidan, laminarin, inulin, glucosamine, ulvan, chondroitin, polyphenol, lignin, alkaloid, and flavonoid.

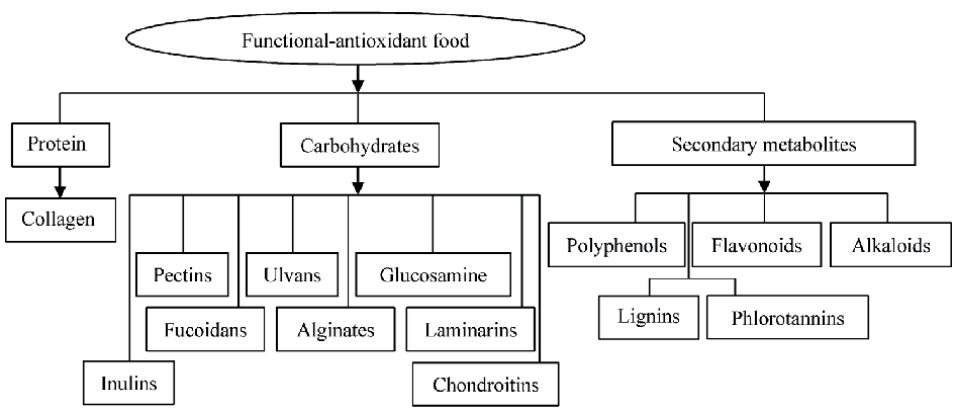

Figure 1.
Classification of functional-antioxidant food.

3. Antioxidants in nature

3.1 Protein (colagens)

Collagen belongs to the endogenous protein group, is found in connective tissues of vertebrates (skin, scales, bones, articular cartilage, blood vessels, and tendons), and contains basal components consisting of glycine, proline, alanine, and hydroxyproline. Glycine plays as the helical center in the structure of collagen. Collagen possesses a molecular weight of about 100,000 daltons, crosses intermolecular bonds, and the repeated glycine-proline-hydroxyproline chains [16]. Tiago *et al.* noticed that the scientists identify at least 28 collagen types, but the main of types I (bones, skin, tendons, and organs), II (cartilage), and III (reticular fibers, blood, and skin). The most prevalent invertebrates contain I to IV types collagens [17]. The structural stability of collagen depends mainly on the ratio of glycine and hydroxyproline that is different between animal species, even in a species. Various structural, chemicals, and amino acid content in glycoproteins lead to the difference. The collagen of fish is similar to mammalian on amino acid composition. Collagen having a molecular weight up to 3–10 kDa possesses antioxidant activity higher than other fractions. The antioxidant activity of collagen in fish is higher than that one in animals [18]. Differences in the structure and the molecular weight of acid-soluble collagens extracting from rainbow trout skins that have grown in the sea and freshwater do not occur. In rainbow trout skins, glycine content is the highest, following alanine, proline, and hydroxyproline with the various chains such as α_1-, α_2-, and β [19]. Denaturation temperature of fish collagen (25–30°C) is low in comparison to mammalian collagen (39–40°C) [20].

3.2 Polysaccharides

3.2.1 Fucoidans

Fucoidans are only found in brown algae, belong to the sulfated anion polysaccharide group. L-fucose is a basic unit in fucoidans. The primary linkage of (1 → 3)-α-L-fucopyranosyl and the linkage of alternating α(1 → 3) and α(1 → 4)-L-fucopyranosyls occur in fucoidans structure [21, 22]. Fucoidans are structurally diverse and different between various brown algae species, even in a season that leads to the difference in their content. Therefore, fucoidans exhibit bioactive diversity, for example, antioxidant [21, 23], antitumor, antibacterial, anticoagulant, anticancer [22], antivirus, immune activation, neuroprotective, and the protection of the stomach and liver [24]. Fucoidan contents in sterile tissue (dry matter) and reproductive tissue of kelp species ranged from 0.5–13% and 1.4–69%, respectively [25]. For brown algae grown in Vietnam, fucoidan content usually ranges from 0.8 to 3.5%, compared to dried algae. Fucoidans that extract from brown algae have a color range from light brown to dark brown, depend on their purification.

3.2.2 Alginates

Alginates belongs to sulfate polysaccharide, exist in brown algae species with the linear structure of copolymers, and the basic units of β-D-mannuronate (M) and α-L-guluronate (G) that links via (1,4)-glucoside linkage. Alginate content is about 15 to 25% of dry algae [26]. Nowadays, over 200 different alginates appear in the market [27]. Different alginates have various G/M ratio and molecular weight that depend on the season, species, and growth sites. G/M ratio and molecular weight play an important role in exhibiting bioactive and application of alginate. Alginates form a

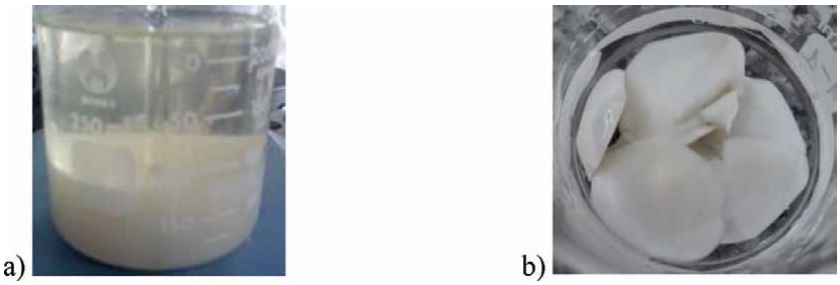

Figure 2.
(a) Alginate in 96% ethanol; (b) pectin from cactus Opuntia dillenii.

robust and rigid gel when low the M/G proportion and large guluronic blocks ratio in their structure, and reverse forming soft and elastic gels [28, 29]. Alginates possess a molecular mass larger than 50 kDa exhibiting the prevention ability of diabetes and adiposity. Numerous studies showed that antioxidant activity is also one of the bioactive characteristics of alginates [30, 31]. Therefore, alginates are useful in the food, cosmetic, functional food, pharmaceutical, even dentistry, and toothpaste such as stabilizers, emulsifying agents, or thickeners. Puried alginate has white color (**Figure 2a**).

3.2.3 Glucosamine

Glucosamine sulfate (2-amino-2-deoxy-D-glucose) is the sulfate derivatives of chitosan that formed after deacetylation for chitin. Glucosamine sulfate is an essential amino monosaccharide in connective tissues (cartilage and ligaments) of marine invertebrates and commonly the material for synthesizing glucosaminoglycans, glucoprotein, and glucolipide [32]. Nowadays, crab, lobster, or shrimp shells are materials for producing glucosamine. Numerous studies showed that glucosamine could exist in glucosamine hydrochloride, glucosamine sulfate, and N-acetylglucosamine and links to chondroitin sulfate in the connective tissues. The biological activity of glucosamine is proven (antioxidant, antiinflammatory, induce ER stress, antigenotoxic, cardioprotective, neuroprotective, O-GlcNAc modification, and antifibrotic) and is in positive proportion to the sulfate groups in their structure [33, 34].

3.2.4 Inulins

Inulins belong to the fructooligosaccharides group, composed of linear fructosyl polymers and oligomers with degree polymerization (DP) (3–65). DP of inulins in chicory consists is from two to approximately sixty units. In inulin, terminal glucose residues unit the non-reducing end via an α-(1,2) glycosidic bond and contains two or more fructosyl moieties that link each other by β-(2,1) bonds. The fructooligosaccharides (fructose oligomers) possess one glucose unit and two to four fructose units. Short fructooligosaccharides compose of 1-kestose, nystose, and 1F-fructofuranosylnystose. Small inulin oligomers (degree polymerization <10) are oligofructose and fructooligosaccharides. Inulins in plants and fungi contain β-(2,1)-D fructofuranosyl units [35]. Antioxidant activity of inulin is higher than simple sugars (fructose, glucose, and sucrose) and stable under the impact of the cooking and digestion processes (pH changes, digestive enzymes). Inulin unaltered better than ascorbic acid that lost from 40 to 90% of antioxidant activity at high temperatures. The antioxidant role of inulins exhibit better than other ROS (radical oxygen system) scavengers (vitamins C and E – the absorbance in the first part of the gut) because inulin is absorbent in the colon that occurs in vitamins C and E.

Inulins against protein oxidation are basing on the protection of the mucosal and the submucosal layers. Fructans (inulin style) respond to a defensive role against oxidative stress, at the same time activating automatic before the endogenous systems of detoxification in rats. Radical oxygen system scavenging capability of oligosaccharides in intra-peritoneal administration in vivo decide the decrease of lipid peroxidation. Besides inulins, levans (high molecular weight polymer) are about 107 Da with type β-2,6 linkages. Galactopyranosyl oligomers possess DP (3–8) with mostly β-(1,4) or β-(1,6) bonds and less β (1,2) or β-(1,3) linkages. Levans can combine various metal nanoparticles such as Levan–Fe^{2+} and levan–Cu^{+} that ROS inhibition up to 88% and 95%, and the combination exhibit antioxidant activity better than 33–40%, compared to single levans [35]. Moreover, levans possess numerous bioactivities, for example, antioxidants, anti-tumor, and anti-inflammatory [36].

3.2.5 Laminarins

Laminarins are found in brown algae, belong to a linear sulfated polysaccharide that soluble in water and 22–49% of the dry algal mass. Laminarins are known as a β sulfated glucan consists of a 3:1 ratio of β (1 → 3) and β (1 → 6) with a molecular weight of 5 kDa and (1 → 3)-β-d-glucopyranose residues [37]. Laminarin structure depends on algae species, growth period, and growth condition, while molecular weight is affected by the polymerization degree. Laminarin could be M chains or G chains, corresponding to the terminal 1-O-substituted D-mannitol or glucose, respectively, depends on the sugar type at the reducing end of laminarin [38]. Numerous studies showed that laminarins as a potential bioactive ingredient in cancer treatment basing on antioxidant activity and inhibition (melanoma cells, colon cancer, and anti-metastatic). The structure, molecular weight, monosaccharide unit number, degree polymerization, and branching length of laminarin control their antioxidant activity [39, 40]. For example, laminarins (15 and 06 kDa) possess antioxidant activity (7.5 and 79.7%), respectively. DPPH scavenging activity of purified laminarin (10 kDa) corresponds to 87.57%. The antioxidant activity of laminarin is basing on the interaction between carbonyl groups and transition metal ions (Cu^{2+} or Fe^{2+}) and carbonyl groups [39]. Laminarins also exhibit the ability to the inhibition of lipid peroxidation. Laminarins also play a role in the inhibition of chain initiation, peroxide decomposition, and binding of transition metal ions.

3.2.6 Ulvans

Ulvans are mainly high sulfated polysaccharide existing in green algae (Ulva and Enteromorpha) and animal (glycosaminoglycans), belongs to heteropolysaccharides group, and water-soluble. Ulvans contain the basic units such as xylose, rhamnose 3-sulfate, iduronic acid, xylose 2-sulfate, and glucuronic acid and account for about 38 to 54% of dry algae mass. The units of α-L-rhamnose-3-sulfate-1,4-β-D-glucuronic acid, α-L-rhamnose-3-sulfate-1,4-α-D-iduronic, and α-L-Rhamnose-3-sulfate-1,4-β-D-xylose are repetition in the structure of ulvans [41]. Ulvans and their-derived oligosaccharides exhibit antioxidant activity via the level decrease of the total and LDL cholesterol and triglyceride reduction in the serum. The molecular weight of ulvans is a positive proportion to their antioxidant capacity. For example, hydroxyl radical scavenging ability and the molecular weight of ulvans ranged from approximately 50 to 90% and 18.2 to 100.5 kDa, respectively. Sulfate group number in ulvans also affect their antioxidant activity. For example, 2.0 mg/mL of ulvan (32.8% w/w sulfate) of *U. pertusa* species arrested 90% hydroxyl radical that higher than native ulvan possessing 19.5% w/w sulfate [42]. Therefore, the over-sulfation of ulvans will have more benefits for antioxidant activity.

3.2.7 Pectins

Pectins exist mainly in cell walls of terrestrial and marine plants, classified into a heterogeneous polysaccharide group. Pectins contain over 65% of 1,4-linked-α-D-galacturonic acid that depends on species, for example, the galacturonic acid content of pectin in mangosteen rind (73.16%), in lime (72.5%), and mango peels (56.67%) [43]. Pectins are diverse on the structure and is usually classified based on esterified galacturonic acid units (methoxylation degree) as well as possesses different biological activities (antioxidant, antitumor, and anti-inflammatory). The main pectins in a plant are homogalacturonan, and unpopular (xylogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, arabinogalactan I, and arabinogalactan II. The structure, the content, molecular weight, and the plant species lead to a difference in the reaction rate constant between pectins and hydroxyl radical scavenging. Pectins in fresh white cabbage, carrot, onion, and sweet pepper exhibit antioxidant activity lower than Opuntia ficus indica. The rating constant for the reaction range from $2.05 \pm 0.56 \times 10^9\ M^{-1}\ s^{-1}$ to $(1.03–1.37) \times 10^9\ M^{-1}\ s^{-1}$. The rate constant also depends on the compositions in pectin compounds such as protein or secondary metabolites. Xanthine oxidase inhibition of pectin is improved when the molecular weight and a Gal residues number in pectin structure are high. Pectin of onion control inhibition of xanthine oxidase better than radical scavenging of DPPH and the hydroxyl [44]. Low etherified pectin helps the stabilization of malonic dialdehyde level and the inhibition of glutathione reductase and glutathione peroxidase [45]. Pectins have the content (2–35%) and the molecular weight (25–360 kDa) depending on plant species. Pectin after purifying is also white color, same purified alginate (**Figure 2b**).

3.3 Secondary metabolites

3.3.1 Polyphenols (phlorotannins, lignins, polyphenols)

Polyphenols are diverse in structure and exist in all different plants as well as marine (sponges). Polyphenol is named phlorotannin in marine. For terrestrial plants, polyphenols are determined, such as quercetin, tannin, gallic acid, mangrin, resveratrol, and lignin. Functional-antioxidant food is commercial in the market, for example, tannins, quercetin, gallic acid, mangrin, phlorotannins, or resveratrol, but not lignins. Lignins are antioxidant polyphenols existing in all plants interesting in the near time, especially their application into functional food and pharmaceuticals. Therefore, phlorotannins, lignins, and polyphenols are focused on the current chapter (**Figure 3**).

Phlorotannins that compose of the phloroglucinol units and the various linkages (ether, phenyl, ether/phenyl, and dibenzodioxin) are polyphenols in all marine plants and some marine animals [46]. Therefore, the structure of phlorotannins is diverse, and the thing leads to other bioactive of them, for example, antioxidant

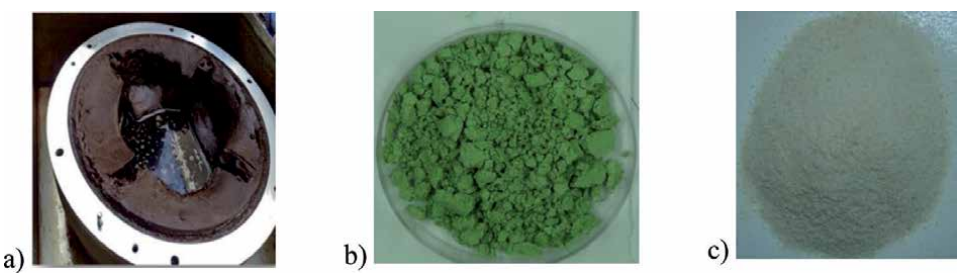

Figure 3.
(a) Crude fucoidan from brown algae Sargassum duplicatum grown in Vietnam; (b) chlorophyll powder from maize leaves; (c) glucosamine from shrimp shell.

[31, 47, 48], antitumor, anticancer, and inhibition of UV radiation [49]. Phlorotannins have two styles of free (existing in membrane-bound vesicles) and cell wall linkage (phlorotannins-alginic acid). Their content in brown algae is more than different marine organisms [50]. Phlorotannins content could reach up to 2% in brown algae growth in temperate regions of Pacific and Atlantic and tropical Atlantic regions [51]. Some studies noticed that phlorotannins in thallus dry weight are up to 25–30% [52]. For brown algae grown in Vietnam, antioxidant phlorotannin content is from 0.1–1.1%, compared to dried algae.

Lignins exist in the matrix of hemicellulose, cellulose, and lignin in the cell wall. Lignins are classified into an irregular polyphenol with monolignols (cinnamyl alcohols (guaiacyl), coniferyl alcohol (syringyl), sinapyl alcohol (p-hydroxyphenyl), and p-coumaryl alcohol) that cross-linked together via the linkages of carbon–carbon, ester, and ether. The ratio of monolignols in lignin structure is different between various plants [53]. Dehydrogenative polymerization of phenyl propanoid units helps to the synthesis of irregular lignin more advantageous. In-plant, lignins are formed via the metabolic pathway of phenylalanine/tyrosine. They account for up 10 to 25% of the dry plant mass and 1 to 43% in lignocellulosic biomass (cellulose, hemicellulose, and lignin). In the sugarcane and corn (stalk, stover, and straw), lignins are up to 25–32% and (6.9, 19.54, and 7.5), respectively [54]. Lignins are known for the free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl, DPPH• and (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS). The antioxidant efficiency of lignins depends on the structure and the solubility of lignins. Antioxidant activity is affected by the carbonyl groups of side chains but free hydroxyl groups and ortho-methoxy substitution in phenol ring impact. The molecular weight, the polydispersity, and the heterogeneity of lignins play a role in controlling their free radical scavenging capacity [55].

Polyphenols are known with one or more hydroxyl groups on the aromatic skeleton ring and possess antioxidant activity (scavenge free radicals, and inactivate prooxidants) [56], heart disease prevention, inflammation-reducing, anti-cancers, and antidiabetic, as well as the rate reduction of mutagenesis in human cells. Polyphenols are different in the structure, content, and antioxidant activity in various plants [57]. Nowadays, based on chemistry characteristics such as chemical structure, simple molecules, and highly polymerized compounds, scientists classify about ten different classes with over 8000 polyphenol structures. The relationship between antioxidant activities and the chemical properties of polyphenol is also noticed and demonstrated very clearly. Polyphenols presented in the current section are free polyphenols and belong to the group that dissolves in an organic solvent and aqueous, not alkaline and acid [58]. Common polyphenols in terrestrial plants are known, such as quercetin, rutin, tannin, gallic acid, catechin, resveratrol, mangiferin, and epicatechin.

3.3.2 Alkaloids

Alkaloids are one of phytochemistry composition in plants, composed of at least one nitrogen atom with hydrogen-carbon groups in an amine-type structure, and accumulate nearly 20% of plant species. They are mainly well-known as pyrrolizidines, pyrrolidines, pyridines, isoquinolines, tropanes, indoles, quinolines, morphine, strychnine, quinine, ephedrine, and nicotine [59]. Alkaloids possess antioxidant activity, such as radical scavenging potential, total antioxidant activity, ferric reducing antioxidant potential, hydroxyl group scavenging ability, and lipid peroxidation inhibition ability. Alkaloids play a controlling role in antioxidant activity better than phenols [60, 61]. Alkaloids content and their activity are a correlation to the species and growth time of plants. For example, alkaloids are the major antioxidants in maca. Hydroxylated alkaloids exhibit antioxidant activity based on the reaction between radicals with high lipophilicity. However, the solvation process causes a decrease in the antioxidant activity of alkaloids [62].

3.3.3 Flavonoids

Flavonoids are secondary metabolisms belonging to the polyphenol group, consist of two phenyl rings and a heterocyclic ring, and abbreviated C_6-C_3-C_6. Flavonoids are commonly flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones [63, 64]. Flavonoids exist in almost various plants and possess high bioactivities in *Morinda* L. [65]. Flavonoids in *Morinda* are antioxidants having the ability against cardiovascular disease and cell components degeneration that age-related [66]. The mainly antioxidant properties of flavonoids are based on a role against free radicals (hydroxyl and superoxide radicals) via scavenging of reactive species and inhibiting biomolecular damage [67]. Besides, flavonoids also exhibit various bioactivities, such as anti-diabetic, anti-inflammatory, cardioprotective activity, anti-age-dependent-neuropathology activity, anti-cancer activity, and anti-viral/bacterial [68]. Flavonoids content corresponds 5 to 10%, compared to the secondary metabolites in plants, and nowadays, there are about 5000 identified flavonoids. Some studies presented an intake dose of flavonoids per day is from 20 mg and 500 mg [69].

4. Extraction process

Nowadays, there are numerous extraction methods of bioactive substances from nature for the application into functional food and pharmaceuticals. For example, maceration, reflux, soxhlet, microwave-assisted, ultrasonic-assisted, enzyme-assisted, and gamma Coban 60-assisted. Different bioactive ingredients will have specifically suitable extraction methods, and the **Figure 4** will present the thing.

4.1 Collagen

The high temperature leads to the degradation of collagen structure, so they are usually pre-treatment, extracted, and purified under cold temperatures (≤ 5°C). After pre-treatment, skins and scales will be dried by using infrared – assisted

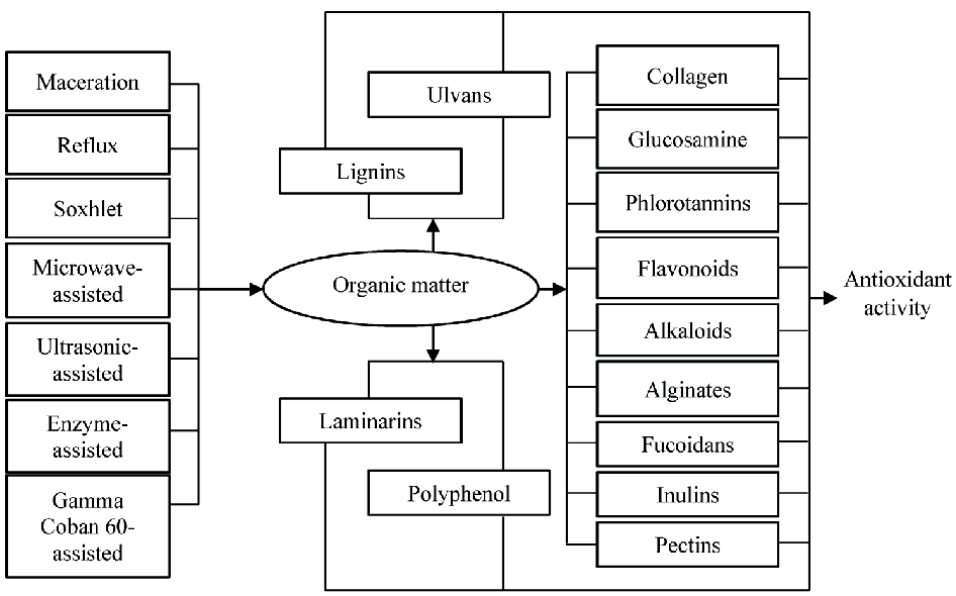

Figure 4.
General extraction schematic of antioxidant ingredients from organic materials.

cold drying and minced. For collagen extraction, the solvent of CH_3COOH is usually useful (**Figures 5** and 6). For fish skins, removing non-protein and lipid are by using 0.1 M NaOH and 10% butyl alcohol, respectively (**Figure 5**). NaCl and ethanol are suitable for the precipitation of collagen from skins and scales, respectively.

Fish skin is scraped, washed to remove impurities, washed with cold water to remove impurities, then chopped with a size of 0.5 x 0.5 cm. After soaking for enough time, the fish skin is removed and rinsed with cold water until neutral pH. Fish skin will be extracted with 0.5 M CH_3COOH solution at 1/15 (w/v) ratio for 48 hours and precipitated in 2.6 M NaCl in 0.05 M tris buffer (hydroxymethyl) aminomethane (pH 7.0) (**Figure 6**). The precipitate was then centrifuged at 20,000 g for 60 minutes at 4°C, then undergone dialysis and finally lyophilized to collect the collagen. The efficiency obtained from the bovine skin with acetic acid

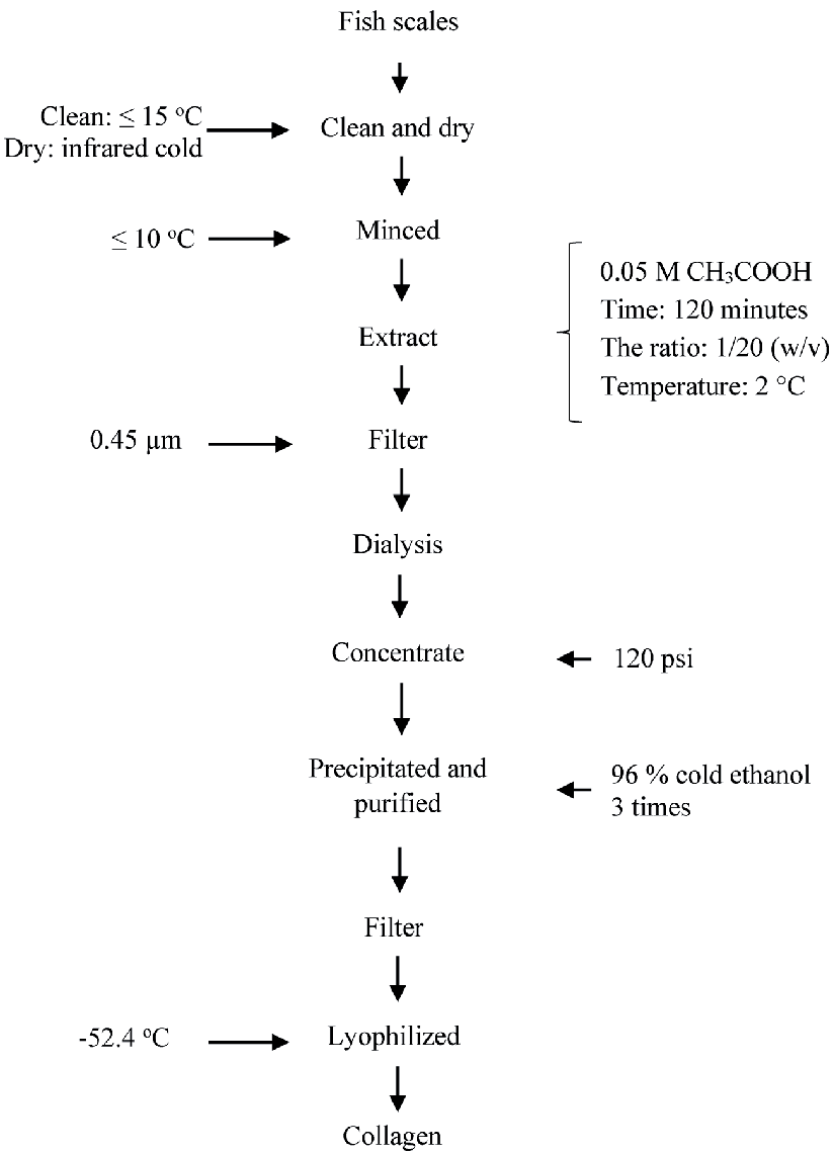

Figure 5.
Extraction schematic of antioxidant collagen from fish scales.

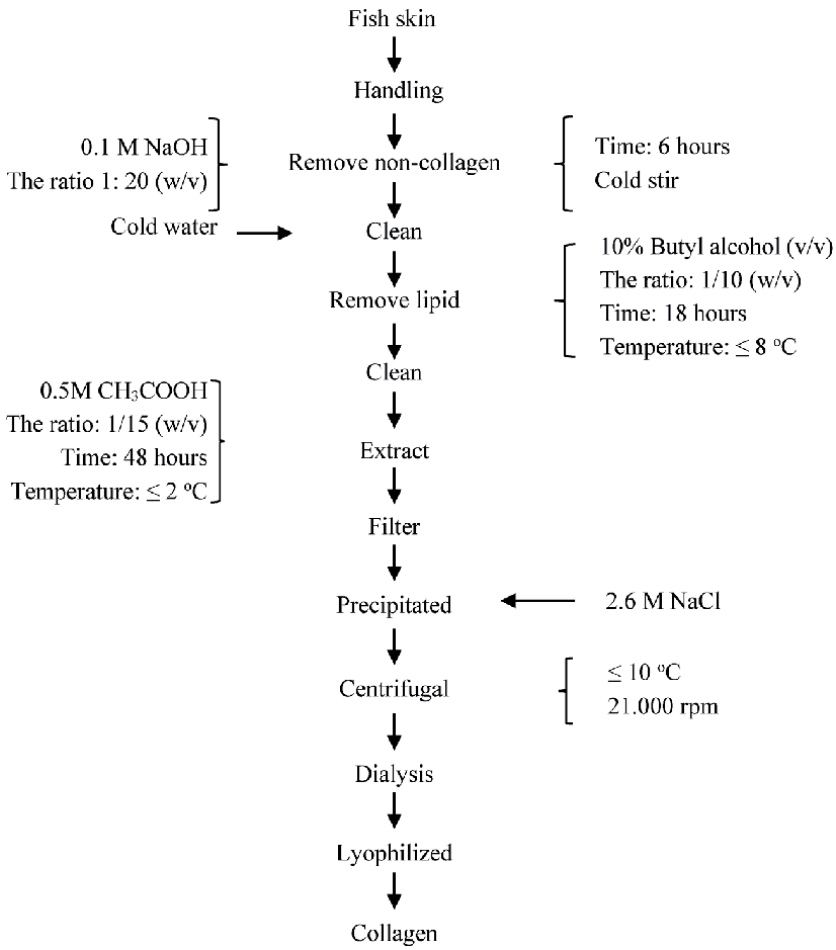

Figure 6.
Extraction schematic of antioxidant collagen from fish skin.

is 4.19% (calculated on the wet weight of the skin), the obtained collagen is types I that consisted of 2 chains α is α_1 and α_2, denaturation temperature is 31.16°C. According to the study, pangasius skin was treated with 0.2 M NaOH for 66 hours, extracted collagen in acetic acid 0.37 M for 2.5 days. Collagen precipitation occurs in 2.05 M NaCl in 4 minutes. The results showed collagen collection efficiency of 31.16%.

4.2 Polysaccharide

All over methods could use for polysaccharides extraction, but only three solvents could use, for example, alkaline, acid, and aqueous.

4.2.1 Fucoidans

For fucoidans extraction, removing the pigment and the lipid out of brown algae is by using an organic solvent such as ethanol/acetone/chloroform and HCl, respectively. Before the fucoidans extraction, separation of polyuronic acid is also via the other steps, such as neutralize (8% $NaHCO_3$), concentration, dialysis (10 kDa membrane), and precipitation. Finally, fucoidans are collected with HCl solvent and running the DEAE cellulose (**Figure 7**).

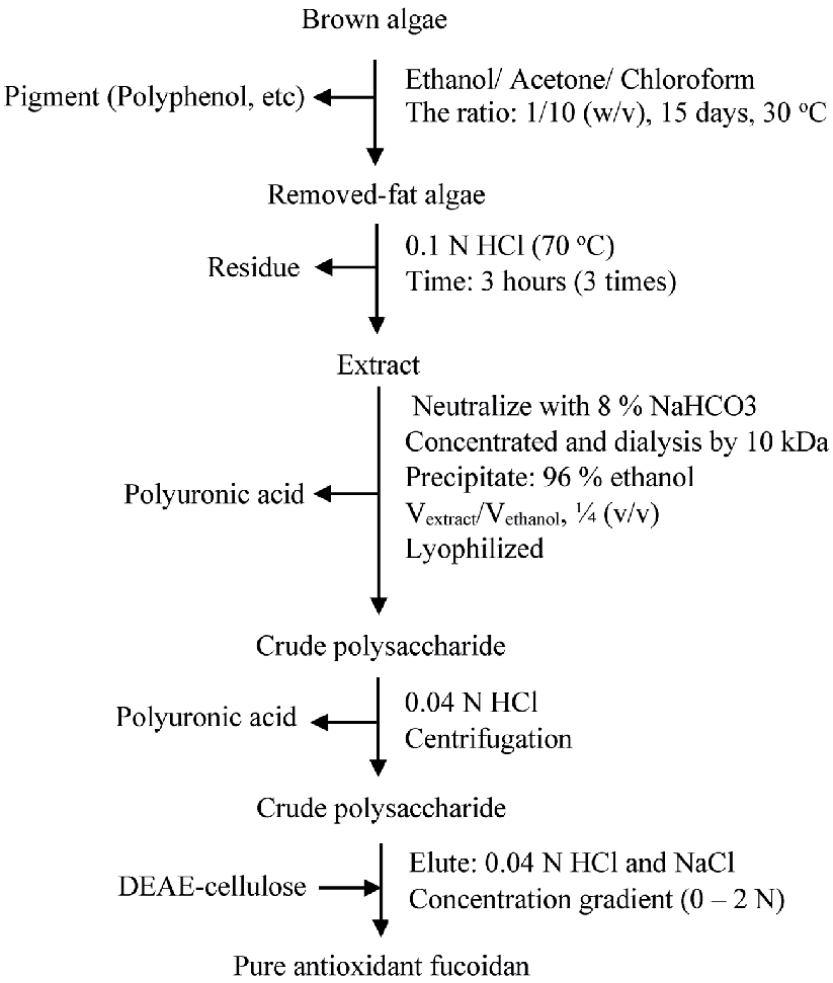

Figure 7.
Extraction schematic of antioxidant fucoidan from brown algae.

4.2.2 Alginates

The removal step of pigment and lipid by using an organic solvent and HCl could not be lack. Na_2CO_3 is a useful solvent for the extraction of alginate. The conversion of sodium alginate to calcium alginate is necessary. Calcium alginate still links to phlorotannins, so bleaching is important. Acidification (pH 2) is phlorotannins removal. Finally, the conversion of sodium alginate is from alginic acid, and the precipitation of them by using 80% ethanol (**Figure 8**) [70].

Based on experiments, the use of a hydrochloric solvent with flexibility in terms of temperature, the material-to-solvent ratio, and time, as well as neutralization, dialysis, concentration, precipitation, centrifugal, and drying, will allow obtaining small units of alginate such as sodium mannuronate and sodium guluronate (**Figure 9**).

4.2.3 Glucosamine

Shrimp shells contain many chitin, protein, and minerals, so removing protein and minerals using HCl and NaOH are indispensable steps. After removing proteins and minerals, continue to wash and soak chitin in HCl solvent to hydrolyze

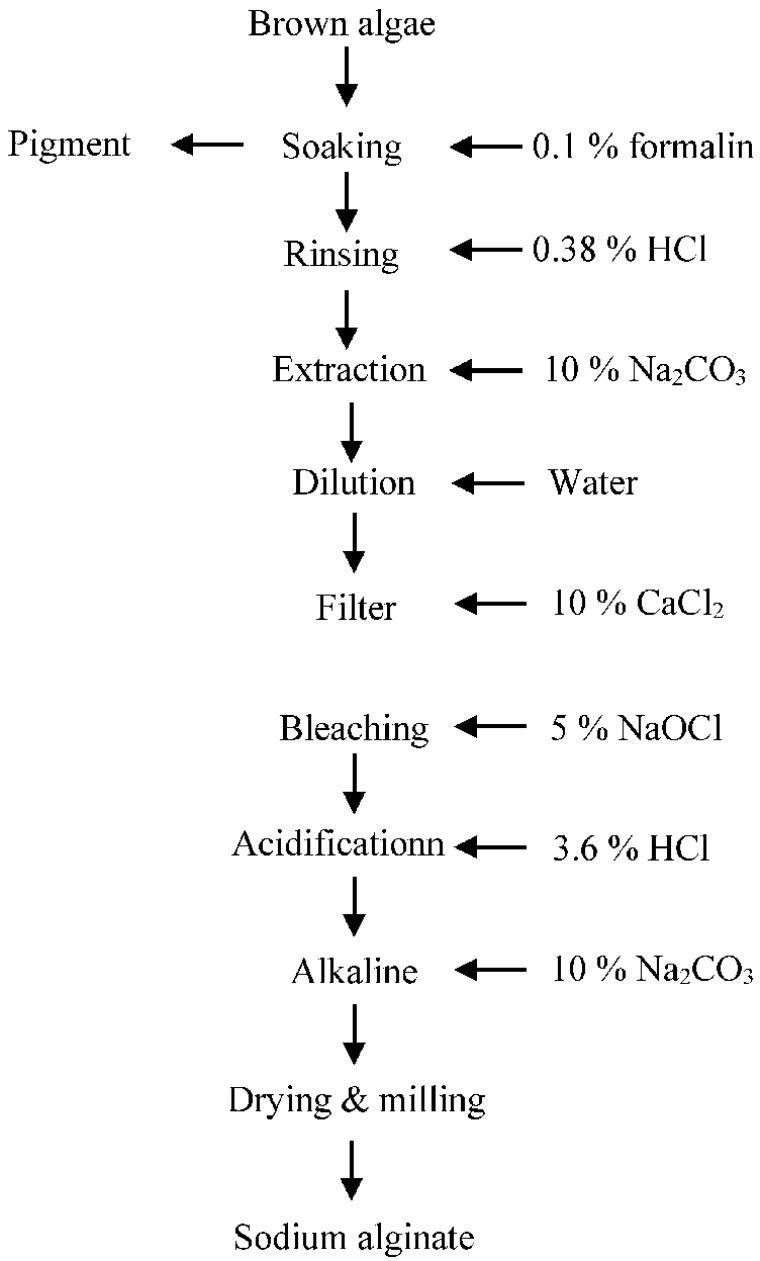

Figure 8.
Extraction schematic of antioxidant alginate from brown algae.

glucosamine. Glucosamine hydrochloride will be collected by crystallization with ethanol and dried at 50°C (**Figure 10**) [71].

4.2.4 Inulins

Extracting and purifying inulin from dangshen, color separation is also a necessary step from the beginning. After color separation, inulin is extracted with water and undergoes filtration, concentration, and purification using ethanol, activated carbon, and $CaCl_2$. The above repetitions will be useful for purified inulin absorption (**Figure 11**).

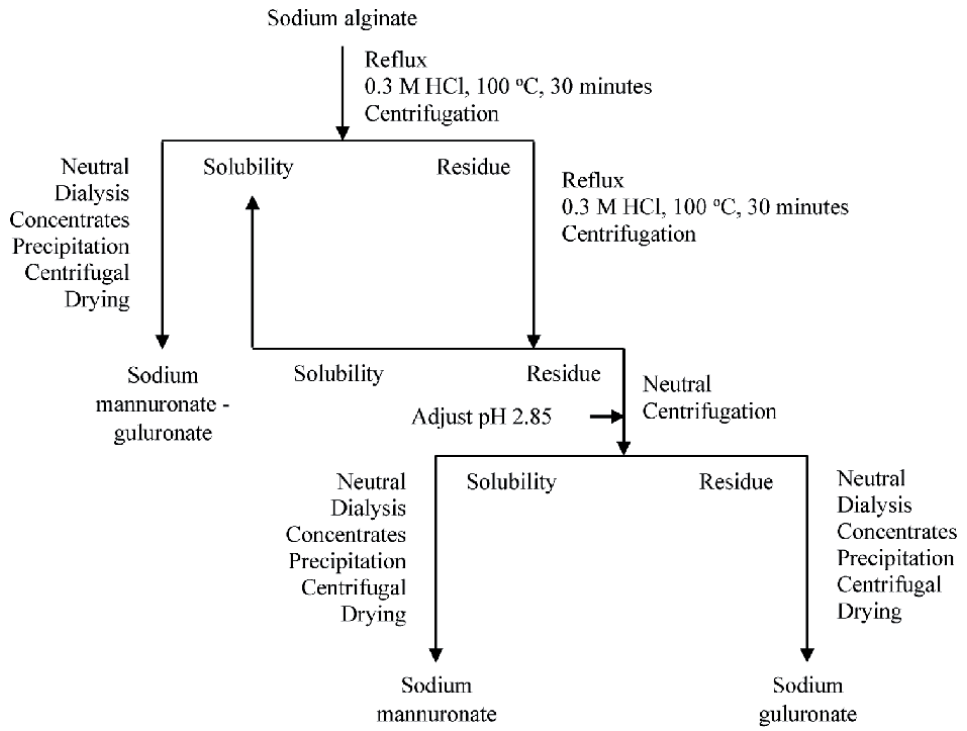

Figure 9.
Extraction schematic of antioxidant sodium mannuronate and sodium guluronate from alginate.

4.2.5 Laminarins

As laminarin is a water-soluble polysaccharide sulfate, several proteins, fucoidan, and alginate also exist in the laminarin extract. Protein removal is using trichloroacetic acid. Eliminate alginate and fucoidan are by adjusting pH and mass fractionation, respectively (**Figure 12**) [72].

4.2.6 Ulvans

Use dichloromethane and ethanol for the most removal of the lipids and pigment that exist in Ulva. Hot-water extractions of the pre-treatment algae are for 7 hours at 75–85°C under continuous stirring. The filtration and centrifugation are for collecting the supernatant. Removal of starch and proteins is continuously by using enzymatic hydrolysis. Afterward, running the solution is via activated charcoal for centrifuging, filtering, and precipitating with absolute ethanol. Finally, the precipitate is ulvan (**Figure 13**) [73].

4.2.7 Pectins

The first step in plant pectin extraction is always the color removal step with organic solvents. After de-colorant from the plants, pectin extraction is by using the solvents with different pH. The filtrate is concentrated and hydrolyzed to remove protein and starch. At this time, semi-pectin was collected and continued to run through activated carbon to remove impurities. Finally, 80% of ethanol is useful for precipitation and purification of pectin (**Figure 14**).

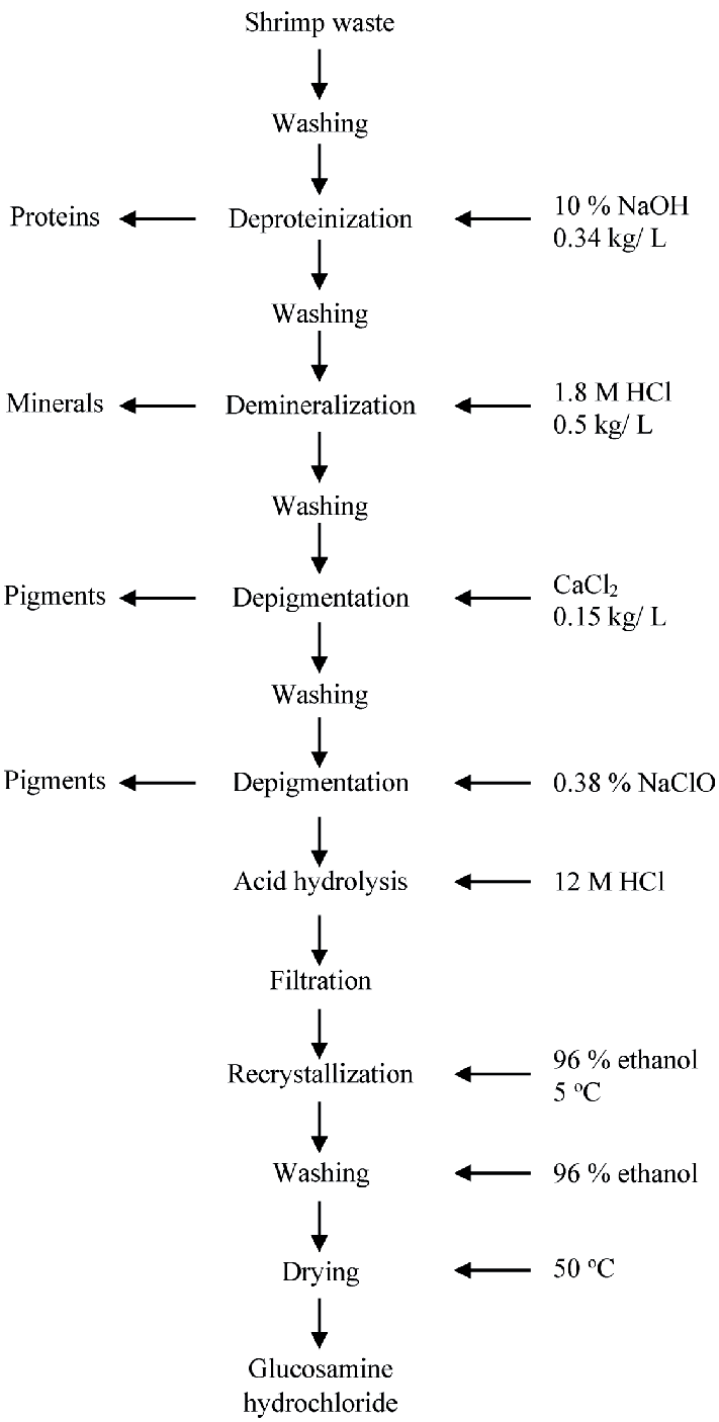

Figure 10.
Extraction schematic of antioxidant glucosamine hydrochloride.

4.3 Secondary metabolites

4.3.1 Polyphenols

Phlorotanins dissolve into the organic solvent, such as ethanol, methanol, ethyl acetate. Phlorotannins in brown algae grown in Vietnam mainly dissolve in ethanol

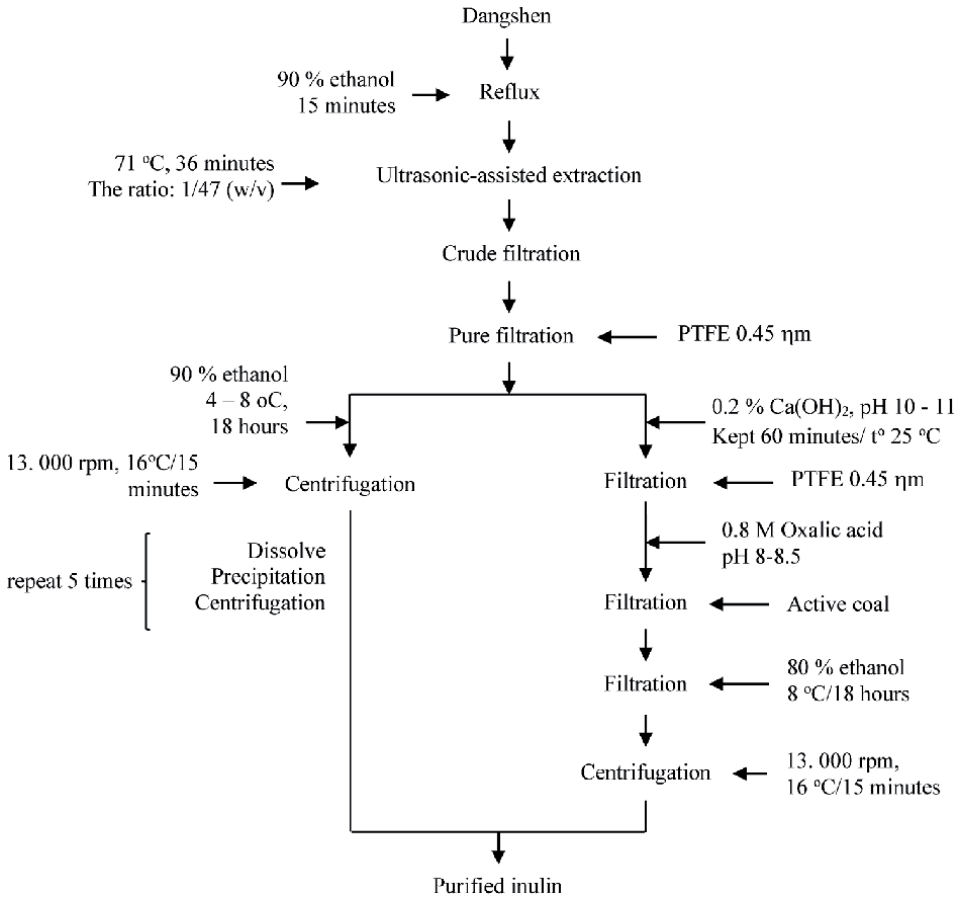

Figure 11.
Extraction schematic of antioxidant inulin from dangshen.

and focus on the fraction ethyl acetate. Sephadex LH20 is useful for the purification of phlorotannins (**Figure 15**). For polyphenol of terrestrial plants also used ethanol as a useful solvent.

According to Neeraj et al., lignins exist in various four styles, such as kraft, lignosulfonate, organosolv, and soda [74, 75].

There are three methods for the extraction of lignin; (i) 7.5% NaOH, (ii) organosolv (85% formic acid/85% acetic acid), and (iii) poly-ethylene glycol (PEG). Method (ii and iii) use heating assistance.

In method (i), the material-to-solvent ratio of 1/10 (w/v) at 90 ± 2°C for 90 min with pH (12) of the black liquor is useful for the extraction of lignin. After hot filtration and allowing to cool for the precipitation is using acidification with 0.5 M H_2SO_4. Meanwhile, the current authors extracted successful kraft lignins from corn stalks according to the **Figure 16**. This difference can be from material differences.

In method (ii), the condition is as follows: Formosolv/acetosolv ratio of 70/30 (v/v), the biomass-to-solvent ratio of 1/8 (w/v) for 2 hours at 98 ± 2°C and allowing to cool for filtration. The residue cleaning is with 80% formic acid and distilled water. The dilution of black liquor is with distilled water, stirred, centrifuged for 1 hour.

In method (iii): Lignin extraction is with the material-to-solvent (1% (w/w) 98% H_2SO_4) ratio of 1/4 (w/v) at 160 ± 2°C for 2 hours and down to room temperature for the collection of the supernatant. The residue washing is with 1,4-dioxane and removed 1,4-dioxane in the black liquor by rotary evaporation.

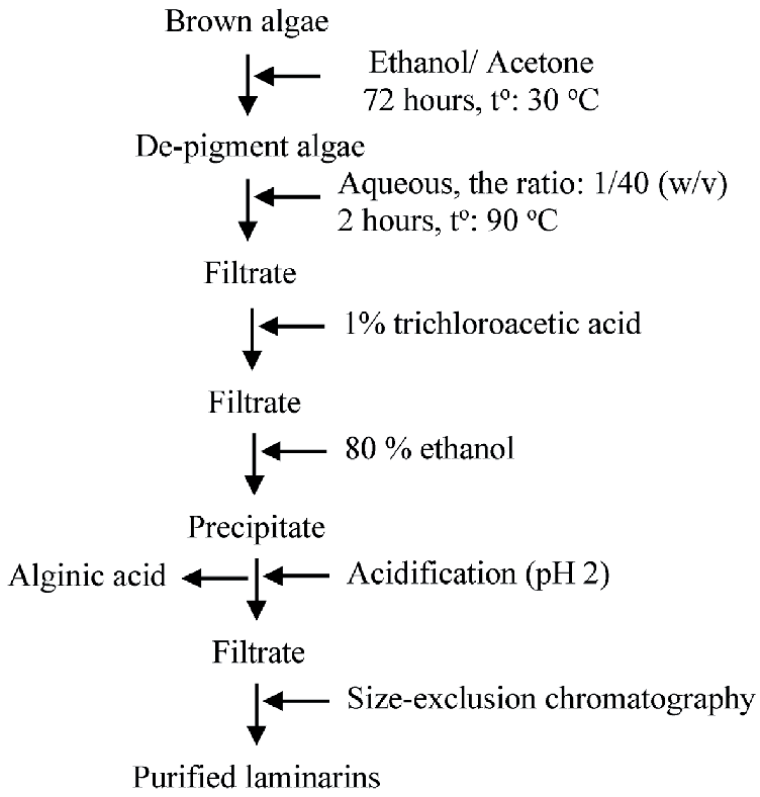

Figure 12.
Extraction schematic of antioxidant laminarin from brown algae.

During the black liquor dilution by using distilled water, stir and centrifuge are for 1 hour. The precipitated lignin(s) is separated, neutral with distilled water, and oven-dried at 50°C for 48 hours.

4.3.2 Alkaloids

Alkaloids are one of the main photochemical components of plants, so they are also extracted with organic solvents, and most claims indicate that alkaloids are soluble in ethanol. According to Surya et al., alkaloid extract after chasing ethanol solvent added 5% CH_3CO_2H, filter, and separated with CH_2Cl_2. The aqueous phase is collected and adjusted to pH 10 for the continuous fraction with CH_2Cl_2. Finally, the obtain of CH_2Cl_2 fraction because alkaloids exist in the CH_2Cl_2 phase (**Figure 17**) [76].

4.3.3 Flavonoids

About 5000 flavonoids have been identified and noticed. Each group of substances in flavonoids can dissolve in different solvents. Apigenin-7-methyl ether and flavone aglycone are among the substances found in 70% ethanol extracts that support the boiling point of water after the plant pre-treatment with Petroleum ether at 40 to 60°C. Ethyl acetate fractionated with ethyl acetate to obtain the ethyl acetate fraction. Then run via the polyamide column, which will select the purified Apigenin-7-methyl ether and flavone aglycone (**Figure 18**) [77].

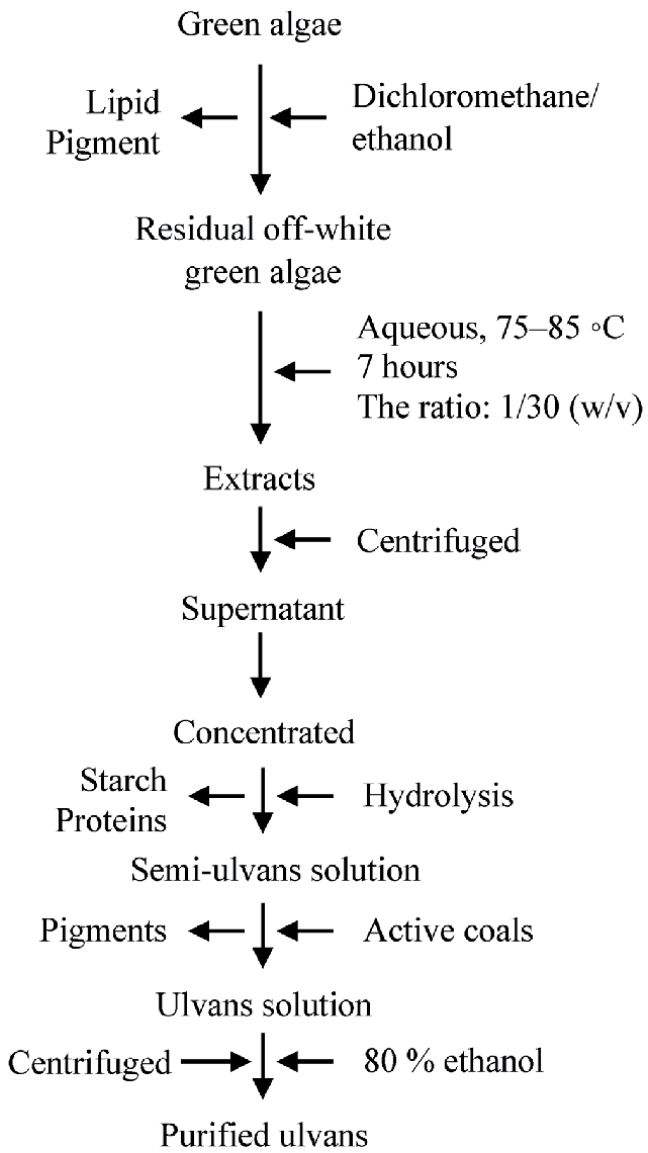

Figure 13.
Extraction scheme of antioxidant ulvan from green algae.

5. Production technology of functional-antioxidant food

5.1 The capsules

In capsules, the preparation of an emulsion system is necessary for filling into the capsules. The emulsion contains antioxidants, surfactants, and excipients that increase the antioxidant effects of functional food. The capsule shells are always composed of gelatin, sorbitol, and colorant ingredients (**Figure 19**).

Some excipients are common in the preparation of the capsule as follows (**Table 1**):

The typical emulsion viscosity is from 50 to 1000 Centipoise (cP). The melting temperature does not exceed 70°C. The suitable particle size in the emulsion is

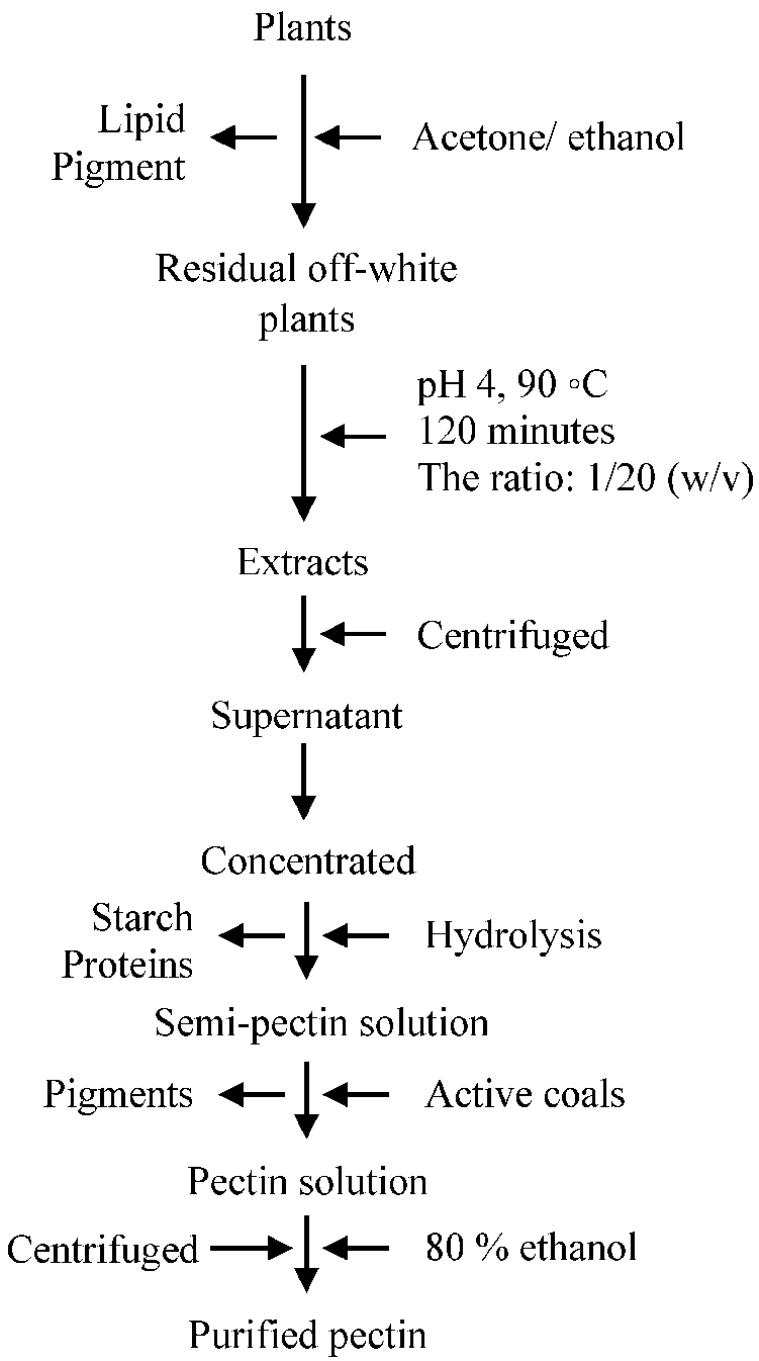

Figure 14.
Extraction scheme of antioxidant pectin from plants (Cactus).

less than 20 μm. The formula gets the standard when Phenomena such as stringing, dripping, splashes, or solidification does not happen at the dosing nozzle. The emulsion should be solidifying below 40 °C. For two-piece capsules, the compaction force is typically useful of 20–30 N, compared to tableting (10–30 kN) (**Figure 20**).

The manufacture of hard gelatin capsules by using a dip-coating method involves four stages. (i) Dipping solution (the gelatin solution preparation), (ii)

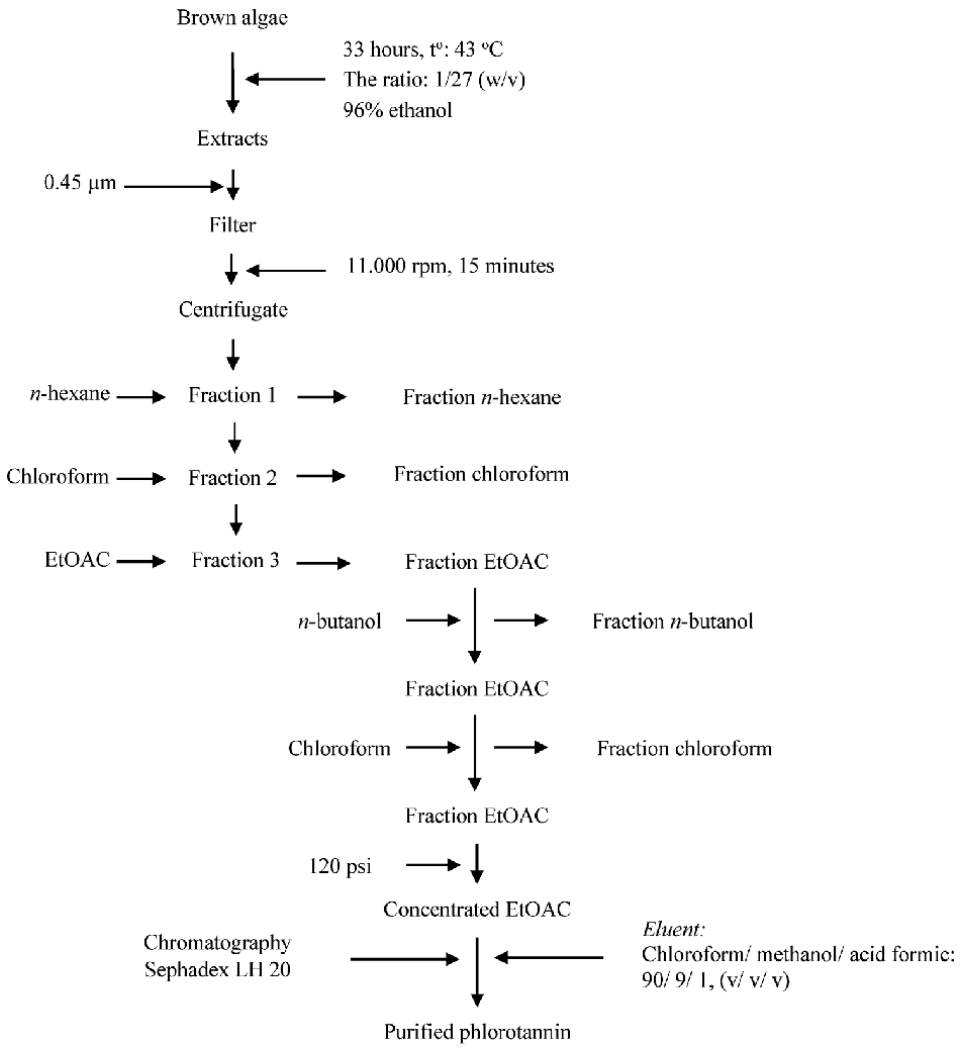

Figure 15.
Extraction scheme of antioxidant phlorotannins from brown algae.

Gelatin-coating on metal pins, rotation, and pins drying, (iii) Stripping, trimming, and the capsule shell joining; (iv) Printing [81].

5.2 The tablets

Functional-antioxidant food tablets contain various powder components that ensure the characteristics of consistency, flow, cohesion, and porosity) for the guarantee of the size, half-life, and swelling capacity of the tablets. All tablets have to get uniforms in the tablet weight, antioxidant content, the indication requirements, and storage time [81].

The actual tablet weights (175 mg) need the target force of 9500 N [82] (**Figure 21**).

5.3 The tubes

For the tube, the compositions of functional-antioxidant food is required in the liquid or the serum and respond all indexes according to the standards.

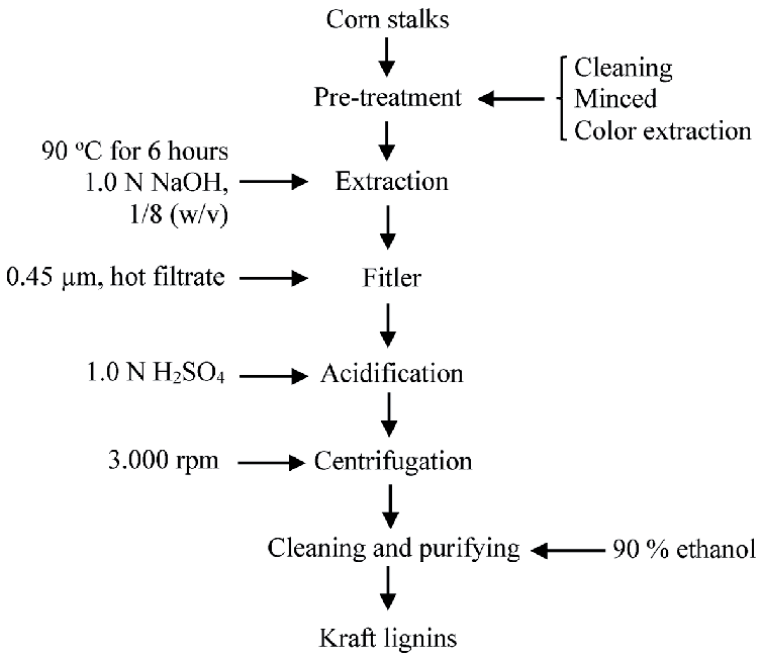

Figure 16.
Extraction scheme of antioxidant lignins from corn stalks.

5.3.1 The effervescent tablets

The effervescent tablet has different compositions such as tartaric acid citric acid, sodium bicarbonate, potassium citrate, mannitol, sorbitol, aspartame, talc. For some formulations, in the granulation process, polyvinylpyrrolidone plays a role as a binder. The wet granulation is suitable for the production of effervescent tablets composed of potassium citrate [83]. The compression and uniformity of effervescent tablets in the wet granulation technique is better and gets less error in the processing such as sticking, capping, and friction than other methods. The strawberry-raspberry flavor is useful for effervescent tablets. All effervescent tablets must contain bicarbonate to make CO_2 [84].

For effervescent tablets of phloroglucinol (dihydrate), the formulation is as follows: phloroglucinol dihydrate (80.0 mg), citric acid (297.2 mg), sodium bicarbonate (362.6 mg), and sodium benzoate (15.2 mg) [85].

5.3.2 The powders and the hard pills

Figures 22 and **23** exhibit the production process for the powder and hard pills of antioxidant polyphenol, chlorophyll from by-product maizes, respectively. The current process is similar, compared to the tablets and the capsules, but their shapes are various. Hard pills are popular in Vietnam.

6. Mechanism of functional-antioxidant food

The antioxidant activity of hydrolyzed collagen depends mainly on the presence of hydrophobic groups in the peptide chains. Histidine and aromatic amino acids play a controlling role in antioxidant activity via two mechanisms for the de-activation of free radicals: (i) hydrogen atom transfer (hydrogen donation in the

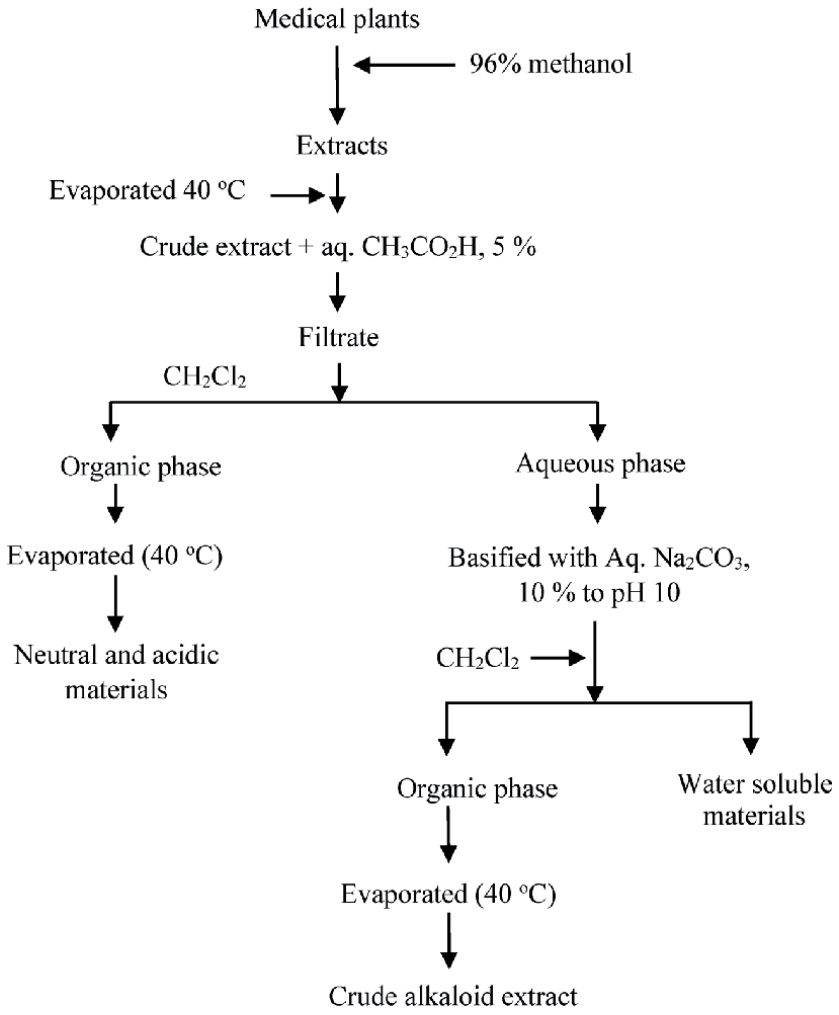

Figure 17.
Extraction scheme of antioxidant alkaloid extract from plants.

assays of ORAC and TRAP), (ii) single electron transfer (one-electron transfer to reducing agent in the assays of DMPD and FRAP assays). The antioxidant capacity of tyrosine is basing on the mechanism (i) and pathway (ii) is mostly for the group (cysteine and histidine) [86, 87].

The mechanism of antioxidant activity is basing on the generation prevention of free radicals via the pathway of chelating ions (ferrous and copper). Transition metal ions take apart to reactive to superoxide and hydrogen peroxide in Fenton reaction for the reactive hydroxyl radicals formation ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^-$). The hydroxyl radical scavenging capacity and the chelating ability of polysaccharide depend on their structure. Some hypotheses on the antioxidant activity of polysaccharides estimate dissociation energy decrease of the hydrogen bond, the abstraction activation of the anomeric carbon, and reduction of molecular weight. For the sulfated polysaccharide, the sulfate groups lead to acidification and weaken the hydrogen bond between other polysaccharides [88, 89].

In general, antioxidant molecules usually take part in the redox reaction as a reducing agent. •NO generation happens under the impact of nitric oxide-synthase on intracellular arginine and combines to O_2 to the formation of $ONOO^{\bullet}$ that cause lipid peroxidation. The peroxidation is also one of the reasons causing autoimmune

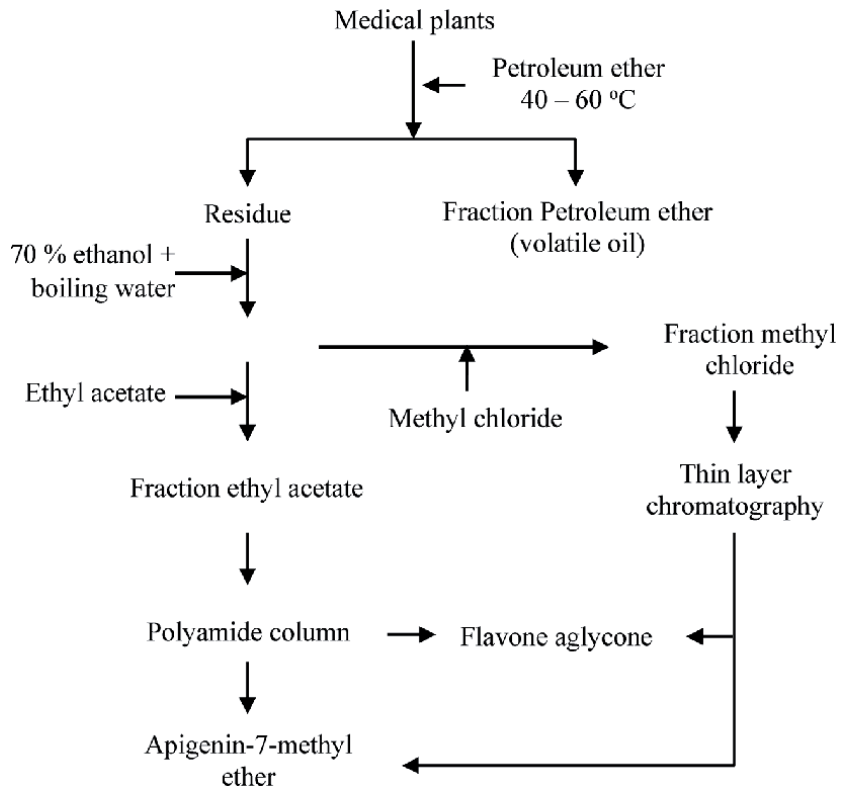

Figure 18.
Extraction scheme of antioxidant flavonoids from plants.

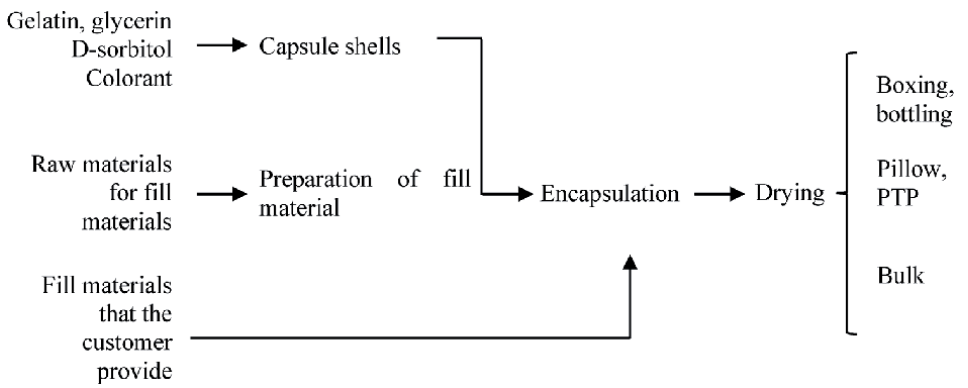

Figure 19.
The production shematic of functional-antioxidant capsules.

diseases (rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, scleroderma, multiple sclerosis, psoriasis, and vitiligo). The metals deactivation and lipid hydroperoxides could be via the antioxidants increase, the singlet oxygen elimination, and the undesirable volatiles minimize. The peroxyl radical ($ROO^{\bullet}$) scavenging mechanism of polyphenol is basing on free radicals getting hydrogen cations of polyphenol and forming hydrogen bonds. The antioxidant activity decrease in a hydrogen-bond-rich medium [90].

Excipient	Emulsion	Substances
Lipophilicity	Vegetable oils	Corn oil, Castor oil, Sesame oil, Olive oil, Hydrogenated vegetable oil, Peanut oil, Soybean oil, Fractionated coconut oil
	Esters	Glycerol Stearate, Isopropyl myristate, Ethyl oleate, Glycol Stearate
	Fatty Acids	Laurie acid, Stearic acid, Oleic acid, Palmitic acid, Oleic acid
	Fatty Alcohols	Stearyl alcohol, Cetyl alcohol
Water-retaining		PEG 3000–6000 MW
Ambipolar		Lecithin, Poloxamers, PEG esters

Table 1.
Liquid excipients compatible with hard gelatin capsule shells [78, 79].

Figure 20.
Some gelatin capsules [80].

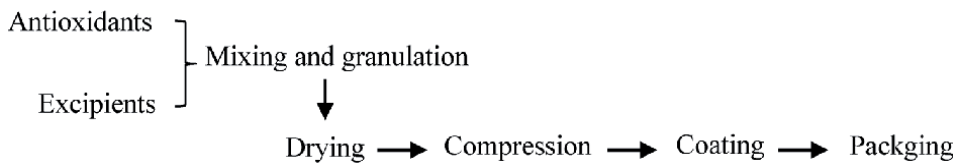

Figure 21.
The production shematic of functional-antioxidant tablets.

7. Opportunity and challenge

Nowadays, in the development trend of the world for surviving and developing in parallel with various functional food and pharmaceutical products in different countries, functional-antioxidant foods have to face numerous opportunities and challenges, such as the technology and science advancement, the aging increase, and expanding global trade. Aging is the biggest threat to humans and causes about 80 different diseases in the world. It is the growth of aging populations, environmental pollution, and life pressures that leads to disease and economic burdens. With

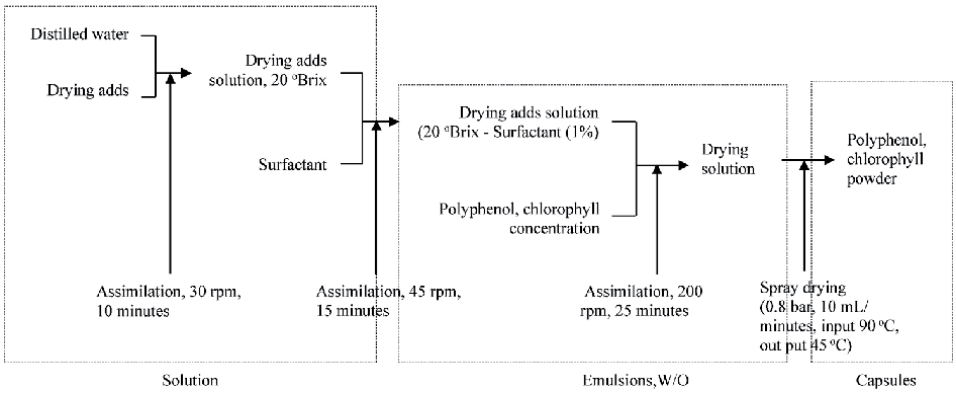

Figure 22.
The production shematic of functional-antioxidant powder from by-product maizes.

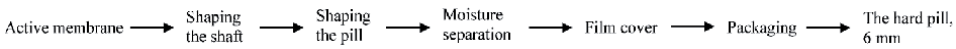

Figure 23.
The production shematic of functional-antioxidant hard pill from by-product maizes.

Beverages	Antioxidant content	Nuts, legumes and grain products	Antioxidant content	Berries, fruit and vegetable	Antioxidant content
Apple juice	0.27	Barley, pearl and flour	1.0	Bilberries, dried	48.3
Apple juice	0.92	Barley, pearl and flour	0.5	Amla (Indian gooseberry), dried	261.5
Orange juice	0.64	Buckwheat, whole meal flour	2.0	African baobab tree, leaves dry, crushed	48.1
Pomegranate juice	2.1	Chestnuts, with pellicle	4.7	Dog rose, products of dried hip	69.4
Prune juice	1.0	Maize, white flour	0.6	Zereshk, red sour berries	27.3
Red wine	2.5	Walnuts, with pellicle	21.9	Chilli, red and green	2.4
Tomato juice	0.48	Pecans, with pellicle	8.5	*Moringa Stenopetala*, dried leaves, stem	11.9

Table 2.
Statistical descriptives of antioxidant content in some food (mmol/100 g) [91].

the development of science and technology, antioxidant bioactive substances are exploited more effectively and thoroughly. However, the uniformity of science and technology in various regions does still not happen. Awareness increase, economy,

and global trade ability lead to the criteria of the goods choice of human more difficult. The difference in antioxidant content in various foods occurs. These are both opportunities and challenges to develop antioxidant supplement products (**Table 2**).

8. Conclusions

This chapter gave an overview of the antioxidant supplements based on the international knowledge and experimentation of the authors, from the basic understanding of the antioxidant active groups (8 groups of polysaccharides, 01 group of proteins, and 05 groups of secondary metabolites) and the origin of these to the technological process of extracting and producing antioxidants to their opportunities and challenges in today's world are all demonstrated.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Physiological and Cellular Targets of Neurotrophic Anxiolytic Phytochemicals in Food and Dietary Supplements

Benjamin S. Weeks, Samuel D. Weeks, Amanda Kim, Landon Kessler and Pedro P. Perez

Abstract

Diet impacts anxiety in two main ways. First anxiety can be caused by deficiencies in antioxidants, neurotransmitter precursors, amino acids, cations and vitamins and other cofactors. Second, anxiety can be reduced by anxiolytic nutraceuticals which are food molecules that bind to molecular targets of the amygdala and the hypothalamus-pituitary–adrenal axis (HPA-axis). Anxiety is a feeling of fear that arises from a perceived threat and can be a beneficial coping mechanism to threats and stressors. However excessive anxiety is a disorder that interferes with healthy responses to stressors. The amygdala is responsible for assigning value to a threat or stressor and triggering the HPA-axis to support the body wide system responses to the threat. The amygdala also communicates with the neuroplastic learning and memory centers of the hippocampus to fix or set a learned value to the threat. Interestingly, many anxiolytic nutraceuticals that show benefits in human clinical trials have neurotrophic activity and increase neuronal plasticity. Moreover, anxiolytic nutraceuticals either act like the neurotrophins, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF and neurotrophin-3 (NT3) by either directly binding to or potentiating the tyrosine receptor kinase (TRK) family of receptors (TRKA, TRKB and TRKC) and activating the ERK1/2 signal transduction pathway associated with neurite outgrowth and neural plasticity. This chapter will explore the neuritogenic activity of clinically proven plant-based anxiolytic nutraceuticals and examine the commonality of TRKA-C receptors and the ERK1/2 signaling pathway in the pharmacological and nutraceutical treatment of anxiety disorders.

Keywords: Anxiety, Anxiolytic, Nutraceutical, Cannabidiol, Neurotrophin, Neurite Outgrowth, NGF, BDNF, NT3, TRKA, TRKB, TRKC, ERK1/2

1. Introduction

In humans, the appropriate and measured behavioral responses to environmental cues are under control of the limbic nervous system which is composed primarily of the amygdala, hippocampus, thalamus, and hypothalamus [1]. In order for

sensory inputs to the cerebral cortex to result in the appropriate responses in the body, sensory inputs relay from the cerebrum, to the limbic system and then from the limbic system to the body either through the brainstem or through the pituitary gland. It is when relaying sensory inputs from the cerebral cortex to the body that the limbic system also assigns emotional value to sensory input and sets or fixes that value by learning and remembering the rewards and punishments associated with specific environmental cues. The amygdala is known for assigning a scaled value to negative threats and stressors which the amygdala then communicates to learning and memory centers in the hippocampus so that human behavioral responses to negative cues can be consistent and appropriate. The amygdala also stimulates the hypothalamus to secrete corticotrophin-releasing hormone (CRH) which in turn stimulates the pituitary to release adrenocorticotropin hormone (ACTH), which in turn stimulates the adrenal cortex to secrete glucocorticoids including primarily, cortisol in what is known as the HPA-axis [2–4]. The hypothalamus can also send signals through the brainstem and activate the adrenal medulla to secrete epinephrine and norepinephrine. Cortisol, epinephrine and norepinephrine are hormones that can signal body wide changes in metabolic rates, breathing, heart rate, blood pressure and a variety of other appropriate body responses to the presence of an environmental threat or stressor [2, 3]. Anxiety is the feeling of fear or worry that arises from the neurochemistry of the amygdala in response to negative environmental cues and the activation of the HPA-axis and the overall preparation of the body to meet the challenges of a threat or stressor and while anxiety is a negative feeling, when it is in proportion to the actual threat a stressor presents, anxiety can be a normal and even healthy part of an adequate response to the stressor [5–7]. However, excessive and prolonged anxiety that is unwarranted by the environmental cue and exaggerated in proportion to the actual threat level leads to inappropriate and prolonged activation of the HPA-axis and cortisol release which is associated with inflammatory damage and other pathophysiologies that further stresses the human body system [2–4]. In these cases anxiety interferes with normal and health everyday life and is considered an anxiety disorder [8, 9].

People suffer from five different types of anxiety disorders; generalized anxiety disorder (GAD, obsessive compulsive disorder (OCD), panic disorder (PD), social anxiety disorder (SAD), and posttraumatic stress disorder (PTSD) [8, 9]. Each of these anxiety disorders can be described by the level of synaptic neurotransmitters and cell surface neurotransmitter receptors in the amygdala [1, 8, 9]. For example, GAD is associated with decreased activity of the inhibitory neurotransmitter, GABA. GABA acts on $GABA_A$ receptors on neurons within the amygdala to inhibit signals and help to assign lower threat values to certain stressor. Down regulation of the $GABA_A$ receptor and the subsequent reduction of GABA signaling in the amygdala leads GAD through elevated valuation of threats [10]. Similarly, PD is also associated with decreased GABAnergic transmission and subsequent over stimulation of neural pathways, however in PD the decrease GABAnergic signaling may be due to reduced level of the GABA neurotransmitter itself and not due to decreased $GABA_A$ receptors as seen in GAD [9, 11, 12]. While GABAnergic pathways in the amygdala are inhibitory and stress reducing, glutamate, the major stimulatory neurotransmitter, when over active in the amygdala enhances stress and can lead to OCD. Pharmacological enhancement of glutaminergic signals in the frontolimbic regions of the brain enhance anxiety and imaging studies have shown increased glutaminergic activity in various structures of the limbic system in the brain [13–15]. PTSD and SAD also appear involve increased glutaminergic activity in the amygdala [9, 16]. GABA and glutamate influence the feeling of anxiety be reducing and enhancing the perceived threats, while the neurotransmitters, serotonin and dopamine are associated with the reward and pleasure pathways of the limbic

system and can influence the overall perception of environmental stressors generally reducing anxiety. For example, SAD is associated with both decreased activity at serotonin receptors and also decreased dopamine levels in limbic neurocircuitry [9, 16, 17]. Taken together, anxiety disorders involve irregularities in the levels of neurotransmitters and neurotransmitter receptors in the neurocircuitry of the limbic system. The inappropriate levels of neurotransmitters and their receptors can lead to hyper activity in regions of the limbic system such as the amygdale and lead to incorrect and unhealthy assessment of the risks and threats associated with stressors or lack of stressors and lead to anxiety and fear potentially even in the absence of threat. Activation of the HPA-axis can contribute to both the clinical signs and symptoms of anxiety and also lead to chronic glucocorticoid induced pathologies which serve and further internal stressors and add to anxiety. Treatments for anxiety disorders have therefore focused on developing drugs that correct and manage the levels of neurotransmitters and neurotransmitters receptors and signaling in the limbic system pathways and particularly in the amygdala.

GABAnergic benzodiazepines are the favored class of anxiolytic medications [10, 11, 18]. The diazepine ring is a seven membered ring structure containing two nitrogens and this diazepine ring and when fuses with a benzene ring forms a benzodiazepine that can bind to GABAA receptors on neurons in the brain [18]. Benzodiazepines are favored due to their lesser side-effects compared to other anxiolytic drugs, although side effects are still concerns [18]. The mechanism of benzodiazepine signaling is binding to either $GABA_A$ or $GABA_B$ receptors and allowing either chlorine ions into the cell at the synapse or stimulating the release of potassium from the cell into the synapse respectively [10, 11, 18]. In the cells of the amygdala, the chlorine influx inhibits the signaling of the pathway and diminishes the level of potential threat assigned to a sensory input or any external or internal stressor. People with GAD and PD express low levels of $GABA_A$ and produce less GABA respectively thereby limiting the patient's ability diminish the signals from stressors is associate with a heightened sense of fear and worry. By being GABAnergic the benzodiazepines help to restore or boost the GABAnergic pathway and the therefore the reduction of anxiety. Alternatively to drugs that act in a GABAnergic fashion, serotonin and dopamine uptake inhibitors, often used for depression, reduce anxiety and fear by increasing levels of these "feel good" neurotransmitters in the limbic neurocircuitry. Low synaptic serotonin and dopamine in the amygdale and nucleus accumbens is associated SAD. Serotonin uptake inhibitors (SSRIs) and noradrenalin and dopamine reuptake inhibitors (NDRIs) increased the level of serotonin and dopamine in the synapse and have been used to treat depression and also provide relief from anxiety and anxiety disorders. [18–22].

In addition to the development of new drugs that interact with the amygdala and HPA-axis, anxiety can also be addressed by diet. The diet can be associated with anxiety in two main ways. First, if a diet is deficient in nutrients such as selenium, lysine, magnesium and inositol, changes in food consumptions or dietary supplementation can replace the deficient nutrient, balance the diet and alleviate anxiety [23]. Further, dietary deficiencies in antioxidants can lead to the buildup of reactive oxygen species (ROSs) that form as a part of normal metabolism and are reactive chemicals that can bind to DNA, lipids and proteins leading to DNA and membrane damage and cellular toxicity. This cellular damage serves as a stress signal and is associated with anxiety [24, 25]. Therefore, increasing dietary antioxidant intake can help with anxiety. Second, food nutrients can directly affect the neurochemistry of the limbic system by either directly boosting GABA or Serotonin levels or by binding to neurotransmitter receptors. For example, GABA is an amino acid is available directly in the diet. Further the amino acid,

5-hydroxytryptophan is a serotonin precursor and is a popular dietary supplement taken to easy feelings of anxiety and stress. While it is not clear if increasing oral consumption of GABA and 5-hydroxytrptophan can increase brain GABA and serotonin levels, clinical studies have shown and relaxing effect of GABA and 5-HTP supplementation [23]. The neurochemistry of the brain can also be altered by food chemicals eaten from bacteria, fungi and plants that have nutraceutical effects by acting in a drug-like fashion as cell signaling molecules and alerting cellular behavior. In this chapter we focus on food nutraceuticals that are anxiolytic in humans and alter the neurochemistry and the amygdala and other limbic structures in the brain. Of particular interest are anxiolytic phytochemicals that in addition to changing the brain neurotransmitter physiology also stimulate neuronal plasticity through the activation and or potentiating of neurotrophin receptors and signal transduction pathways.

Recent studies have revealed that numerous anxiolytic substances, including endogenous neurotransmitters, anxiolytic drugs, and nutraceuticals, are also neurotrophic in that they also activate the brain derived neurotrophic factor (BDNF) pathway, the neurotrophin-3 (NT-3) pathways and the nerve growth factor (NGF) pathway by binding to or potentiating the TRKA – C neurotrophin receptors and directly activating the ERK1/2 signaling pathway leading to neuroplasticity [26–37]. This is important because neurotrophins can regulate neuroplasticity not only during development but also during learning and the establishment of memories [35–37]. Neurotrophins are small soluble signaling molecules that can diffuse between cells to play a role in cell–cell communication [35–37]. These neurotrophic factors include BDNF, NGF and NT3 bind to cell surface molecules on neuronal cells known as the tropomyosin receptor kinases (TRK) A – C respectively [35–37]. Neurotrophin signaling is associated with neuritogenesis or new neurite formation in neuronal cells. The changes in cell shape associated with the establishment of new neurites and therefore potentially new connections is known as neuroplasticity [35–37]. Recent attention has been brought to the idea that in so far as anxiety is related to the memories of trauma and the establishment of a learned threat level in the perception of stressors through neuroplasticity, perhaps anxiolytic phytochemicals with neurotrophic activity can be used to reduce anxiety not only through changes neurotransmitter activity, but also by providing the plasticity required to relearn and reduce the emotional value ascribed to a stressor thereby also facilitating the reduction in anxiety [26–34]. Therefore anxiolytic phytochemical neurotrophins are important because they offer a new area of research into not simply adjusting neurotransmitter activity, but to the development of natural treatments and drugs that can actually reverse the neurocircuitry associated with anxiety through neuroplasticity and relearning. It is important to note however, not all anxiolytic phytochemicals are capable of stimulating neuroplasticity. The following section of this chapter will present all nutraceutical phytochemicals that are anxiolytic in human clinical trials that also show potential for stimulating neuroplasticity either by directly stimulating neuritogenesis or neurite outgrowth neuronal cells or by binding to the TRKA-C neurotrophin receptors and or by the activation of the neurotrophin ERK1/2 signal transduction pathway and others associated with neurite formation.

2. Clinically relevant anxiolytic phytochemicals with neurotrophic activity

In this chapter we present only plants and plant extracts that contain phytochemicals that are both shown to be anxiolytic in human clinical trials and also

possess neuroplastic properties (**Table 1**). The specific anxiolytic nutraceutical or phytochemical in the plant is in most cases not known, in part because neuroactive plants usually contain many nervine agents. Often however there is a suspected phytochemical or group of phytochemicals thought to be responsible for the anxiolytic activity. In some cases the anxiolytic nutraceutical in the plant extract is the same phytochemical that has the neurotrophic activity, while in other cases it may be a different phytochemical in the plant extract. Anxiolytic drugs adjust neurotransmitter and neurotransmitter receptors levels which leads to increased drug insensitivity, extreme withdrawal effects and a return to imbalance neurotransmitter and neurotransmitter receptor levels when and if the drug is removed. In addition to altering neurotransmitter and receptor levels neuroplastic anxiolytics also stimulate the new neurite connections associated with learning and remembering appropriate responses to stressors. If a new response to a threat is learned, then treatment of the anxiety disorder may not require dosage increases and the newly learned healthy perceptions of threats could remain with the patient even if the drug or treatment is removed or reduced. This would represent a tremendous advancement in the treatment of anxiety disorders. **Table 1** is a list of the fourteen clinically supported anxiolytic plants that also have neuroplastic properties.

2.1 Theanine

Theanine is an amino acid that when taken as a green tea extract or in a purified form is able to reduce anxiety in clinical trials [38–40]. When administered in a double blind placebo controlled study, theanine was shown to reduce stress-induced salivary cortisol levels [39]. However in other studies, while theanine did improve the sleep in people with GAD, theanine did not reduced anxiety scores on the HAMA scale [41]. Both animal and in vitro studies have suggested that theanine supplementation increases brain serotonin, dopamine and GABA levels and that the cellular target for theanine includes glutamate receptors to which theanine binds and antagonizes the stimulating action of glutamate on neurons [42–44]. With regard to neuroplasticity, theanine facilitates neuritogenesis in the developing rat hippocampus and enhances object learning memory [45]. Further, dietary theanine increases nerve growth factor (NGF) levels in the developing rat brain [46]. Theanine is not the only green tea molecule that can affect neurotrophin activity. The catechins from green tea have been show to potentiate BDNF binding to TRKB receptors in PC12 cells and enhance neurite outgrowth [47], and potentiate NGF signaling through TRKA receptors and enhance neurite outgrowth also in PC12 cells [48]. Further, the green tea catechin, green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) also stimulates neurite outgrowth in cultured PC12 neurons [49].

2.2 Chamomile

In clinical trials, chamomile has been shown to decrease the symptoms of general anxiety disorder [50, 51], in part by exerting an effect on diurnal cortisol changes [52]. While not yet known, apigenin is a plant flavone component of chamomile which is thought to contribute to the anxiolytic effects of chamomile [53]. Interestingly, apigenin increases neurite formation in murine N2a cells [54] and reverses PTZ induced behavioral impairments in mice by increasing hippocampal levels of brain derived neurotrophic factor (BDNF) [55]. Apigenin also has been shown to increase hippocampal BDNF levels in a chronic corticosteroid treatment model of depression in mice [56]. Apigenin also activates the ERK1/2 pathway in PC12 cells and while not sufficient to stimulate differentiation in PC12 cells [57], apigenin does increases neurite outgrowth in estrogen receptor

Anxiolytic	Neurotrophic Activities.		
Plant/Nutraceutical	**Neurotrophin Pathways**	**Neurite Outgrowth**	**References**
Green Tea/theanine	Increases brain BDNF levels	hippocampal cells	[45, 46]
	Increases brain NGF synthesis	Neural stem cells	
	Potentiates NGF at TRKA		
Chamomile/apigenin	Increases hippocampal BDNF	N2a cells	[55–58]
	ERK1/2 kinase activation		
Lavender/N.D.	Increases brain NGFR	neuronal cells	[67, 68]
Ashwagandha/N.D.	Increases brain GDNF	hippocampal cells	[75]
Passion Flower/ apigenin	Potentiates NGF	PC12 cells	[80]
Cannabis/CBD	Binds TRKA	PC12 cells	[88]
	Activates ERK1/2		
Valerian/ Sesquiterpenes	Increases BDNF secretion	PC12 cells	[102, 103]
	NGF potentiation		
Citrus/limolene	ERK1/2 activation	PC12 cells	[110–113]
Saffron/N.D.	Increases BDNF and GDNF	N.D.	[116, 117]
Bacopa Monieri/ saponins	Increases brain NGF and	N.D.	[120, 121]
	BDNF levels		
Skullcap/baicalin	Increases brain BDNF	N.D.	[126, 127]
	ERK1/2 activation		
Rhodiola rosea/ salidroside	Increases NT-3, BDNF	stem cells	[130, 131]
	and NGF; ERK1/2 activation		
Hops/prenylflavinoids	TRKA signaling	PC12 cells	[135, 136]
		dorsal root ganglia	
Nigella sativa/ thymoquinone	N.D.	hippocampal cells	[141, 142]
		dorsal root ganglia	

All plants listed above have been shown to be anxiolytic in human clinical trials. In some cases the anxiolytic molecules and neurotrophic activities have not been determined (N.D.). Neurotrophic activities are those associated with activating neurotrophin signaling pathways by increasing levels of neurotrophin (NGF, BDNF, NT3, GDBF) synthesis, or by directly binding to neurotrophin receptors (TRKA, TRKB, TRKC and NGFR) or by activating the ERK1/2 signaling pathway. Another neurotrophic activity is the induction of neurite outgrowth or neuritogenesis in neuronal cell cultures and in. In these cases the names of the cells or tissues showing a neuroplastic response is provided.

Table 1.
The neurotrophic activities of anxiolytic plant extracts and phytochemical nutraceuticals.

expressing PC12 cells [58] again linking dietary phytochemicals that are anxiolytic to neural plasticity.

2.3 Lavender

Lavender oil also has anxiolytic effects in clinical trials in which it can both reduce anxiety associated with stressful event such as surgeries and recovery and also reducing anxiety in anxiety disorders [49–63]. Targets for lavender oil include the 5-HT1A serotonin receptor, the NMDA receptor and the serotonin transporter (SERT) [64, 65]. Linalool, a lavender oil terpene in specific can bind to SERT [64]. In a clinical trial where subjects were subjected to stress, linalool helped to reduce stress as measured by salivary cortisol levels, blood pressure and heart rate [66]. While linalool may be responsible for much of the anxiolytic effects of lavender oil, linalool has not been shown to have neurotrophic activity, however, lavender oil has be shown to increase neurite outgrowth and synapse formation in neuronal cell cultures [67] and increase both BDNF and nerve growth factor receptor (NGFR) levels in mouse brain [68]. Activation of NGFR is associated with enhanced TRKA receptor activity in neurons which triggers neurite outgrowth in response to NGF signaling [69, 70].

2.4 Ashwagandha

Ashwagandha is a plant used in Ayeurvic medicine from which the roots and berries have been used as adaptagens and also to relieve stress. In double blind placebo controlled clinical trials Ashwagandha supplementation has been shown to reduce anxiety based reducing both scores on the Hamiltion-Anxiety (HAMA) scale and morning salivary cortisol [71] and reduce anxiety in a variety of other contexts including schizophrenia and sleep disorders [71–73]. With regard to brain neurochemistry, Ashwagandha does not appear to affect sertotonergic, GABAnergic, or glutaminergic pathways but instead increases cholinergic signaling in the cortical and basal forebrain [74]. While the specific bioactive molecule(s) in Ashwagandha that are anxiolytic have not been specifically identified, sominone, an aglycone derivative of Withanoside IV when injected into mice stimulated neurite outgrowth in the hippocampus and increased production of the neurotrophin, Glial Derived neurotrophic Factor (GDNF) [75]. Further injection of sominone into mice enhances spatial memory, again suggesting that anxioloytic phytochemicals that are neurotrophic may ease anxiety by providing signals to enhance neural plasticity and learning [75].

2.5 Passion flower

Passion flower also shows anxiolytic properties in clinical trials that are as effective as midazolam and oxezepam [76, 77] and can reduce anxiety associated with ambulatory surgery and dental extraction [77–79]. The anxiolytic molecule from passion flower has not been identified and the effects of passion flower on brain neurochemistry is not well studied. It is interesting to note however that C-dideoxyhexosyl flavones from passion flower have been shown to enhance NGF-induced neurite outgrowth in PC12 cells [80].

2.6 Cannabidiol

Cannabidiol (CBD) is anxiolytic and has been shown in clinical trials to reduce social stress [81] and reduces anxiety in social phobia patients [82]. CBD

also reduces anxiety associated with drug-craving during recovery from heroin addiction [83]. With regard to brain neurochemistry in clinical trials, CBD reduction in SAD was associated with increased blood flow in the limbic and paralimbic brain areas [84]. CBD is anxiolytic through direct binding of the $GABA_A$ receptor and activating the GABAnergic pathway [85–87]. CBD also bind to the NGF receptor, TRKA which signals the ERK1/2 signal transduction pathway and stimulates neurite outgrowth in PC12 cells [88]. Indeed the mechanism of action of CBD is recognized to help with the neuronal plasticity through autophagy and neuritogenesis and may help not only with anxiety, but also with other psychiatric disorders [88, 89]. Due to the lipophilicity of CBD, there is interest in developing emulsification techniques to increase CBD bioavailability when taken in the diet. For example, nanoemulsification [90] and lipid extractions [91] and lipid-vehicles [92] and piperine nanoliposhperes of CBD [93, 94] have been investigated for better oral absorption and better bioavailability for cellular targeting. Nanoemulsified, versus lipid emulsified CBD were tested for their ability to stimulate neurite outgrowth in PC12 cells (**Figure 1**). Continuous lipid extracted CBD shows greater bioavailability and activity compared to nanoemulsification and piperine nanoliposheperes (**Figure 1**).

2.7 Valerian root

The anxiolytic activity seen in patients supplementing with Valerian root extract [95], is known to be due to the sesquiterpene, valerenic acid [96]. While there is evidence to suggest that valerenic acid activates the GABAnergic pathway [97, 98],

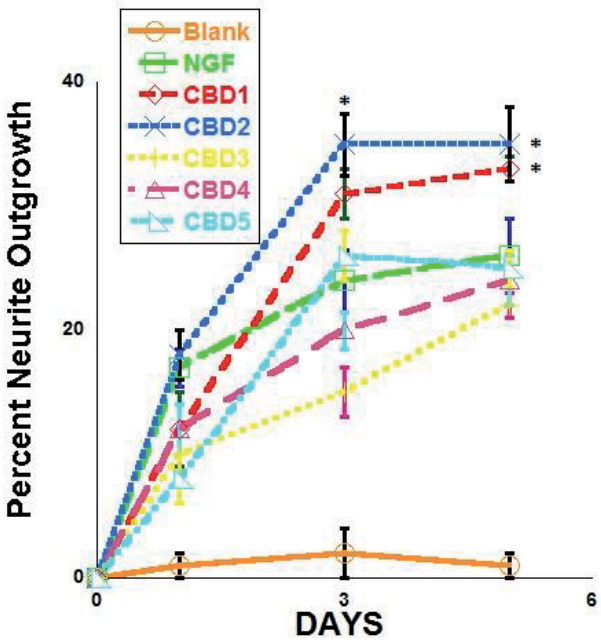

Figure 1.
The effects of CBD on PC12 cell neurite outgrowth. PC12 cells were seeded on tissue culture plastic in a serum free defined medium and the percentage of cells that formed neurites were counted by visual inspection over a five day period. Cells were either untreated (blank) or treated with 100 ng/ml nerve growth factor (NGF) or with 10 uM of five different CBD formulations (CBD1-CBD5). CBD designations are as follows: CBD isolated by continuous lipid extraction, (CBD1), CBD1 + vitamin C (CBD2), nano-emulsion CBD (CBD3), liposomal-emulsion CBD (CBD 4) and piperine nanoliposhpere preparation CBD (CBD5). CBD1 and CBD2 were statistically significantly more neuritogenic at 95% confidence (*) on days three and five ($p < 0.05$, t-test) compared to any of the other CBD formulations.

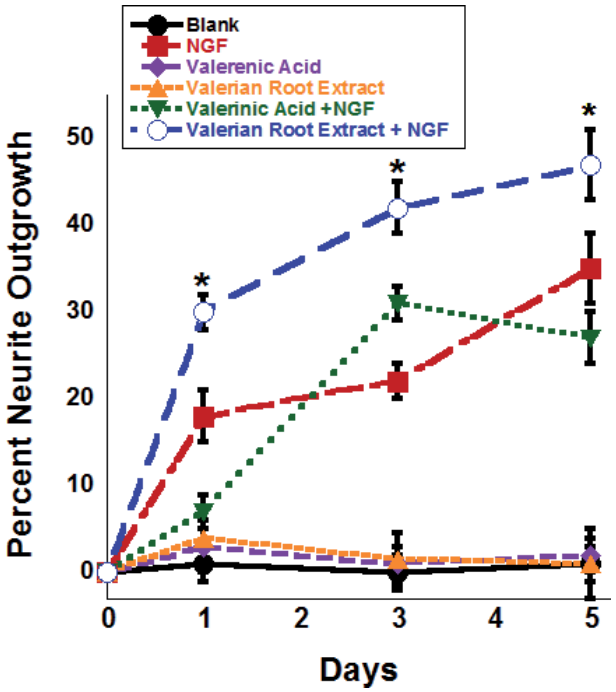

Figure 2.
The effect of valerian root extract and valerenic acid on neurite outgrowth in PC12 cell cultures. PC12 cells were seeded in serum free defined medium and treated as indicated with 10 ng/ml NGF, 100 μM valerenic acid and 50 mg/ml of 4:1 aqueous valerian root extract. The percentage of cells that formed neurites was counted by visual inspection over a five day period. More neurites were seen in PC12 cells treated with the valerian root extract and NGF when compared to NGF alone and these differences were statistically significantly at 95% confidence (*) on days one, three and five ($p < 0.05$, t-test). These data suggest valerian root extract phytochemical can potentiate NGF activity.

growing evidence suggests that valerenic acid mediates anxiolytic effects also by both antagonizing glutaminegric pathways [99, 100] and agonizing the serotonin receptor [101]. Valerenic acid also activates secretion of BDNF in cultured SH-SY5Y neurons [102]. Interestingly, germacrane, another sesquiterpene extracted from Valerian root, while not associated with an anxiolytic activity, has been shown to potentiate NGF and TRKA signaling and neurite outgrowth in PC12 cells [103]. An aqueous extract of valerian root enhances NGF-mediated neurite outgrowth and neuroplasticity but unlike CBD, the valerian extract is not neurotrophic in PC12 cells in the absence of NGF stimulation (**Figure 2**).

2.8 Citrus

Citrus plant extracts, including those from lemon, bitter orange, and bergamot relieve anxiety in clinical trials. For example, lemon inhalation reduced anxiety in myocardial infarction patients [104] and bergamot aromatherapy reduced preoperative anxiety [105] and bitter orange aroma therapy relieves anxiety in patients with acute coronary syndrome [106] and chronic myeloid leukemia [107] and preoperative anxiety [108]. Bitter orange extract contains primarily limolene and b-myrcrene, appear to act on the 5-HT serotonergic pathway [109]. When tested in PC12 cells, citrus phytochemicals such as nobilitin, gardenin A and auraptene all stimulate neurite outgrowth [110–112] and 5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone from sweet orange peel stimulates neurite outgrowth in and NGF-like fashion activating the ERK1/2 signaling pathway suggesting binding to TRKA [113].

2.9 Saffron

Saffron has been shown to be anxiolytic in two double blind placebo controlled clinical trials. [114, 115] and while the active anxiolytic molecule in Saffron has not been identified, crocin, a carotinoid in Saffron, has been shown to increase BDNF and GDNF expression in neuronal stem cells [116] and also increase hippocampal BDNF and protect the murine brain from methamphetamine toxicity [117].

2.10 *Bacopa monnieri*

In double blind placebo controlled trials, *Bacopa monnieri*, an adaptagen of Ayruvic medical tradition, has been show to enhance cognition and reduce anxiety [118, 119]. This anxiolytic adaptagen has been shown to increase nerve NGF expression in rats [120]. A saponin isolated from *Bacopa monnieri*, Bacopacide I, has been shown to have antidepressant activity in mice by modulating the HPA axis and enhancing BDNF mRNA expression in the hippocampus and prefrontal cortex of mice [121].

2.11 Skullcap

Skuttleria is a genus of plants known as the skullcaps that include scutellaria Radix and *Scutellaria lateriflora* (American skullcap). American skullcap has been shown to be anxiolytic in humans as shown by a reduction in anxiety in healthy volunteers using the Beck Anxiety Inventory (BAI) [122, 123]. The Skullcap flavone, baicalin which is found in american sckullcap and other members of the skuttleria genus has been shown to be anxiolytic by binding to $GABA_A$ receptors in mice [124, 125]. Interestingly biacalin increases hippocampal BDNF expression and in doing so protects the hippocampus from corticosterone induced depression in mice [126]. In addition biacalin stimulates neurite outgrowth in C17.2 neuronal stem cells by signaling through the ERK1/2 pathways, the known signal transduction triggered by NGF binding to TRKA [127].

2.12 Rhodeola Rosea

Rhodeola Rosea has been shown to reduce GAD in small pilot and self reporting clinical trials [128, 129]. While the effects of Rhodeola on brain neurochemistry has not been well studied, salidroside, a glycoside from Rhodiola has been shown to increase stem cells expression of neurotrophin-3 (NT-3), BDNF, NGF mRNA and induce differentiation into neurons [130] and also activates the ERK1/2 pathway in NGF treated PC12 cells [131].

2.13 Hops

One study shows Hops to be anxiolytic in clinical trials [132]. Prenylflavinoids from extracted from Hops can both bind to the benzodiazepine binding site on $GABA_A$ receptors [133, 134] and stimulate neurite outgrowth through TRKA a signaling in PC12 cells and cultures of dorsal root ganglia neurons [135, 136].

2.14 *Nigella sativa*

One clinical study shows that *Nigella sativa* seeds are anxiolytic in clinical trials [137]. Oral administration of Nigella extracts increase brain serotonin level in rats [138, 139] and thymoquinone, a terpine from Nigella is anxiolytic through

a GABAnergic pathway when orally administered to mice [140]. Thymoquinone promotes neurite outgrowth in rat hippocampal neurons and dorsal root ganglion neurons [141, 142].

3. Conclusion

Phytochemical nutrients that are used to reduce anxiety may have this affect in part by stimulating neuroplasticity and altering the brain neurocircuitry associated with learned responses to stressors and threats. Fourteen of the roughly forty-five plant and plant extracts proven to reduce anxiety in humans in clinical trials are also able to act like neurotrophins; endogenous molecules that stimulate neuroplasticity in the human brain. Anxiolytic drugs are harsh and symptoms return when the drug is removed because the neurotransmitter chemistry returns to an imbalance. Neuroplasticity offers an opportunity to use food phytochemicals along with drugs or in their place to learn to establish more appropriate responses to perceived threats by reworking neural connections. These new neural connections may not be lost even when the anxiolytic treatment is removed. Using neuroplastic drugs and foods to not only alter brain chemistry, but also the circuitry, would be a tremendous advancement in the treatment of anxiety.

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Acronyms and abbreviations

BDNF	Brain derive neurotrophic factor
NGF	Nerve growth factor
NT3	Neurotrophin-3
GDNF	Glial derived neurotrophic factor
TRK	Tropomyosin receptor kinase
GABA	Gama miniobutyric acid
CBD	Cannabidiol
GAD	Generalized anxiety disorder
PTSD	Post traumatic stress disorder
OCD	Obsessive compulsive disorder
PD	Panic disorder
SAD	Social anxiety disorder

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Chapter 8

Natural Compounds with Antioxidant Activity-Used in the Design of Functional Foods

Petre Săvescu

Abstract

This chapter is intended to describe the main antioxidants used in the design and construction of functional foods. Defining the role of antioxidants, in the main redox processes in which certain oxidoreductases are involved, the best way of monitoring the activity of certain coenzymes of these oxidoreductases, will be established the main criteria in the design of sustainable functional foods. In addition, the importance of some coenzymes (FMN, FMNH + H^+, NAD, NADH + H^+) in preserving the activity of some valuable bio-compounds (with the role of antioxidants) in functional foods will be highlighted. Antioxidants are good disease-fighters, protecting our bodies from free radicals' attacks that would otherwise damage of the human cellular structures. Knowing and supporting the activity of the main compounds (with antioxidant activity) are operations that improve the reaction mechanisms of redox processes and can significantly contribute to achieving good functional foods - able to regulate the acid–base balance of the body and improve the metabolic processes from the consumer body.

Keywords: main antioxidants, functional foods, bio-compounds, coenzymes NAD and FMN, oxidoreductases, healthy products

1. Introduction

The modern man, limited by time resources is often attracted to fast food, regardless of the side effects generated by this diet. These foods prepared and served quickly are the result of previous scientific research - a process aimed at obtaining finished products with minimal effort, from extremely limited natural resources and carefully studied transfer phenomena (heat, mass, impulse). However, the results obtained are in contradiction with a normal and healthy diet and, over time, produce major changes in metabolism for consumers, till severe diseases. Today, our foods must be enriched in antioxidants; these are important in combating free radicals and decreasing of diseases such as cancer, type 2 diabetes, and chronic fatigue syndrome [1].

Consumer education, directing their attention to natural and high-energy diets, personalizing diets according to genetic characteristics, personal, acquired, and developed throughout life are the main goals of any nutritionist. A special role in the development of a safe, healthy diet is associated with the food industry specialist able to study and improve both the quality of raw materials entered in the manufacturing process and all stages of this process.

In order to develop synergetic collaboration between farmer, processor and nutritionist, consumer and all other factors interested in the integrated process "from farm to fork", is necessary a good professional and complementary training, good communication on the production and consumption chain, good promotion of good production practices, hygiene, laboratory, good dissemination of the results of scientific research in the field, promotion of natural bionic, biotechnological, bio-nanotechnological practices, a revaluation of food resources (to combat food waste and encourage the use of all components of the chain in innovative biotechnological sequences) [2].

Antioxidants are among the best natural disease-fighters, protecting our bodies from everyday stresses that would otherwise attack the human cellular structures.

Free radicals are primarily a by-product of oxygen. Through aerobic metabolism, every cell in the body utilizes oxygen to make energy so that it can live. The body creates by-products called oxidants, or free radicals when cells burn oxygen. These unwanted free radicals cause to damage cells in the body as they react to molecules in and outside of cells. The thermodynamics make a moving free radical to seeks another molecule which will be whole, for stability. Unfortunately, when it binds to another molecule, it tears cell walls, these free radicals can rip pieces of DNA, or can changes the chemistry of cell structures [3]. The antioxidants can change these phenomena through blocked the active energy of free radicals. They can neutralize the reactivity of unwanted free radicals and the consumer body will be protected. The formation of free radicals in the body, especially in the catabolism mechanism, is a normal process; it can happen as a result of breathing [4].

Plus, the following factors contribute to the increased level of free radicals in the body: stress, pollution, radiation, the unknow and ultra-processed food, the excess of drugs, the unwanted metals, the weak mentality, and a low level of consciousness. All of these must be changed. The first results come through the use of antioxidants, innocuity foods, functional foods, nutraceuticals, organic products - in the consumers' nutrition.

Very important - on the production chain "from farm to fork" (regardless of the size of the production chain) are the processes that take place with electron exchange (redox processes) - which include extremely complex mechanisms in which participates one of the most important classes enzymatic (oxidoreductases) [5]. The role of functional foods and dietary supplements in supporting and regulating metabolic functions in conditions of a daily life affected by stress and pollution is well known. In plants, there is an important category of compounds with high values of nutritional density and therefore, it is desirable to use as many recognized bioactive compounds as possible, in order to design and develop various functional foods [6]. An important problem arises in the case of preserving the active properties, in the conditions of advanced processing and therefore, it is extremely important to study the application of new protective technologies in the construction of such foods.

Antioxidants - used as food additives - can extend the shelf life and protect food from damage caused by the oxidation process. The oxidation reaction occurs due to the presence of oxygen. Atmospheric oxygen comes into contact with certain foods and can produce a significant number of unwanted compounds. After oxidation, a number of unwanted processes can also occur oxidation and rancidity of fats, peroxidation with changes in color, taste, smell of food.

Antioxidants - as food additives are widely used in the food industry, and additives can be classified into two broad groups.

The first group comprises compounds (acids and their derivatives), which block or delay the colour change in fruits or meat products. These substances include Ascorbic Acid (E 300) and Citric Acid (E330). Although a natural antioxidant

occurring in most fruits and vegetables, E300 (for Australia or New Zealand only "300", without "E") can also be produced in a synthetically way, from the fermentation and oxidation of glucose. It is an acid that is most commonly used in the manufacture of bread, by acting as a flour-treating agent [7].

According to FDA (Food and Drug Administration), citric acid is generally considered safe (GRAS) and can be used in food with no limitations other than current good manufacturing practice [8]. It can be used as an antimicrobial agent, antioxidant, flavouring agent, *p*H control agent, sequestrant in food.

According to EFSA (European Food Safety Authority), citric acid anhydrous and monohydrate (E330) are authorized as food additives in Commission Regulation (EU) No 231/2012 and categorized as "additives other than colours and sweeteners" [9].

The second group of antioxidants is composed of substances that prevent the oxidation of fats and oils. This oxidation leads the rancidity of food by changing its appearance and becoming inedible. In this group of antioxidants, can find Butylated Hydroxy Anisole (BHA, E320), Butylated Hydroxy Toluene (BHT, E321), and Galat (E 310, E 311, E312). However, they are chemicals obtained by synthesis, they are not recommended for use as antioxidants in functional foods (**Table 1**).

No	Food additives (antioxidants)	The characteristic activity	References
1	Citric Acid (E 300)	Important for the healthy development of bones, teeth, and blood vessels	[7]
		An act to reduce wrinkles (support the production of collagen in the skin)	[9]
		Acidulant, preservative, antioxidant and chelating agent in food (can prevent or slow down the oxidation process in foods)	[10]
		Prevents oesophageal cancer cell growth (inhibition of cell proliferation and induction of cell apoptosis)	[11, 12]
		Citrate can suppress tumours growth	[13]
2	*BHA* or *butylated hydroxy anisole* (1,1-dimethylethyl)-4-methoxyphenol)	• Not recommended for use as antioxidants in functional foods • A synthetic antioxidant, used to prevent fats in foods from going rancid and as a defoaming agent for yeast	[14]
		• Anticipated to be a human carcinogen	[15]
3	*Butylated Hydroxy Toluene* (*BHT*, E321) 2,6-di-tert-butyl-4-hydroxytoluene	• Synthetic phenolic antioxidants (SPAs), not recommended for use as antioxidants in functional foods	[16, 17]
		• BHT exposure is linked to cancer, asthma, and behavioural issues in children	[16, 17]
		BHT is tumour promoters, in high quantity	[15, 18]
4	Octyl gallate (E 311) and dodecyl gallate (E 312)	• Are substances authorized as antioxidants in foods as well as in food flavourings	[Annexes II and III to Reg.(EC) No 1234/2007. 1333/2008]
		• Required for a proper assessment of the safety of octyl gallate as a food additive	Reg. UE 2018/1481. [19]

Table 1.
Antioxidants – Food additives.

2. Antioxidants: compounds with antioxidant activity

2.1 Antioxidants: vitamins, provitamins with antioxidant activity

Vitamin A - Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, and several provitamins A carotenoids (most notable beta-carotene) [20–22] (**Figure 1**).

Generally, the three major antioxidant vitamins are beta-carotene (precursor of vitamin A), vitamin C, and vitamin E. We will find them in colourful fruits and vegetables, especially those with purple, blue, red, orange, and yellow hues [23].

The active form (retinol) comes from animal sources such as milk, eggs, meat, and fatty fish, all of which may be high in fat and cholesterol. But it also comes from plants, in the form of beta-carotene and other carotenoids, which are converted into vitamin A in the body [24].

Depending on the environment, vitamin A can be converted to an ester (a) or oxidized to aldehyde (b). The chain can continue to oxidize the aldehyde of vitamin A to the specific acid [25] (**Figure 2** and **Table 2**).

The main carotenes are showed in **Figure 3** (right side) (**Figure 4**).

The group of *xanthophylls* includes (among many other compounds) lutein, zeaxanthin, neoxanthin, violaxanthin, flavaxanthin, and α- and β-cryptoxanthin (**Figures 5** and **6**).

Vitamin E is a fat-soluble vitamins group with 4 tocopherols and 4 tocotrienols. The tocopherol content of animal and vegetable fats (oils) is strictly influenced by animal feed (**Figure 7**).

For alpha(α)-tocopherol each of the three "R" sites has a methyl group (CH3) attached. For beta(β)-tocopherol: R1 = methyl group, R2 = H, R3 = methyl group. For gamma(γ)-tocopherol: R1 = H, R2 = methyl group, R3 = methyl group. For delta (δ)-tocopherol: R1 = H, R2 = H, R3 = methyl group. The same configurations exist for the tocotrienols, except that the hydrophobic side chain has three carbon–carbon double bonds whereas the tocopherols have a saturated side chain [43]. For alpha(α)-tocotrienol each of the three "R" sites has a methyl group (CH3) attached.

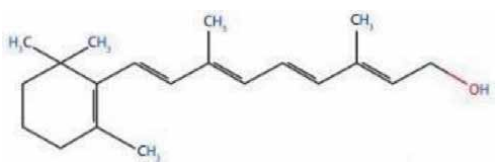


Figure 1.
Structure of vitamin A (Retinol).

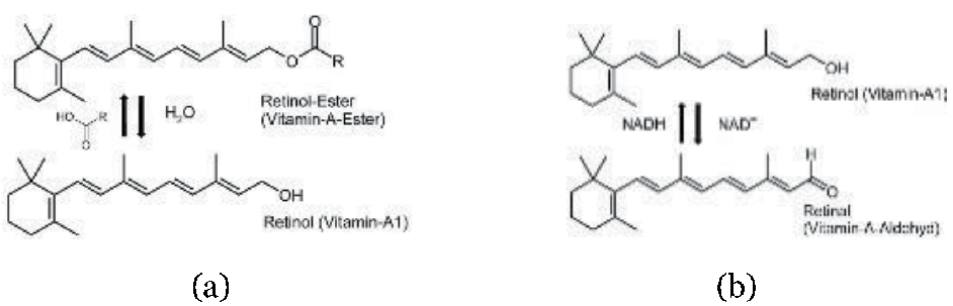


Figure 2.
Changes of vitamin A (to ester (a) or to aldehyde (b)).

No	Vitamins A, Provitamins	The characteristic activity	References
1	*Vitamin A (Retinol or retinyl ester – in tissues)*	• Important for growth, for the maintenance of the immune system, and for good vision	[23, 26]
		• In the food classical technology, produced and administrated as esters such as retinyl acetate or palmitate	[27]
		• Fat-soluble vitamin that maintains healthy soft tissue, bones, and mucous membranes, and produces pigment in the retina of the eye.	[24]
		• Retinol promotes healthy reproduction in women, fights cancer, and prevents premature aging	[27]
		• No pieces of evidence that beta-carotene or vitamin A supplements increase longevity in healthy people or in people with various diseases	[21]
2	*The carotenes:* alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll beta-cryptoxanthin (*all of which contain beta-ionone rings*)	• Function as provitamin A in organisms which possess the enzyme beta-carotene 15,15′-dioxygenase in the intestinal mucosa	[24, 28]
		• Cleave and convert provitamin A to retinol	[29].
		• β-carotene supplements may increase the risk of lung cancer for smokers	[30, 31]
		• β-carotene is a true antioxidant	[31]
		• The synthetic β-carotene can increase mortality by 1–8%	[32]
		• Lutein and zeaxanthin protect the body's proteins, fats, and DNA from stressors and can even help recycle glutathione	[33]
		• Consumption of lutein and zeaxanthin may protect against AMD (Age-related macular degeneration) progression to blindness	[34]
3	Other carotenoids, including lycopene (without *beta-ionone rings*),	• Have antioxidant activity and thus biological activity in other ways	[32]
		• Encapsulation increases the chemical and thermal stability of carotene molecules (and preserve the antioxidant activity)	[35, 36]
		• Lycopene having antioxidant effects in humans, particularly in the skin, heart function, or vision protection from ultraviolet light	[37]
		• Lycopene is a key intermediate in the biosynthesis of many carotenoids	[38]
		• During the processing of fruits, increases the concentration of bioavailable lycopene	[39–42]

Table 2.
The Vitamins A, provitamins with antioxidant activity.

R1 = methyl group, R2 = H, R3 = methyl group in beta(β)-tocotrienol. R1 = H, R2 = methyl group, R3 = methyl group – in gamma(γ)-tocotrienol. R1 = H, R2 = H, R3 = methyl group, in delta(δ)-tocotrienol. Palm oil is a good source of alpha and gamma tocotrienols (**Figures 8** and **9** and **Table 3**) [58].

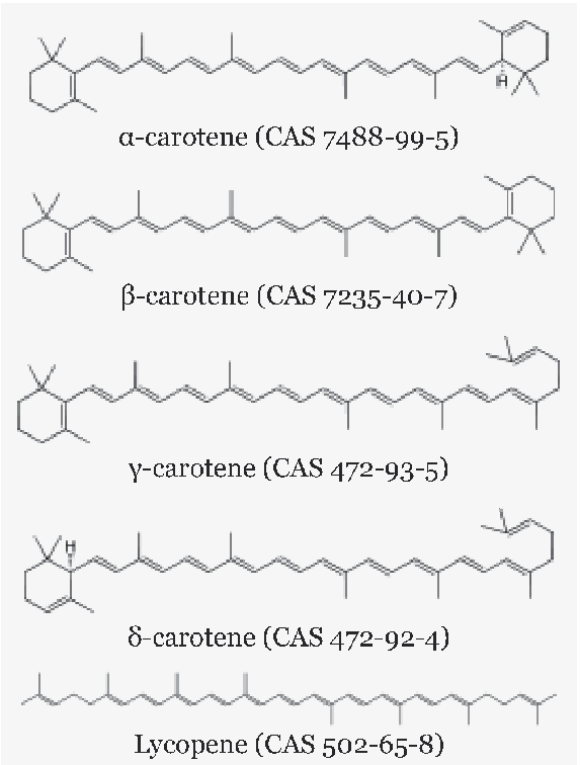


Figure 3.
The main carotenes.

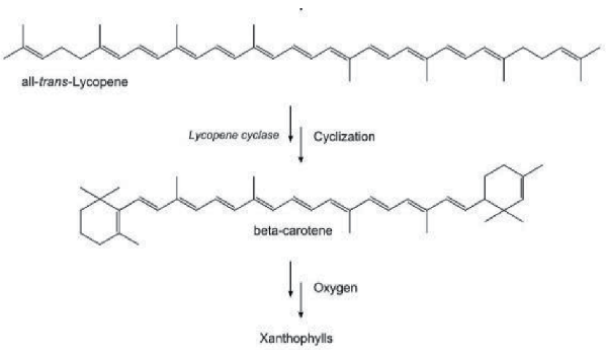


Figure 4.
Lycopene is a key intermediate in the biosynthesis of many carotenoids.

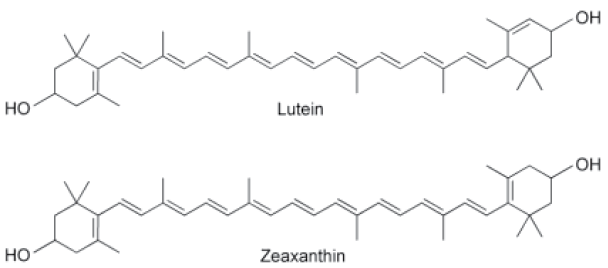


Figure 5.
The Chemical Structure of Lutein and Zeaxanthin.

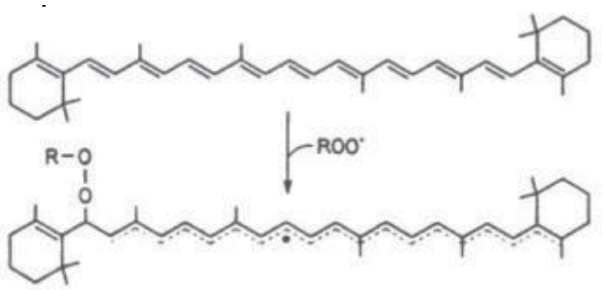


Figure 6.
The action of carotenoids on free radicals.

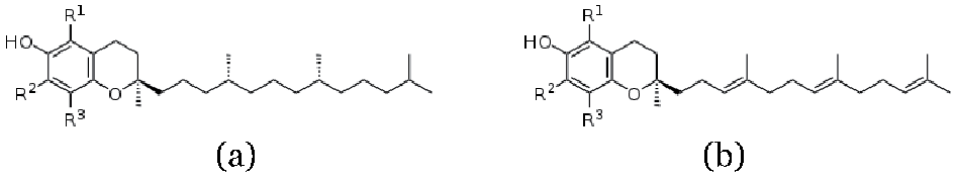


Figure 7.
General chemical structure of tocopherols (a) and tocotrienols (b).

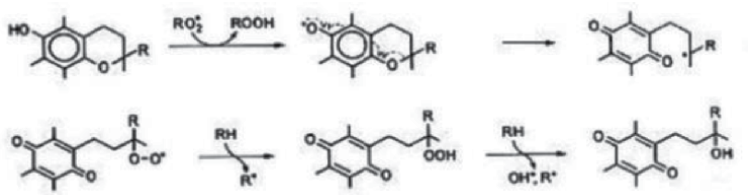


Figure 8.
Conversion of α-tocopherol to hydroxy alkyl quinone [44].

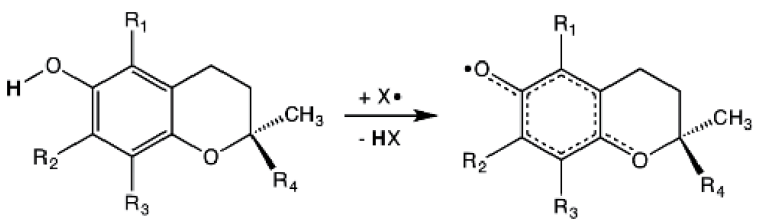


Figure 9.
Tocopherols function by donating H atoms to radicals (X) [45].

Oxidation of L-ascorbic acid to dehydroascorbic acid (**Figures 10** and **11**) depends on several parameters: oxygen partial pressure, pH, temperature and the presence of metal ions (**Figure 12**). Traces of metal ions - especially Cu^{2+} and Fe^{3+} – result from losses or transfers of substances (insufficiently controlled reaction media, insufficiently protected packaging).

2.1.1 Vitamins K

Actively participates in cellular oxidations, by reversing the transition from oxidized to reduced form, ensuring the transport of hydrogen non-enzymatically (**Figure 13**).

No	Vitamins E, Vitamins C	The characteristic activity	References
1	Vitamins E (tocopherols and tocotrienols)	• Fat-soluble antioxidant protecting cell membranes from reactive oxygen species	[46]
		• The antioxidant activity of tocopherols increases from alpha to delta; α-tocopherol is a good inhibitor of peroxide radicals formed during oxidation than γ-tocopherol • α-tocopherol can also generate alkyd radicals, which can initiate the self-oxidation of unsaturated fatty acids	[46]
		• Alpha-tocopherol is a lipid-soluble antioxidant functioning within the glutathione peroxidase pathway	[47]
		• Protect cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction	[48]
		• The oxidized α-tocopheroxyl radicals produced in the lipid peroxidation chain may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.	[49]
		• Vitamin E is implicated in the maintenance of normal cell function of cells lining the inner surface of arteries and generates anti-inflammatory activity and inhibition of platelet adhesion and aggregation	[50]
		• Can slowing the progression of age-related macular degeneration (AMD)	[51]
2	Vitamin C *(ascorbic acid*	• Protect of connective tissue, bones, cartilage, and blood vessels, as well as in healing injuries and forming collagen	[52]
		• The main biochemical role of vitamin C is to act as an antioxidant (a reducing agent) by donating electrons to various enzymatic and non-enzymatic reactions	[53]
		• Ascorbic acid acts as an antioxidant, thereby reducing the adverse effects of chemotherapy and radiation therapy	[54, 55]
		• Ascorbic acid functions as a cofactor for enzymes involved in photosynthesis, synthesis of plant hormones, as an antioxidant and also regenerator of other antioxidants	[56, 57]

Table 3.
The Vitamins E and C activity.

A number of other vitamins (B vitamins, vitamin K) together with their precursors are active in redox processes in the body and can be very good antioxidants, especially in reduced forms (hydrogenated forms). These bio-compounds are the first to oxidize, protecting the cellular environment from free radical attack.

2.2 Other antioxidants

2.2.1 Phenols and Polyphenols with antioxidant activity

Flavonoids (or bioflavonoids; from the Latin word flavus, meaning yellow, their colour in nature) are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in diets. Flavonoids are a well-known family of plant polyphenolic compounds. Flavonoids are represented by 6 major subclasses, present in the basic diet in humans: anthocyanidins, flavan-3-ol, flavonols, flavanones, flavones and isoflavones, flavonols.

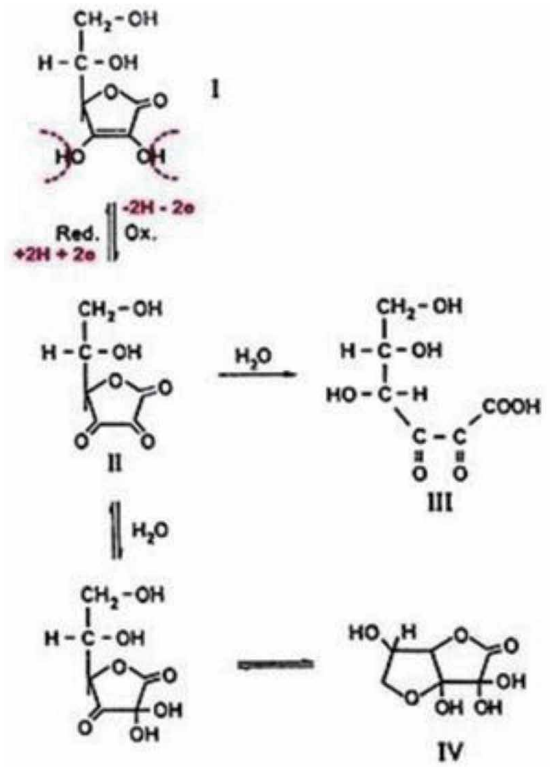


Figure 10.
Forms of ascorbic acid: I-L-Ascorbic acid, II-Dehydroascorbic Acid, III-2,3-Dicetogulonic acid; Hydrated IV-hemiacetal.

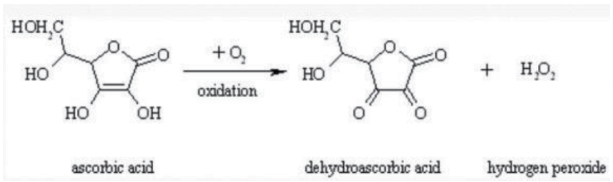


Figure 11.
L-ascorbic acid is a powerful reducing agent.

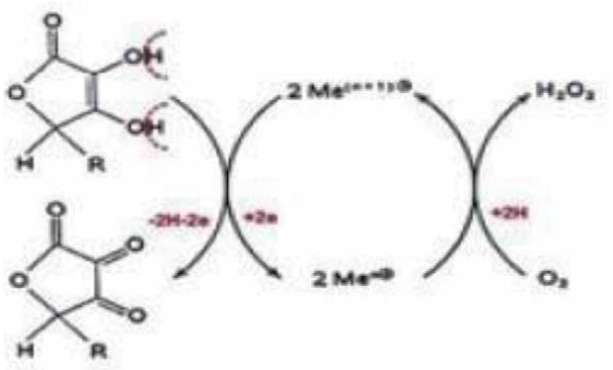


Figure 12.
The electron transfer during metal catalysis.

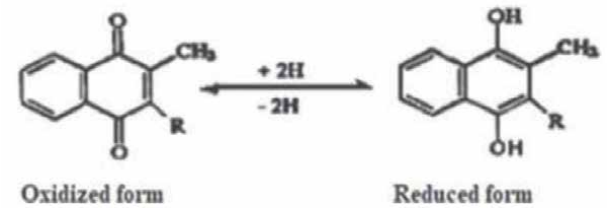


Figure 13.
The redox system of vitamin K.

Anthocyanidins are vegetable pigments, similar to anthocyanins but lacking in the carbohydrate side (**Table 4**). Their activity is based on that of the flavylium cation and the oxonium ion, which have various replacement groups of hydrogen atoms. Depending on the pH, these pigments can have various colours: red, purple, blue, and bluish green [59] (**Figures 14** and **15**).

Flavan-3-ols (sometimes referred to as flavanols) are derivatives of flavans that possess a 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton.

These compounds include catechin, epicatechin-gallate, epigallocatechin, epigallocatechin gallate, pro-anthocyanidins, theaflavins, thearubigins (**Figure 16**).

Until 2013, both the Food and Drug Administration and the European Food Safety Authority did not issue restrictions on the use of catechins, nor did they approve any catechin-based medicines. [60] (**Figure 17**).

Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Their diversity stems from the different positions of the phenolic -OH groups. Flavonols are present in a wide variety of fruits and vegetables. In Western populations, estimated daily intake is in the range of 20–50 mg per day for flavonols. Individual intake varies depending on the type of diet consumed [61]. The most used flavonols: Isorhamnetin, Kaempferol, Myricetin, Quercetin (**Figure 18**).

Anthocyanidin	R3′	R5′	R5	R6	R7
Cyanidin	−OH	−H	−OH	−H	−OH
Delphinidin	−OH	−OH	−OH	−H	−OH
Malvidin	−OCH3	−OCH3	−OH	−H	−OH
Pelargonidin	−H	−H	−OH	−H	−OH
Peonidin	−OCH3	−H	−OH	−H	−OH
Petunidin	−OH	−OCH3	−OH	−H	−OH

Table 4.
The main anthocyanidins and their substitution radicals.

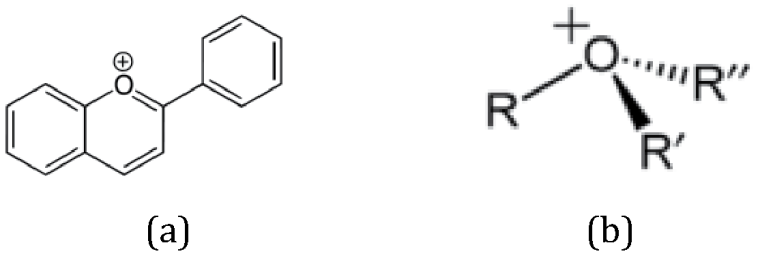


Figure 14.
Flavylium Cation (a) and general pyramidal oxonium ion (b).

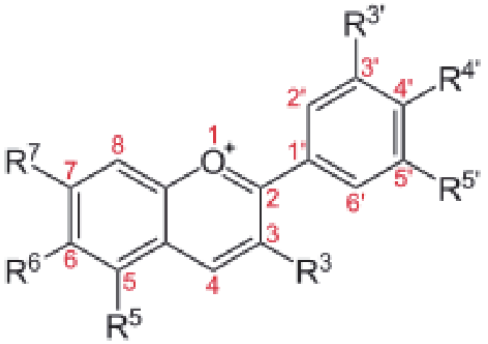


Figure 15.
Basic Structure of Anthocyanidins (R3' and R4' = -OH).

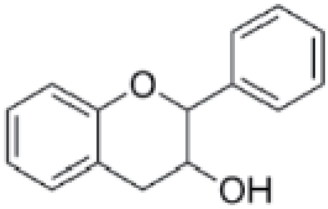


Figure 16.
Chemical Structure of Flavan-3-ols.

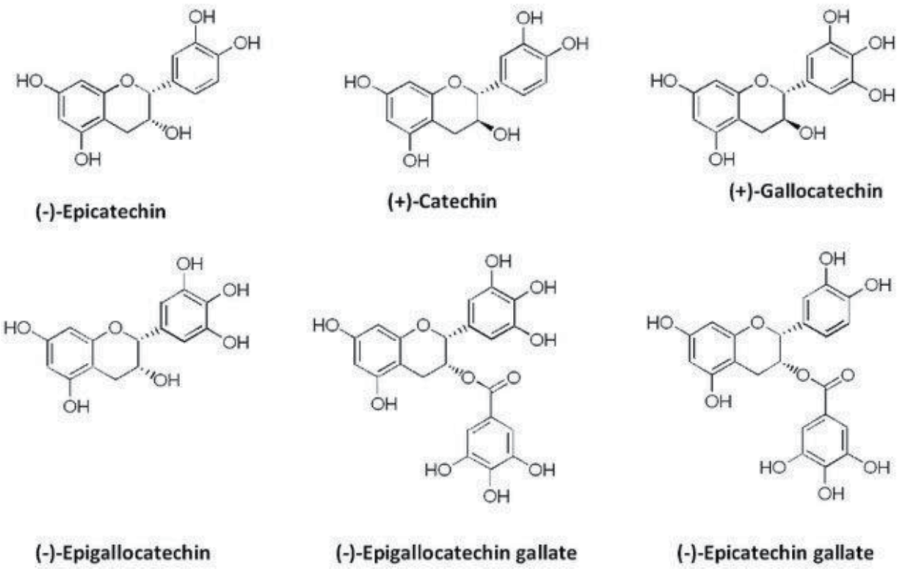
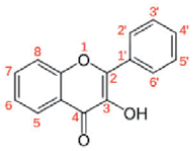


Figure 17.
Chemical Structure of Catechins.

Flavones (derived by the Latin flavus "yellow") are a class of flavonoids based on the nucleus of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Apigenin (4 ', 5,7-trihydroxyflavone), luteolin (3', 4 ', 5,7-tetrahydroxyflavone), tangeritine (4', 5,6,7,8-pentamethoxyflavone), chrysin (5,7-dihydroxyphlavone)) and 6-hydroxyflavone are compounds that belong to the class of flavones [62].



Flavonols	The substitution radicals								
Name	5	6	7	8	2′	3′	4′	5′	6′
Isorhamnetin	OH	H	OH	H	H	OCH3	OH	H	H
Kaempferol	OH	H	OH	H	H	H	OH	H	H
Myricetin	OH	H	OH	H	H	OH	OH	OH	H
Quercetin	OH	H	OH	H	H	OH	OH	H	H

Figure 18.
Chemical Structure of the main Flavonols and their substitution radicals.

In plants, a number of flavonoid glycosides often appear, which are in fact colourless aromatic ketones, derived from flavone (flavanone). [63].

Isoflavones are substituted derivatives of isoflavone, a type of naturally occurring isoflavonoids [64] many of which act as phytoestrogens in mammals [65]. Isoflavones are produced almost exclusively by the members of the bean family, Fabaceae (*Leguminosae*) (**Figures 19** and **20**).

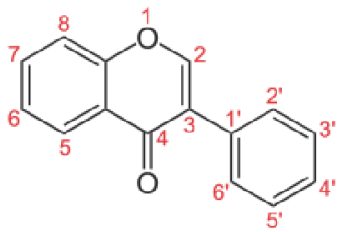


Figure 19.
Isoflavone, numbering. Genistein (5-OH, 7-OH, 4'-OH) or daidzein (7-OH, 4'-OH).

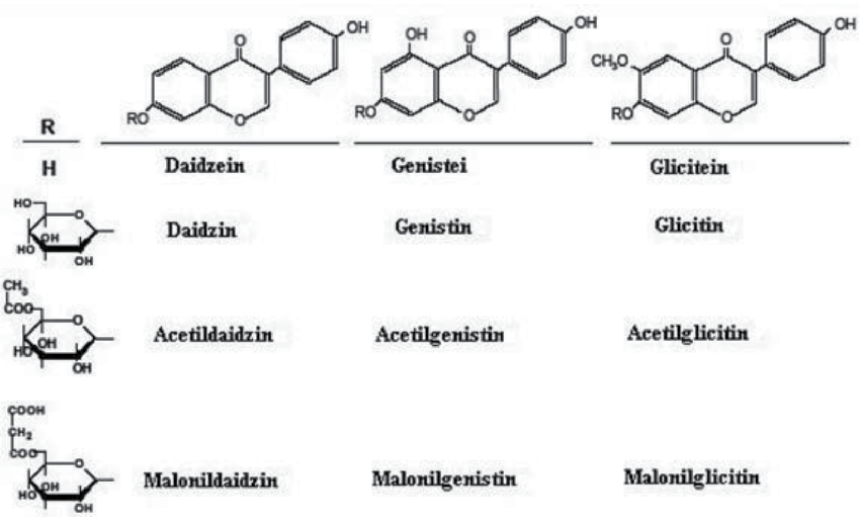


Figure 20.
Chemical Structure of the main isoflavones.

The consumption of isoflavones-rich food or dietary supplements is under preliminary research for its potential association with lower rates of postmenopausal cancer [66, 67] and osteoporosis in women [68]. Use of soy isoflavone dietary supplements may be associated with reduction of hot flashes in postmenopausal women [67, 68] (**Figure 21**).

2.3 Antioxidants: acids, amino acids and other compounds with antioxidant activity

2.3.1 Lipoic acid

Lipoic acid (LA) is an organo-sulfurized compound of caprylic acid (octanoic acid). It is also known - in the technical literature and as α-lipoic acid (ALA) and thioctic acid [69] (**Figures 22** and **23**).

In cells, α-LA can be reduced to dihydrolipoic acid, the more bioactive form of LA, involved in antioxidant processes that lead to decreased redox activities of iron and copper ions in solutions. [70]. Recent research has shown that the anti-aging and cellular disease prevention effects are mainly due to genetic mechanisms that improve the antioxidant state of the cell. However, this likely occurs via pro-oxidant mechanisms, not by radical scavenging or reducing effects [71–73]. α-Lipoic acid is an antioxidant that acts in both forms (both oxidized and reduced) on tissues and lipo- and water-soluble substances. It can be easily reduced by breaking the disulfide bridge with the formation of sulfhydryl groups. The di-hydrolipoic form of α-lipoic acid is regenerated by the redox mechanisms of vitamins C and E.

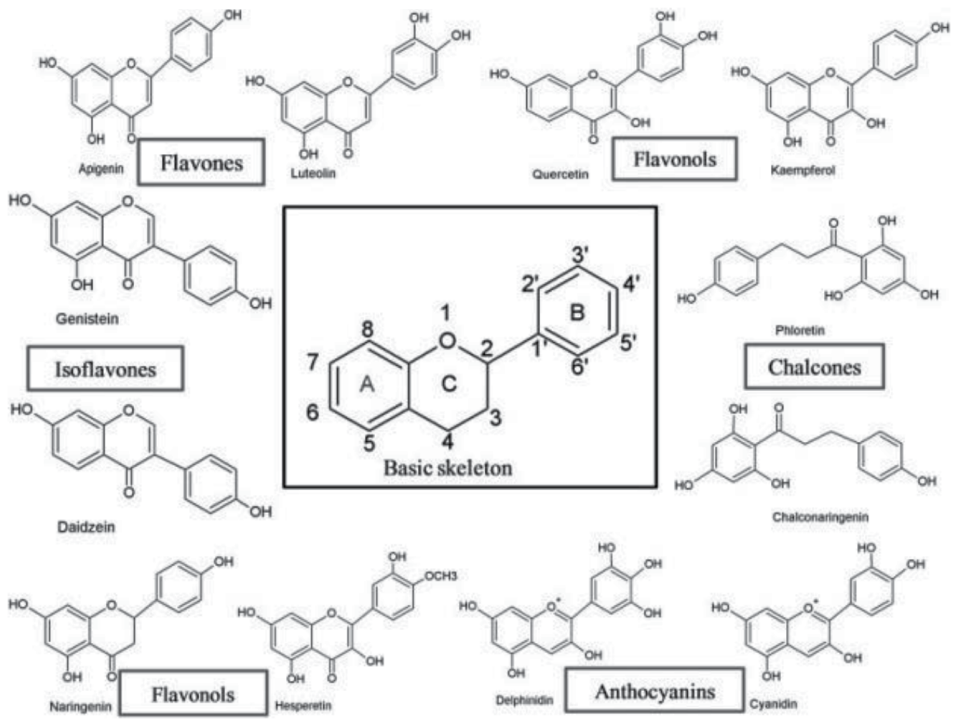


Figure 21.
The main structures of flavonoids.

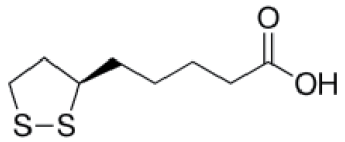


Figure 22.
Structure of α-Lipoic Acid.

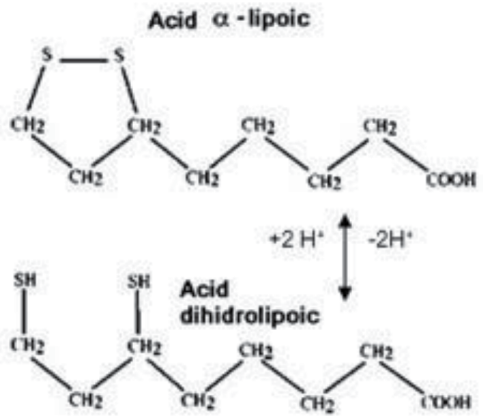


Figure 23.
α-lipoic acid (α-LA) and the two forms in which it is found (oxidized and reduced).

2.3.2 Folic acid

The tetrahydrofolate (II) derivative of folic acid (I) is the enzymatic cofactor that can transfer a carbon unit in various oxidation states (such as in formyl or hydroxymethyl residues) (**Figure 24**).

Folate contributes major to spermatogenesis. In women, folate is important for oocyte quality and maturation, implantation, placentation, fetal growth and organ development [74].

2.3.3 Cysteine

Cysteine (symbol Cys) [75] is a semi essential [76] proteinogenic amino acid with the formula HOOC-CH-(NH_2)-CH_2-SH. The thiol side chain in cysteine often participates in enzymatic reactions, as a nucleophile. Due to the ability of thiols to undergo redox reactions, cysteine has antioxidant properties. Its antioxidant properties are typically expressed in the tripeptide glutathione, which occurs in humans and other organisms. The systemic availability of oral glutathione (GSH) is negligible; so, it must be biosynthesized from its constituent amino acids, cysteine, glycine, and glutamic acid [77]. While glutamic acid is usually sufficient because amino acid nitrogen is recycled

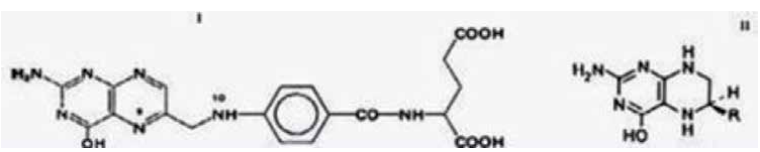


Figure 24.
Folic acid (I) and tetrahydrofolate derivative (II).

through glutamate as an intermediary, dietary cysteine and glycine supplementation can improve synthesis of glutathione [78]. Cysteine and cystine - form an important redox system, whose steady-state depends on oxidation conditions (**Figure 25**).

2.3.4 Glutathione

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is found in both animals, plants, and microorganisms (**Figure 26**).

The active group of glutathione is -SH, through which glutathione can participate in redox reactions, having a reduced form marked with G-SH and an oxidized one (with disulfide bridge, G-S-S-G, according to the Eq. (1)):

$$\underset{\text{Reduced form}}{2GSH} \longleftrightarrow \underset{\text{Oxidized form}}{G\text{-}S\text{-}S\text{-}G + 2H} \qquad (1)$$

GSH protects cells by neutralizing single reactive oxygen species [79–81]. This transformation is found in the reduction of peroxides:

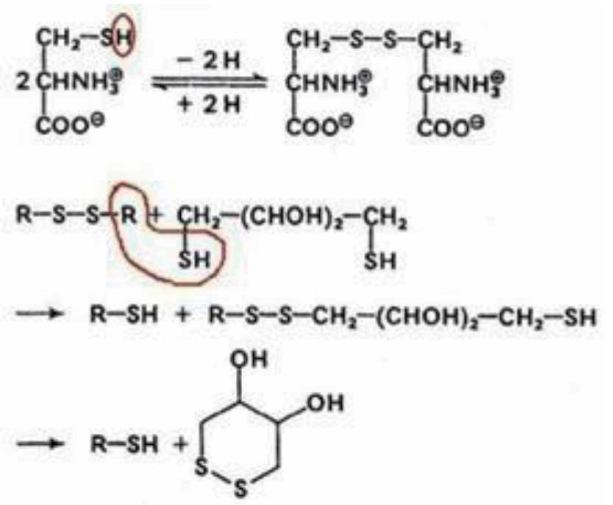


Figure 25.
The redox mechanism Cysteine-Cystine.

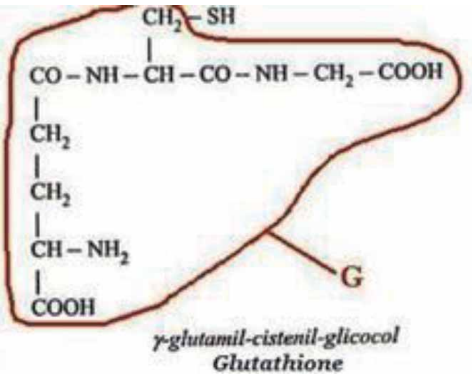


Figure 26.
Structure of Glutathione.

$$2\,GSH + R_2O_2 \rightarrow GSSG + 2\,ROH\ (R = H, alkyl) \quad (2)$$

and with free radicals:

$$GSH + R. \rightarrow 0.5\,GSSG + RH \quad (3)$$

It maintains exogenous antioxidants such as vitamins C and E in their reduced (active) states [81].

2.4 The main oxidoreductases with antioxidant activity

a. *FAD-dependent oxidoreductases* are enzymes of a heteroproteinic nature from the group of aerobic dehydrogenases having as active groups derivatives of vitamin B2 (riboflavin or 7,8-dimethyl-10-ribithyl-isoaloxazine), namely: flavin adenine mononucleotide (FMN) and flavin dinucleotide (FAD). Flavin enzymes (FMN, FAD) are involved in electron and proton transfer reactions mediated by the isoalloxazine nucleus. They accept either an electron or a pair of electrons (unlike NAD and NADP which only accept electron pairs) (**Figure 27**).

$$FAD\ (FMN) + 2\,H + +2e - \leftrightarrows FADH_2\ (FMNH_2) \quad (4)$$

Flavin-enzymes have the standard redox potential E_o between +0.19 V (oxidants stronger than NAD +) and − 0.49 V (reducing agent stronger than NADH), which shows a wide range of variation of redox properties depending on environmental conditions and the nature of the substrate (**Figure 28**). For some flavin -enzymes that also contain a metal (molybdenum or iron) in their molecule, it can stabilize the semi-quinone form by pairing the electron alone with unpaired electrons existing in metal ions; the metal can transport electrons to the respective flavin enzymes.

b. *NAD-dependent oxidoreductases* are enzymes from the class of anaerobic dehydrogenases and have as coenzymes, Nicotinamide Adenine Dinucleotide (NAD+) or reduced (NADH + H+) and Nicotinamide Adenine Dinucleotide Phosphate Oxidate (NADP+) or reduced (NADPH). These coenzymes consist of a derivative of vitamin PP, nicotinamide and an adenine-derived nucleus (**Figure 29**).

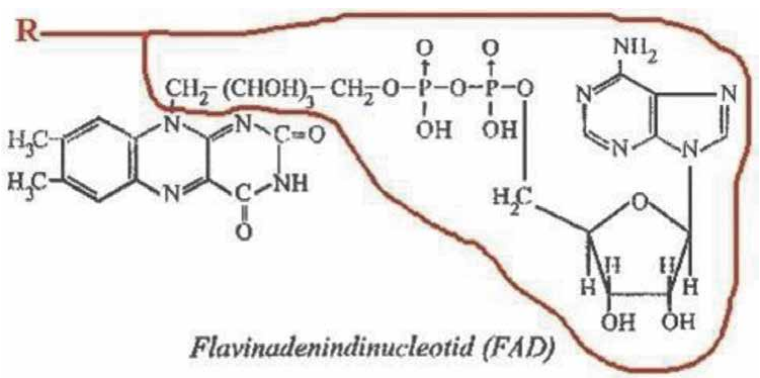


Figure 27.
Structure of Flavin Adenine Dinucleotide (FAD).

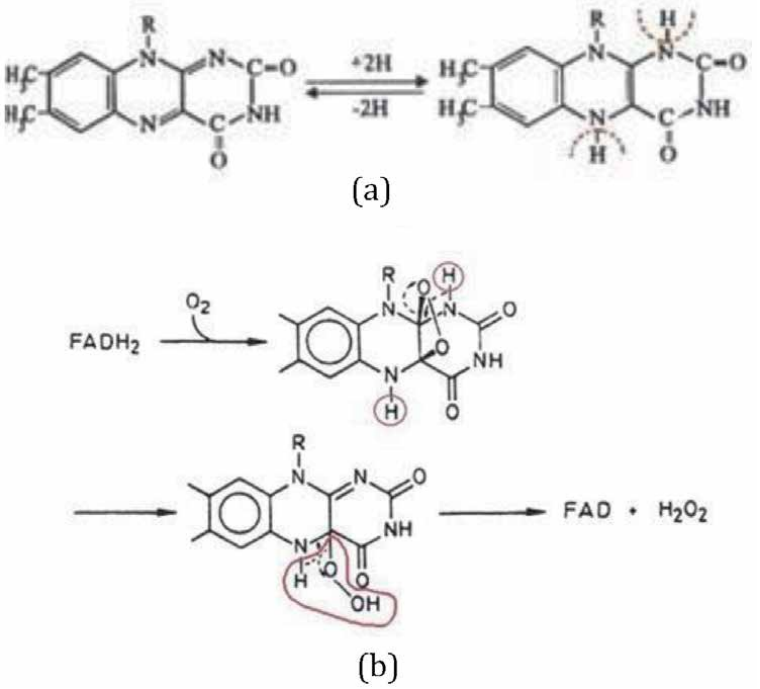


Figure 28.
Mechanisms of FAD (a-left) and (b-down).

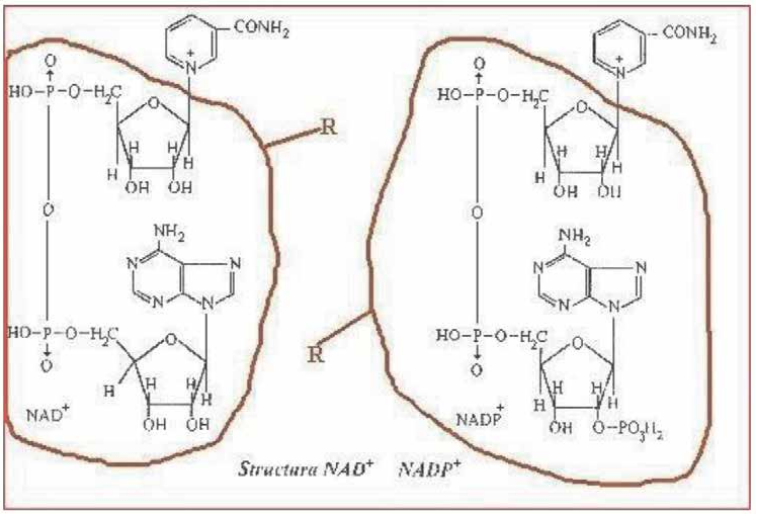


Figure 29.
Structure of NAD and NADP.

NAD^+ and $NADP^+$ are anaerobic, because the transferred hydrogen acceptor is not oxygen, but another element. They catalyze redox reactions by the generally reversible transfer of protons. The transfer of hydrogen in the redox reactions catalyzed by NAD^+ and $NADP^+$ is carried out at the level of the nicotinamide component in the structure of these coenzymes (**Figure 30**).

Preservation of antioxidant characteristics can be achieved by using special techniques: Mild Food Processing, Supercritical Fluid Extraction (SFE), separation in active plasma field, separation in magnetic and gravitational field.

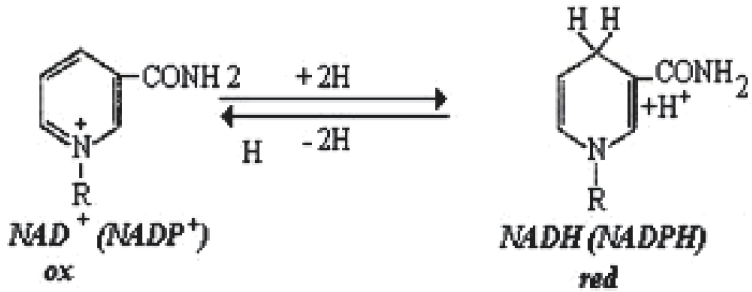


Figure 30.
NAD (P) – redox mechanism.

Figure 31.
Separation a synthetic food preservative from a liquid food using, nano-plasma field, SFE and antioxidant agent (©).

Using the properties of compounds with antioxidant activity (from certain redox systems in food), an improved SFE process at the nanomolecular level - with the help of a nano-plasma field, Professor Savescu Petre succeeded in separating (in the form of crystals) a synthetic food preservative from a liquid food (**Figure 31**). The advanced separation was performed by a personal technique (under innovative patent by PhD. Habil. Professor Petre Săvescu), within the INCESA Research Hub of the University of Craiova, Romania.

3. Conclusions

Antioxidants are valuable bio compounds that can increase both the nutritional value of the functional food and the therapeutic value of this important product.

For dietary supplements and functional foods, it is important to use only natural antioxidants. Synthetic antioxidants can cause a number of consumer health problems. In the design and construction of a functional food it is important to use only inoculated and even organic raw materials. All used raw materials, food additives, and technological adjuvants must be analysed before processing the food supplement - to avoid unwanted reactions and the appearance of compounds with a potential risk to the health of the consumer.

It is forbidden to use raw materials, food additives, technological auxiliaries which can contain traces of antibiotics, plant or animal hormones, pesticides, heavy

metals. For their analysis will be used complex chromatography techniques (GC, LC), advanced separation techniques (using supercritical fluids and plasma fields), optical methods of analysis (UV-Viz, NIR, FT-IR) with Certified Reference Materials and Pure Analysis Substances and modern standardized methods of electrochemistry. Antioxidants can have the functions of immune-modulatory compounds, food preservatives, and food colouring, sequestering/chelating agents for heavy metals.

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Conflict of interest

The author declare no conflict of interest.

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Chapter 9

Theoretical Studies on Anti-Oxidant Activity of the Phytochemical, Coumestrol and Its Derivatives

Puttanveedu Vinduja and Karuvanthodi Muraleedharan

Abstract

Free radical-induced changes in cellular and organ levels have been studied as a possible underlying cause of various adverse health conditions. Important research efforts have, therefore, been made to discover more powerful and potent antioxidants/free radical scavengers for the treatment of these adverse conditions. The phytoestrogen coumestrol intensively attracted scientific interest due to their efficient pharmacological activities. In this scenario, DFT studies were carried out to test the antiradical activities of coumestrol and its derivatives. The results obtained from FEDAM plots demonstrated that the coumestrol derivatives pointed out were good radical scavengers relative to the parent molecule in the gas phase. The derivatives whose 16^{th}position substituted with electron-donating groups like -NH_2, -OCH_3 and -CH_3 showed good antioxidant capacity. Three antioxidant mechanisms, including hydrogen atom transfer (HAT), electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET), were investigated by measuring thermodynamic parameters.

Keywords: phytochemical, coumestrol, anti-oxidant activity, global descriptive parameters, donor acceptor map, full electron donor acceptor map

1. Introduction

Extreme production of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) with half-lives of just a few nanoseconds is the source of the harmful process called oxidative stress, the effects of which can significantly alter cell structures (e.g. membranes) and destroy bio molecules such as lipids, lipoproteins, proteins, and nuclei [1–3]. Our body has natural defence mechanisms provided by secondary metabolites called antioxidants to neutralise these ROSs. In the respiratory chain, the electron transfer to molecular oxygen takes place and the electron transport chain is located on the mitochondria, suggesting that the ROS is mainly formed in mitochondria [4]. Natural products reflect a diverse community of different kinds of antioxidants that inhibit or postpone the oxidation of essential cell macromolecules by scavenging certain free radicals [5, 6]. Antioxidants are commonly dispersed in different parts of plants, such as fruits, leaves, flowers, etc., and cow milk and honey milk contain a number of antioxidants [7–9].

Various antioxidant techniques have involved either the increase of endogenous antioxidant enzyme defences (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) or the enhancement of non-enzymatic defences (e.g., glutathione, vitamins) by dietary or pharmacological means in order to counteract and neutralise the deleterious effects of ROS/RNS. By scavenging free radicals and decreasing oxidative stress, antioxidants may slow, inhibit or prevent the oxidation of oxidizable substrates. The defence against ROS is, however, impaired or harmed in disease conditions and the oxidant load increases. Under such circumstances, the external supply of antioxidants is sufficient to mitigate the adverse effects of oxidative stress [10]. It is generally recognised that the presence of one or more conjugated -OH groups or -COOH groups, which increases the capacity of such a molecule to quench free radicals, is the most important structural feature that facilitates successful antioxidant activity. Therefore Studies have shown that polyphenols (both natural and synthetic) are promising antioxidants [5, 6].

Coumestrol is a phytoestrogen belongs to the coumestan family of compounds, in plants. Coumestrol exhibit estrogenic and antiestrogenic activity based on oestrogen levels in the body. It has a similar structure to isoflavones and estradiol. It was first isolated from ladino clover in 1956 by E.M. Bickoff. Coumestrol is widely distributed in plants like clover, alfalfa, soya beans, peas, brussels sprouts, spinach, strawberries and a variety of legumes. Coumestrol can easily pass through cell membranes due to its low molecular weight and stable structure. Coumestrol exhibit a neuroprotective effect via cerebral ischemia prevention. Coumestrol exert beneficial effects in cancer, menopause, osteoporosis, atherosclerosis, and cardiovascular disease. In addition to this, coumestrol shows Anti-ageing, Neuroprotective, Anti-adipogenic, Depigmenting activity, Anti-oxidant and Anti-cancer properties. A detailed mechanistic (radical scavenging mechanism) study on coumestrol and its derivatives is needed to describe the antioxidant characteristics in a satisfactory manner, so the current work is an attempt to provide a theoretical exploration of the antioxidant property of the molecules under study.

2. Materials and methods

2.1 Materials

The present study mainly focussed on the anti-oxidant property of coumestrol and its derivatives. The three-dimensional structure of the parent molecule is downloaded from the PubChem database. Using the Gaussview-5.0 graphical user interface, the input structures of coumestrol derivatives were drawn and assigned to the Gaussian 09 software package for computational calculations.

2.2 Computational methodology

2.2.1 Frontier molecular orbital (FMO) analysis

In particular, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) form the frontier molecular orbitals (FMOs). FMOs are strongly involved in the study of the electrical and chemical properties of substrates. Analysis of frontier molecular orbitals of coumestrol and its derivatives have been carried using density functional theory and their energy gaps were computed. A lower energy gap indicates the reactivity of the molecule. An anti-oxidant ‘s working mechanisms are derived from HOMO, as a weak electron donor represents a lower HOMO, and vice versa. In addition, electron transfer is

involved in hydrogen abstraction, and therefore the study of HOMO-LUMO is important.

2.2.2 Global descriptive parameters

Global descriptive parameters are parameters that give information's about the reactivity of coumestrol derivatives and also give the relation between the reactivity of derivatives and responses to the changes in external conditions. So, by calculating these parameters, we can compare the reactivity of coumestrol with its derivatives. It is an attractive method for understanding the reactive nature of all the products [11]. Global parameters include ionisation potential (I), electron affinity (A), hardness (η), softness (S), electronegativity (χ), chemical potential (μ) and electrophilicity index (Ω) [12]. These parameters depend upon the number of electrons and electron density due to the external changes [13]. Global descriptive parameters can be calculated by two methods; they are according to Koopman's theorem and the Energy vertical method. These methods have particular relevance in the comparison of different molecules. Low ionisation potential, high electron affinity and high electronegativity contribute to high reactivity. So, by analysing the values of these parameters' reactivity can be studied.

According to energy vertical, difference in total electronic energy of the neutral molecule and its corresponding anion and cation were considered. The equations for finding ionisation potential (I) and electron affinity (A) are given below;

$$I = E_{cation} - E_{neutral} \tag{1}$$

$$A = E_{neutral} - E_{anion} \tag{2}$$

According to Koopman's theorem of closed shell compounds;

$$I = -E_{HOMO} \tag{3}$$

$$A = -E_{LUMO} \tag{4}$$

Where E_{HOMO} is the energy of the highest occupied molecular orbital (HOMO) and E_{LUMO} is the energy of the lowest unoccupied molecular orbital (LUMO). The global properties were computed by using the equations given below;

$$\text{Hardness } (\eta) = (I - A)1/2 \tag{5}$$

$$\text{Electronegativity } (\chi) = (I + A)1/2 \tag{6}$$

$$\text{Softness } (s) = 1/(2\eta) \tag{7}$$

$$\text{Chemicalpotential } (\mu) = -\chi \tag{8}$$

$$\text{Electrophilicityindex } (\omega) = \mu^2/2 \tag{9}$$

2.2.3 Donor acceptor map (DAM)

A donator-acceptor map is a useful tool for a qualitative comparison among substances. DAM can be used for classifying molecules in terms of their electron accepting and donating capacity (with respect to coumestrol). Graphical representation of DAM plot is shown in **Figure 1**. DAM also provides information's regarding anti-radical capability of molecules and also gave us a base for antioxidant studies. Single-point calculations (Energy vertical) were used to compute ionisation potential (I) and electron affinity (A). Ionisation potential was calculated as the difference between the energy of the cation and that of the neutral molecule.

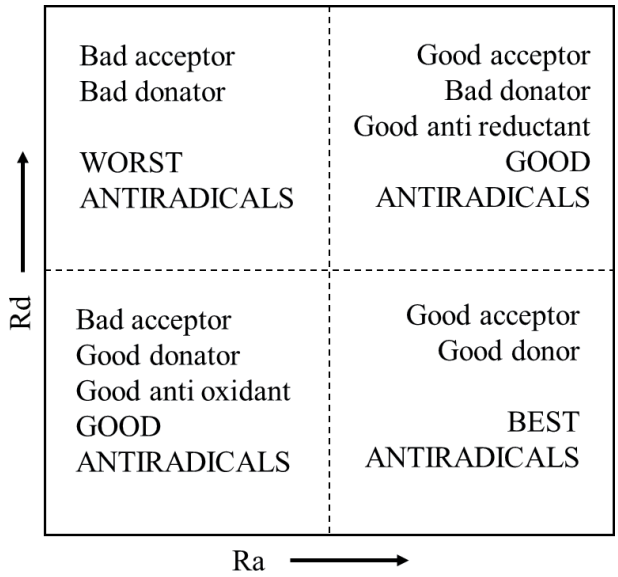

Figure 1.
Graphical representation of DAM.

And electron affinity was calculated as the energy difference between the neutral and the anion, and both were assumed to have ground state nuclear configuration of the neutral molecule.

According to J.J. Gázquez's approximation, the tendency to donate charge, or electron donating power, maybe defined as;

$$\omega- = (3I + A)^2/16(I–A) \tag{10}$$

whereas, the tendency to accept charge, or electron accepting power, maybe defined as;

$$\omega+ = (I + 3A)^2/16(I - A) \tag{11}$$

I and A donate or accept a single electron whereas, ω- and ω + refer to fractional charges. Lower values of electron donating power indicate the greater capacity for donating charge and higher values of electron accepting power indicate the greater capacity for accepting charge. So, it is a simple charge transfer model expressed in terms of chemical potential and hardness. Chemical potential gives more importance for ionisation potential in the context of charge donation and give more importance on electron affinity in the context of charge acceptance.

2.2.4 Full electron donor acceptor map (FEDAM)

FEDAM is a plot of electron donation index (RI) vs. electron acceptance index (RA), which gives information about the radical scavenging activity of different molecules. The ionisation enthalpy (I) and electron affinity (A) were obtained through DFT-B3LYP/6–31 + G(2d,2p) using energy vertical method. The electron donating and accepting indexes of the coumestrol derivatives were calculated with respect to the parent molecule, coumestrol, by using the equations given below;

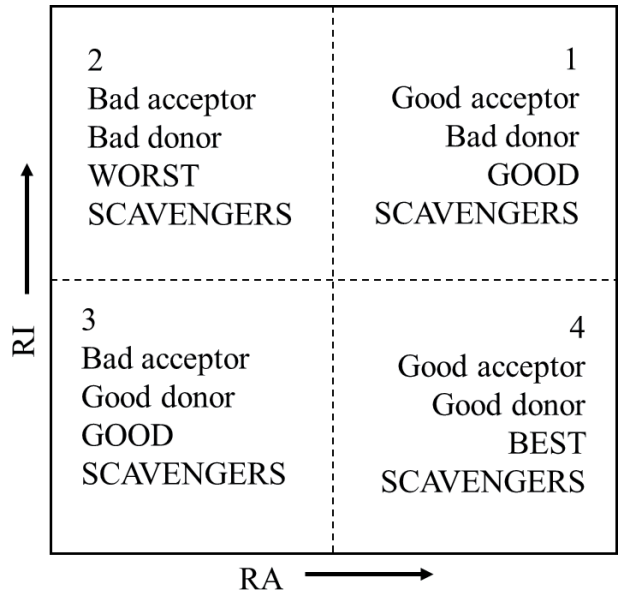

Figure 2.
Graphical representation of FEDAM.

$$RI = I_L/I_{Cou} \tag{12}$$

$$RA = A_L/A_{Cou} \tag{13}$$

Where, L = Ligand (Derivatives).
Cou = Coumestrol.

The graphical representation of FEDAM is shown in **Figure 2**. It is used for evaluating the single electron (SET) transfer processes. Generally, the electron transfer takes place from region-3 (good donor) to region-1 (good acceptor). From this graph, it's vivid that the molecules with low I value and high A value exhibits the best scavenging activity.

2.2.5 Antiradical activity

To clarify the radical scavenging potential of phenolic anti-oxidants, three main mechanisms have been proposed. Consequently, antioxidants can deactivate free radicals according to the following mechanisms [14, 15].

3. HAT (*hydrogen atom transfer*) mechanism

$$ArOH + X^{\cdot} \rightarrow ArO^{\cdot} + XH \tag{14}$$

The phenolic anti-radical interacts directly with a free radical that is neutralised, according to this mechanism, and a radical form of phenolic antiradical develops. The hydrogen atom is transfered (HAT, Eq. (14)) from antioxidant molecules (ArOH) to radicals. Bond dissociation energy(BDE) is a numerical parameter connected to this mechanism. A better anti-radical property is defined by the lower BDE parameter.

$$BDE = H(ArO^{\cdot}) + H(H) - H(ArOH) \tag{15}$$

4. SET (*single electron transfer*) mechanism

It takes place through two steps. Initially, a free radical cation is formed by the transfer of anelectron from a neutral species.

$$ArOH + X^{\cdot} \rightarrow ArOH^{\cdot+} + X^{-} \tag{16}$$

Neumerical parameter associated to this step is AIP.

$$IP = H\left(ArOH^{\dot{+}}\right) + H(e^{-})\text{–}H(ArOH) \tag{17}$$

In the next step, phenolic radical cation decomposes into phenolic radical and proton.

$$ArOH^{\cdot+} \rightarrow ArO^{\cdot} + H^{+} \tag{18}$$

PDE is the neumerical parameter related to this step.

$$PDE = H(ArO^{\cdot}) + H(H^{+})\text{–}H\left(ArOH^{\dot{+}}\right) \tag{19}$$

5. SPLET (*sequential proton loss electron transfer*)

In SPLET mechanism, The phenolic antioxidant dissociates into an anionic form and proton in the first step, and then ions formed in the first reaction react with the free radical.

$$ArOH \rightarrow ArO^{-} + H^{+} \tag{20}$$

$$ArO^{-} + X^{\cdot} + H^{+} \rightarrow ArO^{\cdot} + XH \tag{21}$$

The first step corresponds to the PA and it can be calculated using Eq. (15):

$$PA = H(ArO) + H(H^{+})\text{–}H(ArOH) \tag{22}$$

The numerical parameter for the second step ETE can be calculated by the equation,

$$ETE = H(ArO^{\cdot})\text{–}H(ArO^{-}) \tag{23}$$

6. Results and discussion

6.1 Optimisation of structures

Coumestrol is a polycyclic aromatic compound containing a coumestan moiety, which consists of a benzoxole fused to a chromen-2-one to form 1-Benzoxolo[3, 2-c] chromen-6-one. The lowest energy conformer of coumestrol is obtained through potential energy scanning and is used for further analysis. The derivatives were drawn by substituting 16th position of coumestrol with electron donating groups like -OH, -NH_2, -OCH_3, -CH_3, -Ph, -$CHCR_2$, -OCOR and -NHCOR and electron withdrawing groups like -F, -CL, -BR, -CN, -NO_2, -SO_3H, -CHO, -COR, -COCL, -COOR and -COOH (where 'R' is a methyl group). All the structures were optimised through DFT-B3LYP/6–31 + G(2d,2p). The optimised structure of coumestrol is shown in **Figure 3**.

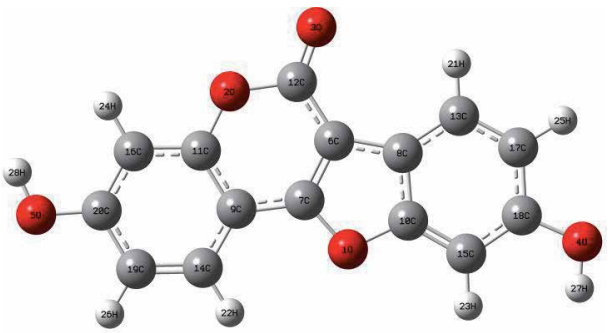

Figure 3.
Optimised lowest energy conformer of coumestrol.

Method	I	A	H	X	S	μ	ω
Energy vertical	7.3311	0.4221	3.4545	3.8766	0.1447	−3.8766	2.1751
Koopman's theorem	5.8556	1.8844	3.9712	3.87	0.1259	−3.97	1.8857

Table 1.
The global descriptive parameters of coumestrol.

6.2 Global descriptive parameters

Global descriptive parameters were calculated for comparing the chemical reactivity of coumestrol derivatives with parent molecule.

The global descriptive parameters of coumestrol are shown in **Table 1**. It can be calculated in two different methods, energy vertical method (single point energy calculations) and Koopman's theorem.

Table 2 indicates the global descriptive values for coumestrol substituted at the C-16th position according to koopman's theorem. Generally, derivatives of coumestrol substituted with an electron withdrawing group showed a common trend; Ionisation potential, electron affinity, electronegativity, softness and electrophilic index increases with increase in electron withdrawing power. Hardness and chemical potential decreases with increase in electron withdrawing power. The trend followed by derivatives of coumestrol substituted with electron donating group is given by; ionisation potential, electron affinity, hardness and electronegativity decreases with increase in electron donating power. And softness, chemical potential and electrophilic index increases with increase in electron donating power. As the electro negativity increases reactivity increases.

Table 3 indicates the global descriptive values for coumestrol substituted at C-16th position calculated by vertical energy method. The derivatives substituted with electron withdrawing groups showed the same trend as in Koopman's, i.e. Ionisation potential, electron affinity, electro negativity, softness and electrophilic index like parameters generally increases with increase in the electron withdrawing power. Hardness and chemical potential decreases with increase in electron withdrawing power. The general trend followed by derivatives of coumestrol substituted with electron donating group was given by; ionisation potential, electron affinity, hardness and electronegativity decreases with increase in electron donating power. And softness, chemical potential and electrophilic index increases with increase in electron donating power. Derivatives substituted with electron donating groups also showed same trend as in Koopman's.

Derivatives	I	A	η	X	S	M	ω
16-F Coumestrol	5.992	2.03	1.981	4.011	0.2524	−4.011	4.0606
16-Cl Coumestrol	5.9903	2.0466	1.9718	4.0184	0.2536	−4.0184	4.095
16-Br Coumestrol	5.9811	2.0387	1.9712	4.0099	0.2536	−4.0099	4.0777
16-CN Coumestrol	6.2015	2.4324	1.8846	4.317	0.2653	−4.317	4.9443
16-NO_2 Coumestrol	6.2243	3.205	1.5096	4.7146	0.3312	−4.7146	7.3617
16-SO_3H Coumestrol	6.1247	2.2855	1.9196	4.2051	0.2605	−4.2051	4.6058
16-CHO Coumestrol	6.0877	2.652	1.7178	4.3698	0.2911	−4.3698	5.5586
16-COR Coumestrol	6.0023	2.4077	1.7973	4.205	0.2782	−4.205	4.9191
16-COCl Coumestrol	6.1607	2.661	1.7498	4.4108	0.2857	−4.4108	5.5592
16-COOR Coumestrol	5.8951	2.0988	1.8981	3.9969	0.2634	−3.9969	4.2082
16-COOH Coumestrol	5.9664	2.2316	1.8674	4.099	0.2678	−4.099	4.4987
16-OH Coumestrol	5.879	1.9369	1.971	3.908	0.2537	−3.908	3.8746
16-NH_2 Coumestrol	5.8148	1.8591	1.9779	3.8369	0.2528	−3.8369	3.7217
16-OCH_3 Coumestrol	5.833	1.871	1.981	3.852	0.2524	−3.852	3.7452
16-CH_3 Coumestrol	5.7974	1.8109	1.9932	3.8042	0.2508	−3.8042	3.6302
16-Ph Coumestrol	5.7849	1.824	1.9804	3.8044	0.2525	−3.8044	3.541
16-$CHCR_2$ Coumestrol	5.7508	1.7879	1.9815	3.7693	0.2523	−3.7693	3.585
16-OCOR Coumestrol	6.07	2.142	1.9645	4.1055	0.2545	−4.1055	4.2899
16-NHCOR Coumestrol	6.0431	2.1162	1.9634	4.0797	0.2546	−4.0797	4.2394

Table 2.
Global descriptive parameters of coumestrol substituted at C-16th position according to Koopman's method.

Analysing the reactivity based on ionisation potential, electronegativity and electron affinity; reactivity increases with increase in electron affinity and electron negativity and decrease in ionisation potential. According to this relation, the derivatives with more reactivity are 16-NO_2 Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol.

6.3 Dam plot

The **Figure 4** shown the DAM plot of coumestrol substituted at C-16th positions. The derivatives like 16-F Coumestrol, 16-Cl Coumestrol, 16-Br Coumestrol, 16-CN Coumestrol, 16-NO_2 Coumestrol, 16-SO_3H Coumestrol, 16-CHO Coumestrol, 16-COR Coumestrol, 16-COCl Coumestrol, 16-COOR Coumestrol, 16-COOH Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol were good anti-reductants with large size and they were good acceptors. Most of the derivatives substituted with electron withdrawing groups were anti-reductants and bad donors. The derivatives substituted with electron donating groups like 16-NH_2, 16-OCH_3 Coumestrol and 16-CH_3 Coumestrol showed good antioxidant capacity through their electron donating power and they were of small size. 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol were exceptional derivatives with electron donating substitution and anti-reductant capacity. Therefore, all the derivatives were good anti-radicals.

Derivatives	I	A	η	χ	S	μ	ω
16-F Coumestrol	7.4689	0.5578	3.4556	4.0133	0.1447	−4.0133	2.3306
16-Cl Coumestrol	7.4425	0.6080	3.4173	4.0253	0.1463	−4.0253	2.3707
16-Br Coumestrol	7.4210	0.6148	3.4031	4.0179	0.1469	−4.0179	2.3719
16-CN Coumestrol	7.6594	1.0007	3.3294	4.3300	0.1502	−4.3300	2.8157
16-NO_2 Coumestrol	7.6796	1.5158	3.0819	4.5977	0.1622	−4.5977	3.4295
16-SO_3H Coumestrol	7.5222	3.2371	2.1426	5.3796	0.2334	−5.3796	6.7537
16-CHO Coumestrol	7.5348	1.1064	3.2142	4.3206	0.1556	−4.3206	2.9039
16-COR Coumestrol	7.4306	0.9353	3.2477	4.1830	0.1540	−4.1830	2.6939
16-COCl Coumestrol	7.6040	1.1919	3.2061	4.3980	0.1560	−4.3980	3.0165
16-COOR Coumestrol	7.3310	0.6910	3.3200	4.0110	0.1506	−4.0110	2.4229
16-COOH Coumestrol	7.4149	0.7913	3.3118	4.1031	0.1510	−4.1031	2.5418
16-OH Coumestrol	7.3332	0.4735	3.4298	3.9034	0.1458	−3.9034	2.2211
16-NH_2 Coumestrol	7.2667	0.4105	3.4281	3.8386	0.1459	−3.8386	2.1491
16-OCH_3 Coumestrol	7.2750	0.4329	3.4210	3.8539	0.1462	−3.8539	2.1708
16-CH_3 Coumestrol	7.2491	0.3721	3.4385	3.8106	0.1454	−3.8106	2.1115
16-Ph Coumestrol	7.1791	0.4754	3.3519	3.8272	0.1492	−3.8272	2.1850
16-$CHCR_2$ Coumestrol	7.1559	0.4109	3.3725	3.7834	0.1483	−3.7834	2.1221
16-OCOR Coumestrol	7.5087	0.7162	3.3963	4.1124	0.1472	−4.1124	2.4898
16-NHCOR Coumestrol	7.4808	0.6957	3.3926	4.0882	0.1474	−4.0882	2.4633

Table 3.
Global descriptive parameters of coumestrol substituted at C-16th position according to energy vertical method.

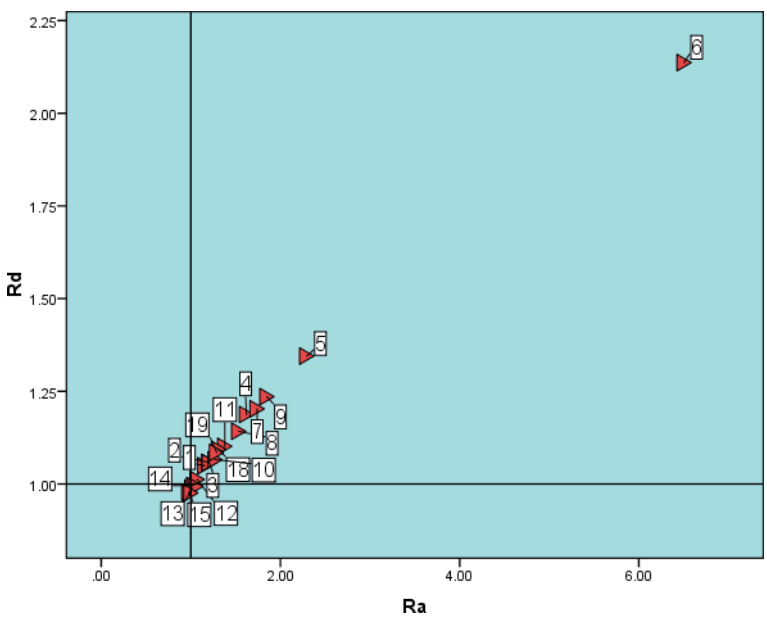

Figure 4.
DAM plot of coumestrol substituted at C-16th position.

6.4 FEDAM plot

All the coumestrol derivatives pointed out were good radical scavengers relative to coumestrol. From the **Figure 4**, it was clear that the derivatives like 16-F Coumestrol, 16-Cl Coumestrol, 16-Br Coumestrol, 16-CN Coumestrol, 16-NO_2 Coumestrol, 16-SO_3H Coumestrol, 16-CHO Coumestrol, 16-COR Coumestrol, 16-COCl Coumestrol, 16-COOR Coumestrol, 16-COOH Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol and 16-NHCOR Coumestrolexhibit good accepting capacity and 16-NH_2 Coumestrol, 16-CH_3 Coumestrol, and 16-$CHCR_2$ Coumestrol exhibit good donating capacity of electron. Therefore, all these derivatives were good radical scavengers while 16-PhCoumestrol and 16-OCH_3 Coumestrolwere best radical scavengers with both electron donating and electron accepting capacity. The substitution of electron withdrawing groups on coumestrol imparts electron accepting and donating groups impart electron donating capacity on the derivatives. The size distribution says that good electron acceptors are large in size and good electron donors are small in size (**Figure 5**).

6.5 Anti-oxidant capacity

Numerical parameters corresponding to all the possible anti-oxidant mechanism of coumestrol and selected coumestrol derivatives in gas phase are shown in **Table 4**.

Logically speaking, free energy (ΔG) decides the thermodynamically preferred mechanism. The calculated free-energy equation is $\Delta G = \Delta H - T\Delta S$. In accordance with this equation ΔG is defined by ΔH and ΔS. However the absolute values of the entropic term, TΔS, reach only a few units or tens of kJ/mol, in the case of studied reactions. Free energies are thus predominantly influenced by the enthalpy term ΔH. The mechanisms of HAT, SET-PT and SPLET are primarily regulated by BDEs, IPs and PAs, respectively, and the BDEs, IPs and PAs can therefore specify the thermodynamically preferred reaction pathway involved in the free radical

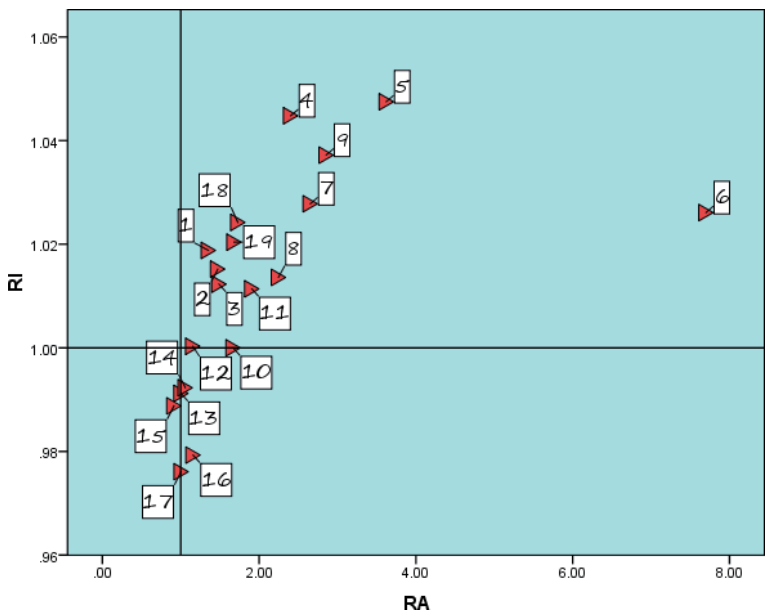

Figure 5.
FEDAM of Coumestrol substituted at C-16th position in gas phase.

Molecule	Bond	BDE	AIP	PDE	PA	ETE
Coumestrol	20-OH	82.9768	166.6985	230.1977	325.5727	71.3235
	18-OH	80.7975		228.0184	332.9728	61.7441
16-NH_2Coumestrol	20-OH	71.3662	125.3958	259.8898	325.0048	60.2808
	18-OH	79.7603		268.2839	333.0714	60.6083
16-OCH_3Coumestrol	20-OH	78.6458	163.4813	229.0839	323.8452	68.7200
	18-OH	79.8399		230.2781	332.7745	60.9848
16-CH_3Coumestrol	20-OH	79.7163	125.1574	268.4784	326.0258	67.6099
	18-OH	79.7063		268.4683	333.6405	59.9852

Table 4.
Numerical parameters corresponding to all the possible antioxidant mechanism of coumestrol and selected coumestrol derivatives in gas phase.

scavenging method. From the table, the measured IPs and PAs of coumestrol and its derivatives in the gas phase have been found to be substantially higher than BDEs and thus, from a thermodynamic point of view, HAT is the most desirable method in the gas phase.

BDE is the numerical parameter that characterises the stability of hydroxyl group and it is related to HAT mechanism. The lower BDE value indicate the lower the stability of the O-H bond, and high antioxidant capacity. Coumestrol contain two different hydroxyl groups which can transfer hydrogen to the free radical present in biological systems. From the table it is observed that, the derivatives substituted with electron donating groups like 16-NH_2, 16-OCH_3 and 16-CH_3 showed good anti-oxidant capacity. In gas phase, the antioxidant power all the selected coumestrol derivatives were higher than that of parent molecule.

For coumestrol, the BDE value observed at 18-OH was less than that of 20-OH which means that 18-OH forms most stable radical. The more stable radical can imply the stronger antioxidant abilities of the compound. Among the three selected coumestrol derivatives, 16-NH_2 showed lowest BDE value implies its higher anti-oxidant potential. In the case of 16-NH_2 Coumestrol, the bond 20-OH showed lowest BDE value compared to that of 18-OH. It may be due to the presence of intra molecular hydrogen bonding between -NH_2 with nearby oxygen radical. 16-OCH_3 coumestrolshowed low BDE value than Coumestrol but higher than 16-NH_2 coumestrol because there is no hydrogen bonding interaction possible between oxygen radical and -OCH_3 group near to it. 16-CH_3Coumestrol, also showed a lower BDE value than parent molecule. -CH_3 group being a weakly electron donating one, only a slight difference in BDE value was observed at 20-OH and 18-OH.

6.6 Frontier molecular orbital analysis

Energy and distribution of frontier orbitals are also significant parameters that correlate with the antioxidant activity of the polyphenols. The calculated frontier orbital distributions and energies in the gas phase for Coumestrol and its derivatives like 16-NH_2Coumestrol, 16-OCH_3Coumestrol, and 16-CH_3Coumestrol are present in **Figure 6**.

The molecule's electron donation potential is linked to the energies of HOMO. Higher HOMO orbital energy molecules have a greater capacity to donate electrons [16, 17]. It can be observed from **Figure 6** that 16-CH3 coumestrol provided the highest HOMO energy (−5.797eV), followed by 16-NH2 coumestrol (−5.815eV), 16-OCH3 coumestrol (−5.833 eV), and coumestrol (−5.855eV). This demonstrates

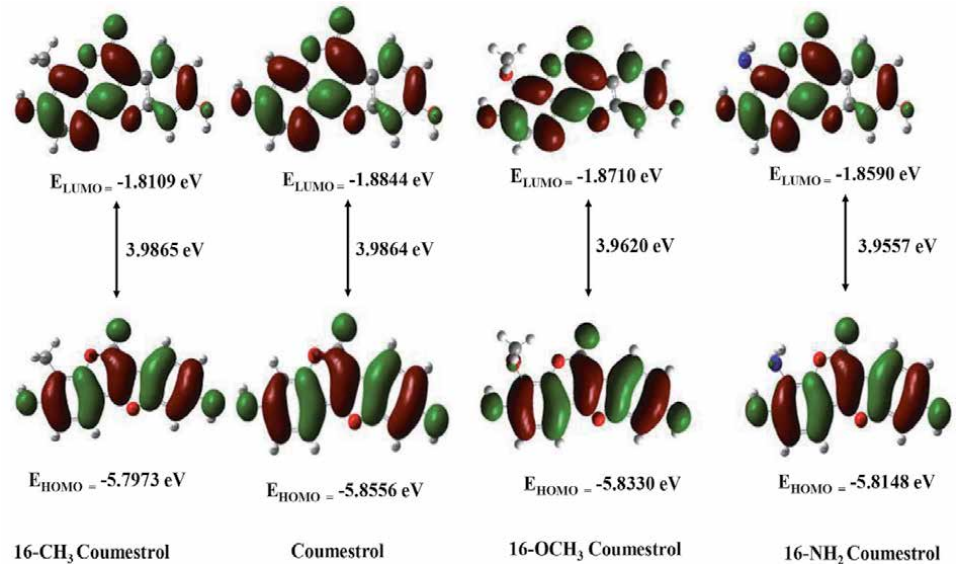

Figure 6.
The energy and distribution of HOMO and LUMO for coumestrol and its selected derivatives.

clearly that all the compounds studied, pocess best electron-donating potential which is in good agreement with IP values. Even though 16-CH3 coumestrol showed high HOMO energy its lectron donating power is less due to the large band gap.

7. Conclusion

The present work explained the antioxidant properties of coumestrol and its derivatives from a theoretical point of view. Since the measured ionisation potential and proton affinities in gas phase are significantly higher than the phenolic O-H group BDEs, we can infer that from a thermodynamic point of view, homolytic O-H bond splitting-off is the most likely process in the gas phase. All the selected derivatives 16-NH_2 Coumestrol, 16-OCH_3 Coumestrol, and 16-CH_3 Coumestrol showed high antiradical activity than parent molecule. Among the selected derivatives, 16-NH2 Coumestrol showed the best antioxidant activity. The calculated molecular properties (electronegativity, ionisation potential, electron affinity, hardness and electrophilicity index) of coumestrol derivatives substituted by electron withdrawing groups at 16^{th} position indicated that, Ionisation potential, electron affinity, electro negativity, softness and electrophilic index increases with increase electron withdrawing power and hardness and chemical potential decreases with increase in electron withdrawing power. In the case of derivatives substituted with electron donating groups, ionisation potential, electron affinity, hardness and electronegativity decrease with increase in electron donating power while the softness, chemical potential and electrophilic index decreases with increase in electron donating power.

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Section 3

Medicinal Based Functional Foods

Chapter 10

Functional Foods for the Management of Non-Alcoholic Fatty Liver Disease

Venkateish V. Palanisamy, Nivya Vijayan, Vani Vijay, Baskaran Vallikannan and Madan Kumar Perumal

Abstract

Non-alcoholic fatty liver disease (NAFLD) is increasingly evolving and a critical public health concern, raising the likelihood of liver cirrhosis, type 2 diabetes and cardiac problems. Existing epidemics of obesity and sedentary life style have lead to NAFLD's elevated prevalence. In recent years there is profound change in the diet pattern, particularly the hypercaloric fat and carbohydrates for preventing or treating chronic liver disorders such as NASH and NAFLD. Functional and nutritional foods have contributed significantly to NAFLDimprovement and management. The justification for exploring functional foods as anti-NAFLD candidates for the chronic liver disease prevention is derived knowledge from *in vitro* and *in vivo* models. The findings from the *in vitro* and *in vivo* studies confirmed that these compounds are healthy, efficient, reversible inhibitors, when sufficiently consumed over a lifetime without severe toxicity, suitable for clinical trials and potentially becoming low-cost medication.

Keywords: non-alcoholic fatty liver disease, functional foods, phenolics, flavonoids, treatment, management

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the primary liver disease posing severe health and economic burden worldwide [1]. NAFLD is characterized with excessive fat storage in the liver, constituting up to 10% of the total liver weight [2]. NAFLD is typically caused due to reasons other than excessive alcohol intake such as obesity, insulin resistance, diabetes mellitus, high triglycerides, dyslipidemia, etc. [3]. Based on liver histology, NAFLD is further classified into the non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is characterized by retention of fat in the liver without hepatocyte injury, whereas in NASH, significant inflammation, hepatocyte injury and liver steatosis are observed [4]. The progression of NASH is quite complicated and if unattended, will lead to liver fibrosis, cirrhosis, hepatocellular carcinoma and organ transplantation. At present, there is no FDA approved drug for the management of NAFLD. Functional foods are being prepared using scientific intelligence to provide the required amount of macro and micronutrients for once health. Compared to conventional foods, functional foods deliver potentially nutritious components that reduce the risk of several

chronic diseases [5]. Nutraceuticals are any food substance or part of a diet that afford significant health benefits [6]. This chapter will emphasize the importance of functional foods and nutraceuticals for the management of NAFLD.

2. Prevalence and pathogenies of NAFLD

NAFLD, the primary cause of chronic liver disease seen in developed countries, is presently highly prevalent among the Asian population. According to recent estimates, the global prevalence of NAFLD is reported at 25 percent and the combined prevalence rate is 27.4 percent in Asia [7]. Two hypotheses are framed for the pathogenesis of NAFLD. The first hit is a two-hit theory where fat accumulation in the liver is caused due to diet, obesity and insulin resistance and the first hit further exposes the liver to more insults called second hit which activate inflammatory pathways and fibrogenesis [8]. In continuation, a multi-hit hypothesis was suggested where numerous factors like environment, dietary habits and genetic led to the development of liver damage [9].

3. Key factors involved in the progression of NAFLD

3.1 Lipid accumulation and insulin resistance

Triglyceride accumulation in hepatocytes is a significant factor in the development of NAFLD. Glycerol and fatty acids undergo esterification to form triglycerides (TGs), which are usually stored or secreted. The fate of the fatty acids is to either undergo esterification or enter the β-oxidation pathway. Under normal conditions, TGs are not toxic; where they maintain free fatty acids [10]. Studies have shown increased de-novo lipogenesis and expression of transcription factors such as sterol regulatory element binding protein-1c (SREBP-1c), carbohydrate response element-binding protein (ChREBP) and peroxisome proliferator-activated receptor-γ (PPAR-γ) in NAFLD [11]. Insulin resistance is another critical factor in NAFLD that drive the activation of de-novo lipogenesis. Insulin receptor substrate-2 (IRS-2) is known to regulate SREBP-1c negatively. Insulin resistance also lead to decreased oxidation of free fatty acids; hence fat accumulate in the hepatocytes. Free fatty acids in hepatocytes also inhibit the insulin signaling through the serine-kinase pathway leading to insulin resistance [12]. Accumulation of fat in the liver also contribute to stress and dysfunction to mitochondria and endoplasmic reticulum (ER). Dysfunctional mitochondria lead to increased reactive oxygen species (ROS) generation and activation of inflammatory pathways leading to hepatic necro-inflammation and further damage of mitochondria (**Figure 1**) [13].

3.2 Cytokines

Studies have shown the involment of cytokines during liver inflammation, liver fibrosis, liver regeneration and hepatocyte apoptosis [14]. In obese individuals, adipose tissue is enlarged and release various adipokines, which further recruit macrophages resulting in the secretion of pro-inflammatory adipokines [15]. Increased leptin levels play a crucial role in NAFLD progression by inducing insulin resistance and steatosis development [16]. Adiponectin, an anti-inflammatory adipokine secreted exclusively by the adipocytes, plays a protective role in the liver by preventing lipid accumulation through enhanced β-oxidation of free fatty acids [17]. Lipid accumulation, insulin resistance, mitochondrial stress, ER stress

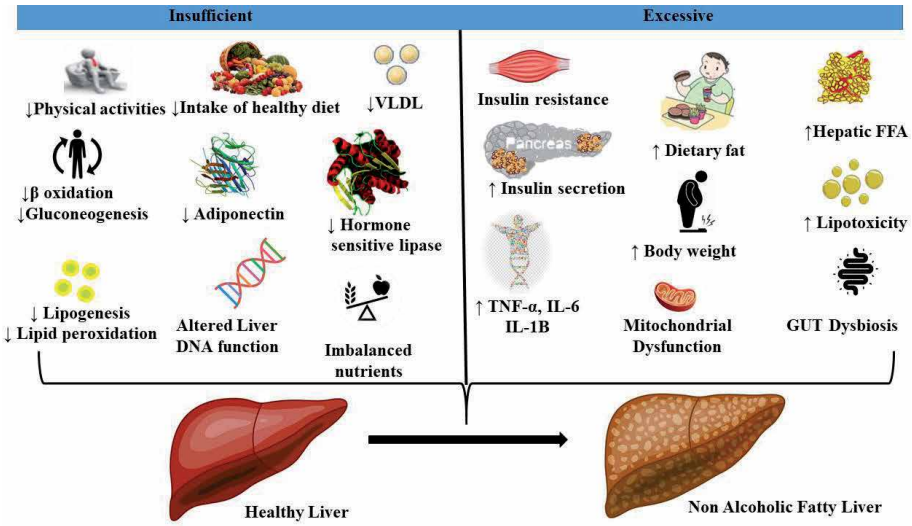

Figure 1.
Pathogenesis of NAFLD.

and fatty dysfunction have contributed to the generation of pro-inflammatory cytokines. c-Jun N-terminal kinase/activator protein 1 (JNK/AP-1), tumor necrosis factor-α (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) are some of the crucial players in the development of inflammation in NAFLD. JNK pathway lead to apoptosis and progression of NAFL to NASH. NF-κB activation lead to chronic hepatocyte inflammation and insulin resistance [18].

3.3 Genetic, epigenetic and dietary factors

Gene mutations like single nucleotide polymorphisms (SNPs) influence the free fatty acids secreted from hepatocytes to release cytokines, further stimulating NAFLD progression [19]. An example of SNP that affect NAFLD progression is the patatin-like phospholipase 3 (PNPLA3) gene. Lipid accumulation in hepatocytes of a PNPLA3 gene mutant carrier is associated with lower lipoprotein secretion, which is rich in liver TGs [20]. Pirazzi et al. (2012) showed that humans with PNPLA3 mutation exhibited increased steatosis and fibrosis. Transmembrane 6 superfamily member 2 (TM6SF2) mutation is also associated with NAFLD [21]. Wild type TM6SF2 protein promote VLDL secretion, while its variant is associated with hepatic steatosis caused by lower VLDL secretion and high ALT levels [22].

Epigenetic modifications generally occur at the transcriptional levels, such as DNA methylation, histone modifications and microRNA (miRNA) expression. Studies have shown that aberration in epigenetics is known to increase susceptibility to NAFLD [23]. DNA methylation has a crucial epigenetic determinant in NAFLD progression, which is influenced by dietary methyl donor deficiency. Betaine, choline and folate are common methyl donors [24]. Sirtuins (SIRTs) are a group of proteins with deacetylase activity mainly involved in epigenetic modification. SIRTs have been implicated in regulating proteins involved in metabolic processes like lipid metabolism, oxidative stress, glucose metabolism and inflammatory pathways. Research findings in animal and human models showed that NAFLD is associated with lowered SIRT1 levels [25]. Recent studies demonstrated the association between miRNA levels and NAFLD pathogenesis. Krützfeldt et al. (2005) showed inhibition of miRNA-122 led to decreased plasma cholesterol levels and reduced expression of genes involved in hepatic cholesterol and fatty acid synthesis [26].

The pathogenesis of NAFLD is also dependent on dietary factors. The quantity of calorie intake and the diet's quality concerning nutrients decide a healthy diet. Diet rich in fructose is associated with NAFLD. Fructose is a lipogenic dietary factor with pro-inflammatory activity, causing oxidative stress and increased expression of TNF-α. Studies demonstrated that fructose intake in patients with NAFLD leads to increased fibrosis [27]. Diet rich in mono-unsaturated fatty acids showed protective effects by improving steatosis and insulin resistance in NAFLD patients [28]. Studies conducted in ob/ob mice showed less steatosis and lowered liver enzyme levels upon a moderate alcohol administration level [29].

3.4 Gut microbiota

Recent studies showed the involvement of the gut-liver axis in the pathogenesis of NAFLD [30]. Bacterial toxins like lipopolysaccharides (LPS) are the potent toxins released by gut bacteria. LPS activate inflammatory response by activating stress-activated protein kinase, JNK, p38 affecting insulin resistance, obesity, hepatic fat accumulation and NASH development [31]. Patients with NAFLD have increased gut permeability and higher bacterial growth than normal subjects [32].

4. Functional foods for NAFLD

Human history's survival has always been highly reliant on food to avoid or battle the diseases. Hippocrates, the renowned physician, quoted, "Let food be thy medicine and medicine be thy food." Functional foods should not contradict the scientific advancements made to treat degenerative diseases over the last two centuries. Due to modern nomadic lifestyle and the shift in natural resources, traditional agricultural practices and dietary habits are not standard in this decade. Anti-NAFLD compounds, in general, must be discerning and innocuous. They should look at molecular and metabolic levels to reduce fat deposition in the liver. Flavones such as quercetin, hesperetin from onions and citrus fruits were shown to reduce fatty acid deposition in the liver. Anti-NAFLD compounds' mechanism must be reversible in the event NAFLD needs, for example, to promote the tissue darning subsequent injury. Most food-derived compounds show reversible activity [33]. The flavonoid, naringenin dose-dependently and reversibly inhibited transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), thereby modulated the fatty acid oxidation and trafficking dependent liver inflammation [34].

Pre-clinical and clinical models have proven their worth to test natural compounds for anti-NAFLD activities. Many anti-NAFLD compounds such as flavones, anthraquinones, stilbenes, naphthols and polysaccharides showed potency at cellular levels, phase I and II clinical trials [35]. Epidemiological studies illustrated that populations gobble such bioactive substances have low disability rates as their main diets. Since these compounds are food endogenous, the fortifying meal is a relatively cheap way of delivering them throughout a lifetime, as many people tend to forget pills over long periods. It was suggested that the use of non-alcoholic steatohepatitis inhibitors might not prove relevant, as these inhibitors may be more efficient against the progression of disease at an early stage as comparing when a metastatic illness has progressed to advanced stages [36]. Meanwhile, the onset or evolution of the process of non-alcoholic steatohepatitis and cirrhosis generally take months or years; continual intake of anti-NAFLD compounds might be the ultimate approach to inhibit NAFLD-related molecular and metabolic stimulators chronically.

Most NAFLD inhibitors of diet can inversely inactivate more than one stimulator of fatty liver disease, making them favorable over irreversible inhibitors with one specificity and substantial lethal side effects. Inhibitors of nutrition sources, such as silymarin and morin, are reversible and appear to target multiple activators and inhibitors of NAFLD [37]. NAFLD inhibitors seem immune or could delay drug resistance in standard therapy to develop drug resistance by prolonging cells [38]. These assets converse to these bioactive composites to bypass drug resistance and be effective against several types of non-alcoholic fatty liver dependent diseases. NAFLD is a widely down-regulated co-fetal mechanism in healthy populations. NAFLD targeting does not lead to side effects even after chronic exposure to naturally occurring and physiological anti-NAFLD compounds.

Low molecular weight anti-NAFLD functional foods such as antioxidants, pre- or probiotics, tangeritin and lycopene may offer novel strategies in NAFLD dependant insulin resistance, obesity and abnormal fatty acid metabolism-related hepatocellular carcinoma [39]. Orally administered lycopene was detected in the liver and plasma and brain cortex of the rat model of various diseases with numerous concentrations [40]. Reviews on the work of several types of research on a diversity of anti-NAFLD functional foods, the non-alcoholic fatty liver-associated enzymes they inhibit, the right sources of NAFLD compounds, the molecular mechanisms of inhibition and references for additional information. The list is not meticulous. Minerals have some negative role on NAFLD and the existed clinical data showed that Zinc (Zn), selenium (Se) and Copper (Cu) have some negative impact on anti-NAFLD [41].

4.1 Phenolic compounds

4.1.1 Catechins

Oolong tea polyphenols markedly inhibited the formation of oxidized lipids, reduced body fat and the risk of developing arteriosclerosis [42]. A double-blind placebo-controlled study by Sakata et al. (2013) demonstrated the health benefits of green tea extracts for NAFLD [43]. The green tea polyphenols showed higher bioavailability in the serum following its consumption. Antioxidant, anti-inflammation, lipid metabolism-related biomarkers, as well as SREBPs and related genes, are critical mediators of NAFLD. Epigallocatechin-3-gallate (EGCG), a critical bioactive from green tea, prevented NAFLD in several experimental models (**Table 1**) [57].

4.1.2 Curcumin

Curcumin, a yellow-pigmented bioactive of *Curcuma longa*, is most commonly used as a dietary spice. Numerous investigations described its pharmacological activities, including antioxidant, lipid-modifying, anti-inflammatory, anti-cancer effects [58]. Oral administration of curcumin (50 mg/kg body weight) synergistically regulated both endogenous and exogenous Nrf2/LXRα pathways in high sucrose diet-induced NAFLD rats [44]. Jazayeri et al. (2019) showed curcumin regulated the PPAR-γ activity, inhibited cyclooxygenase controlled inflammation and improved NAFLD [44, 59]; Curcumin (100 mg/kg body weight) administration for three weeks to methionine and choline-deficient (MCD) diet-fed mice significantly upregulated superoxide dismutase 1 (SOD1), SIRT1 levels and inhibited O-GlcNAcylation pathway [60]. Saadati et al. (2019) conducted a randomized placebo-controlled clinical trial with 52 NAFLD subjects where they showed curcumin (1500 mg) administration for 12 weeks significantly reduced serum cholesterol, glucose and liver fibrosis [61].

Bioactive compound	Disease effect reported	NAFLD biomarkers and enzymes activities investigated	Sources	Mechanisms of action	References
PHENOLICS i. **Catechins (EGCG, ECG)** i. **Curcumin** ii. **Genistein (Isoflavones)** iii. **Daidzein (Isoflavones)** iv. **Resveratrol**	Antioxidant, anti-inflammatory, anti-fibrotic, antitumor and anti-arteriosclerotic	upregulate Insulin-Degrading Enzyme; downregulate AMP, SREBPs; promote glycogen synthase kinase	Fresh tea leaves, black grapes, strawberries, apples, blackberries, broad beans, pears and raspberries	Reduced liver fat deposition in liver. Prevention of NAFLD related liver tumorigenesis	[43]
	Anti-oxidant, antimicrobial, anti-inflammatory, anti-cancer, lipid-modifying,	activate Nrf2; upreulate FXR, LXR-α; reduce serum levels of total cholesterol and TG	Turmeric	Suppressed fat accumulation and hepatic injury	[44]
	Antioxidant, anti-inflammatory, anti-fibrotic, anti-tumorigenesis	activate AMPK; upregulate PPAR-α; downregulate COX-1, hepatic thromboxane A_2 receptor; reduce serum ALT	Soybeans, Soy milk and soy-based beverages	Normalize hepatomegaly and liver steatosis Control of aminotransferase abnormalities and glucose tolerance	[45, 46]
	Anti-cancer, anti- osteoporosis, anti- ischemic heart disease and hepatoprotective	Downregulate ChREBP, Akt activation, Improve phosphatidylinositol 3-kinase; glucose transporter-2, SOD-2 and glutathione S-transferaseα3	Leguminous Plants, Textured soy proteins, Herbal teas	Hepatic de novo lipogenesis, inhibition of hepatic fat accumulation	[47]
	Anti- obese, anti-diabetic, anti- cancer	downregulate TNFα, COX-2, IL-6; reduce ALT and AST, inhibit (PI3K)/Akt pathway, NF-κB pathway	Skin of grapes, Peanuts and Berries	Autophagic mediated fat depletion	[48]
PROANTHOCYANIDINS i. **GSP** ii. **Tannins** iii. **Silybin**	Lipid-lowering, antioxidant.	Downregulate SREBP1c, HMG CoA reductase	Grape seeds	Prevented oxidative liver injury	[49]
	Antioxidant, Anti-lipogenic, antimicrobial	Inhibit histone acetyltransferase, FAS, ACC; upregulate Carnitine palmitoyltransferase-1	Coffee, Tea, Cocoa, Sorghum grain	Reduced liver steatosis	[50]
	Antioxidant, anti-inflammatory, antipyretic, anti-fibrotic	Inhibit NFkB activation, SCD1, AOX, Insulin resistance; reduce AST & ALT	Milk thistle	Reduced liver steatosis	[51]

Bioactive compound	Disease effect reported	NAFLD biomarkers and enzymes activities investigated	Sources	Mechanisms of action	References
SAPONINS & TERPENES i. **Ginsenoside** ii. **Andrographolide** iii. **Glycyrrhetinic acid**	Antioxidant, antimicrobial, antifungal, antidiabetic, hepatoprotective	Upregulate PPAR-α induced fatty acid oxidation; activate SIRT6; Downregulate inflammatory cytokines, SREBP-1C	Panax species	Alleviated hepatic lipid accumulation	[52]
	Anti-inflammatory, anti-platelet aggregation, antineoplastic.	Inhibit CYP3A and CYP2C9; downregulate HNF4α,	*Andrographis paniculata*	Improved glucose metabolism and liver function	[53]
	Antiviral, anti-inflammation, analgesia, anti-tumor, Immunomodulation, hepatoprotective.	Inhibit AKR1B10, Upregulate Glycogen synthesis, PDase and GSK3β	Herbliquorice	Decreased hepatic lipogenesis	[54]
PHYTOSTEROLS	Antioxidant, Lipid-modifying	Downregulate TGF-β, IL-6, IL-10, C-reactive protein, lipoprotein cholesterol (LDL-C) and hepatic TG	Unrefined plant oils, Nuts	Increased liver lipid metabolism	[55]
CAROTENOIDS	Antioxidant, anti-inflammatory	Downregulate Malondialdehyde, TNF-α	Carrots, guava, mangoes, collard greens	Decreased fat accumulation and bleedings	[56]

Table 1.
Mechanism of action of functional foods against NAFLD.

4.1.3 Isoflavones

Isoflavones exhibited an excellent therapeutic effect for NAFLD through de novo lipogenesis via ChREBP and anti-adipogenic Wnt signaling [62]. Genistein derived from soybean is the most investigated isoflavone with higher potency against NAFLD. Genistein supplementation (2 and 4 g/kg diet) for 12 weeks markedly reduced serum and liver lipids and downregulated SREBP-1c, PPAR-γ in NAFLD mice [63]. Genistein significantly suppressed the expression of cyclooxygenase-1 and hepatic thromboxane A2 receptor expression through the thromboxane A2 (TXA2) pathway [46]. Daidzein, a naturally occurring phytoestrogen occurring in soybean and legumes, reduced NAFLD risk and inhibited hepatic fatty acid β-oxidation in high fat supplemented mice [47]. Liu et al. (2017) showed administration of soy isoflavone (10 or 20 mg/kg) to NAFLD animals inhibited fatty acid synthesis. It promoted fat oxidation in the liver by regulating the expression of SREBP-1c and PPARα [64].

4.1.4 Resveratrol

Resveratrol (3, 5, 4′-trihydroxystilbene) is widely present in the skin of grapes, berries and peanuts. Oral administration of resveratrol (50 mg/kg body weight) to high-fat diet (HFD)-induced C57BL/6 J mice reduced inflammation and fibrosis risk by modulating the IκBα-NF-κB and autophagic pathway [48]. Resveratrol by upregulating SIRT1 decreased liver lipogenesis markers and improved lipid metabolism in HFD fed mice [65]. Intragastric administration of resveratrol improved hepatic steatosis mediated by a SIRT1/ATF6-dependent mechanism in HFD fed mice [66]. Theodotou et al. (2019) showed supplementation of trans-resveratrol as a micronized formulation to NAFLD subjects reduced TG accumulation and improved insulin resistance via activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) and SIRT1 [67].

4.2 Proanthocyanidins and flavonoids

The oligomeric and polymeric components of the flavonoid biosynthetic pathway are proanthocyanidins, also known as condensed tannins. Proanthocyanidins are widely distributed in seeds, fruits, flowers, nuts, barks of several plants and are typically made up of catechin and epicatechin [68]. The number of anti-NAFLD proanthocyanidins published studies is less, but findings are similar to those of other flavonoids (**Figure 2**). The grape seed proanthocyanidins (GSP) exhibited anti-NAFLD effect mainly by lipid-lowering and high antioxidant activities [49]. In another study, GSP suppressed high calorie diet-induced hepatic injury in animals [69]. A significant number of studies demonstrated the hepatoprotective and anti-fibrotic effect of morin in NAFLD models, mainly by modulating the key signaling pathways associated with fibrosis [70]. Administration of polymethoxylated flavones enriched Daoxianyeju extracts (0.2% and 0.5%) to HFD fed mice prevented liver inflammation and steatosis by activating nuclear factor erythroid-2 related factor 2 (Nrf2) signaling [71]. Tannic acid supplementation to the western diet-fed mice for 12 weeks suppressed histone acetyltransferase activity and prevented lipid accumulation [72]. Silybin, also known as silibinin, derived from Silybum (milk thistle) plant extracts in combination with tangeretin (75–150 mg/kg) showed potent antioxidant, anti-inflammatory and anti-fibrotic activities [73]. Citrus peel extract composed of hesperidin, narirutin, synephrine and tangeretin prevented *in vivo* lipid accumulation and fatty liver development by regulating AMPK activation [74].

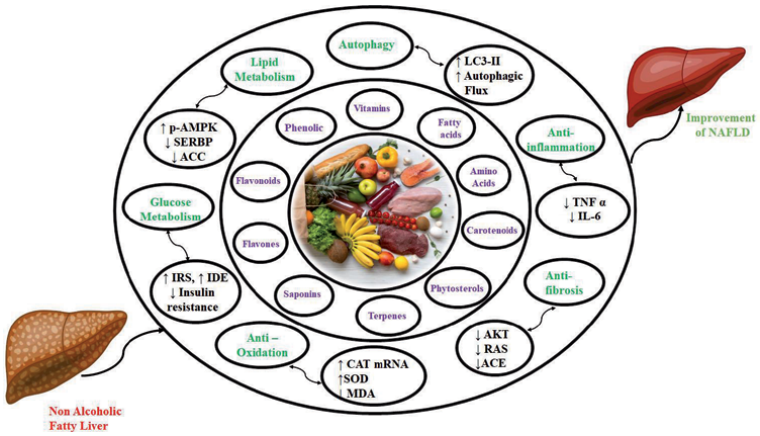

Figure 2.
Significance of functional foods for NAFLD.

4.3 Saponins and terpenes

Saponins consist of a broad family of structurally similar substances with steroid or triterpenoid glycone (Sapogenin) containing more than a fraction of oligosaccharides. The saponins and their derivatives are reported in several edible legumes. Several *in vivo* studies documented legume saponins' health benefits, including antioxidant, antidiabetic, hepatoprotective, hypocholesterolemic, anti-cancer, antitumor, antiviral [75]. Treatment of Akebia saponin D (100 μM) to oleic acid-induced BRL cells reduced lipid accumulation, increased BNip3 levels and mitophagy [76]. Hou et al. (2020) demonstrated oral administration of ginsenoside-Rg1 (30 mg/kg/day) reduced SREBP-1c expression, lipid accumulation and alleviated liver inflammation in NAFLD rats [52]. Sea cucumber-derived saponins echinoside A (EA) ameliorated orotic acid induced-NAFLD mainly by inhibiting lipogenesis genes. Andrographolide, a diterpene lactone present in *Andrographis paniculata* treatment to choline-deficient amino acid-defined mice, prevented liver inflammation, reduced macrophage infiltration and inflammation activation [53, 77].

Wang et al. observed glycyrrhizic acid (a natural triterpene glycoside) administration to MCD diet-fed mice significantly inhibited hepatic stellate cell activation and collagen deposition [54]. In a similar study, glycyrrhizic acid suppressed lipid accumulation and reduced the levels of SREBP-1c, FAS, SCD-1 in HFD fed mice [78]. Glycyrrhetinic acid, a bioactive triterpenoid from licorice, reduced the inflammation and fat content in the mouse liver and inhibited AKR1B10 activity [79].

4.4 Phytosterols

Phytosterols or plant sterols are cholesterol-like molecules which perform vital structural functions in plants. Phytosterols are best known for their cholesterol-lowering effects and recent investigations highlighted their anti-fibrotic developments in key NAFLD models [80]. Plant sterol and stanol ester supplementation significantly reduced plasma lipids and prevented HFD induced inflammation in experimental animals [81]. In a high-fat Western-style diet-induced mice study, stigmasterol and β-sitosterol markedly reduced the liver TGs, cholesterol, intestinal bile acid levels and alleviated NAFLD [55]. Intragastric administration of phytosterol esters for 12 weeks to HFD fed rats reduced liver size, lipid content and improved intestinal flora [82]. In combination with EPA and DHA, phytosterol

esters significantly reduced the levels of TGs, cholesterol, LDL cholesterol and decreased the pro-inflammatory cytokines in NAFLD subjects [83]. β-sitosterol supplementation for 12 weeks mitigated high-fructose diet-induced macrovesicular steatosis and progression of steatohepatitis [84].

4.5 Carotenoids

Carotenoids are a family of poly-isoprenoid structured and fat-soluble pigments that occur naturally in plants and microbes. The primary sources of carotenoids in the human diet are yellow, orange and red-colored fruits and vegetables. In the last few decades, carotenoids have been the main focus of research mainly due to their potent antioxidant, anti-inflammatory and anticancer properties [85]. Besides, carotenoids were also reported for their anti-fibrotic effect in several experimental NAFLD models [86]. Several clinical studies revealed circulatory carotenoid levels to NAFLD risk [56, 87]. β-carotene is reported for strong antioxidant potential and a vast number of *in vitro* and *in vivo* studies revealed the hepatoprotective and anti-fibrotic effect of β-carotene [88–90]. Lycopene, a non-provitamin A carotenoid, mainly exhibited hepatoprotective effect through scavenging ROS. Supplementation of lycopene significantly lowered steatosis and obesity-induced inflammation in NAFLD animals [91–94]. The xanthophyll carotenoid, astaxanthin is reported for various biological effects such as free radical scavenging, ocular protective, hepatoprotective, anti-aging, anti-diabetic, anti-inflammatory, anticancer, etc. [95–97]. In experimental liver fibrosis models, astaxanthin offered hepatoprotection by reducing liver pro-inflammatory cytokines, attenuating insulin resistance, downregulating key signaling pathways [98–102]. Other carotenoids like α-carotene, lutein and zeaxanthin also exerted hepatoprotection in experimental NAFLD models [103–105].

4.6 Functional foods from plant/animal origin/carbohydrates

Oats (*Avena sativa*) rich in β-glucan, a polysaccharide responsible for its functional properties and other active compounds such as antioxidants, vitamins, minerals and phenolic compounds and dietary fibers. A study conducted in Sprague–Dawley rats found that a diet rich in oats increased liver LDLR, reduced liver TGs and cholesterol, thereby preventing NAFLD development to liver cirrhosis [106]. Flaxseed (*Linum usitatissimum*) is a highly nutritional functional food due to active components such as polyunsaturated fatty acid (PUFA), α-linolenic acid, proteins, lignans, soluble and insoluble dietary fibers, antioxidants and phytoestrogens. In a clinical trial conducted with 50 subjects, supplementation of flaxseed diet significantly reduced the body weight, liver enzymes, insulin resistance, hepatic fibrosis and steatosis. A significant difference was observed between control and flaxseed groups in ALT, AST, GGT, fibrosis score and steatosis score, etc. [107].

Choline is an essential nutrient present in eggs, liver, soy wheat and vegetables. Choline is either produced in the body or is absorbed from a diet rich in phospholipids such as phosphatidylcholine. Phosphatidylcholine is a significant component of cell membranes and present in egg yolk and soy. Choline and betaine supplementation effectively alleviated NAFL in dairy cattle, PEMT- deficient mice by increasing AMPK, reducing mRNA levels of DGAT2 and lipid accumulation, decreased expression of genes such as acyl-CoA synthase-1 and -4, mitochondrial glycerol phosphate acyltransferase, etc. [108]. Studies conducted in humans showed that betaine supplementation reduced serum concentrations of ALT and AST and lowered hepatic steatosis [109, 110]. In Balb/c mice, administration of betaine increased serum ALT, decreased hepatic and visceral mass accumulation by reducing glucose

production through inhibiting gluconeogenesis and promoting the use of glucose in glycogen production leading to improved serum glucose levels. Thereby, betaine reversed insulin resistance by promoting IRS1 phosphorylation and enhanced downstream pathways of gluconeogenesis and glycogen synthesis and effectively alleviated NAFLD [111].

4.7 Functional foods from microbial origin

Monascus is a fungi class that includes *M. purpureus, M. pilosus* and *M. ruber* relevant in the field of functional food due to the presence of bioactive metabolites such as monascin and ankaflavin. These compounds possess pharmacological properties such as antioxidant, anti-inflammatory, antidiabetic, immunomodulatory and anticancer [112]. A study conducted in FL83B hepatocytes and male C57BL/6 J mice observed that both monascin and ankaflavin inhibited fat accumulation in hepatocytes by preventing fatty acid uptake, lipogenesis and accelerating fatty acid β-oxidation. Monascin and ankaflavin also improved AMPK phosphorylation and downregulated expression of steatosis related genes. Treatment with monascin and ankaflavin suppressed expression of SREBP-1c, FAS, ACC and upregulated FXR, PGC-1α and PPAR-α. This result suggested that monascin and ankaflavin are potential bioactives for NAFLD [113, 114].

Sargassum serratifolium is a brown macroalga that possess several bioactive compounds such as sargahydroquinoic acid, sargachromenol, sargaquinoic acid, etc. This seaweed is widely used in culinary preparations of Korea and China. *S. serratifolium* have many pharmacological properties such as anti-inflammatory, anti-obesity, lipid-lowering, etc. A review of C57BL/6 J mice treated with a rich ethanol fraction of *S. serratifolium* demonstrated lipid-lowering effects by activating AMPK-mediated fatty acid oxidation signaling and prevented SREBP-1c signaling related lipogenesis in the liver and fatty tissues. The extract was also able to downregulate FAS and SCD-1 along with SREBP-1c and inhibited TG synthesis and cholesterol and activated fatty acid oxidation by promoting AMPK. This showed that *S. serratifolium* is a practical, functional ingredient for alleviating NAFLD by controlling lipid accumulation in liver [115].

Freshwater clams (*Corbicula fluminea*) is well-known hepatoprotective used in Chinese traditional medicine. The major active components are brassicasterol, camesterol, stigmasterol, α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid and carotenoids. In HepG2 cells, ethanolic extract of residual clam meat significantly decreased lipid accumulation by suppressing FAS. In tilapia and mice models, the clam extract reduced AST levels, ALT, total cholesterol, accumulation of triglycerols, etc. The extract was also able to downregulate SCD-1 index, promoted PUFA n3/n6 ratio and reduced ballooning, PGE2, total fatty acids, triacylglycerol level, hepatocyte size and inflammation, etc. This result showed that freshwater clam extract is a useful functional component for developing hepatoprotective supplements against NAFL [116].

5. Role of functional foods for NAFLD

There may be limitations to their use in disease prevention in Anti-NAFLD functional foods, while there are opportunities to assess functional foods' ability as anti-NAFLD compounds.

1. It will be essential to address the suitable intake of anti-NAFLD compounds that confer prevention without toxicity.

2. Knowledge about the kinetics of functional foods or their blood metabolite levels, which may differ from individuals and diseases, would be required over the individual's lifespan for a sustained level of NAFLD inhibitor in the blood.

3. If clinical evidence shows that a dietary component is successful in disease prevention and broad intervention trials prove effective in reducing those disease-related biomarkers, then the medical community can embrace the element.

4. How early in life is anti-NAFLD food being consumed?

5. When does one stop using anti-NAFLD functionally in the event of liver regeneration, such as fat dissipation?

6. How does one assess whether the prevention of disease progression with anti-NAFLD functional foods has been affected?

7. Nevertheless, the use of anti-NAFLD compounds, some from natural sources and others from synthesis, such as LPSF/GQ-02, have shown successful clinical efficacy. However, Thiazolidinediones (TZD) were addressed by suggesting that acting as an insulin sensitizer effective against insulin resistance and fat accumulation could be good anti-NAFLD agents at an early stage NAFLD. Anti-NAFLD functional foods can, therefore, be beneficial at the early stages of the non-alcoholic fatty liver cycle [117].

8. Data on the toxicity of the anti-NAFLD compound will be needed for diseases such as diabetes, where both excessive and insufficient NAFLDs exist, compounds that may inhibit excessive NAFLDs and worsen insufficient NAFLDs may need to be established.

9. Most human NAFLD or liver steatosis cells are deficient in PNPLA3 (Patatin like phospholipase domain-containing gene. This deficiency in PNPLA3 indicates that high-fat deposition activity after eating and decreases during periods of fasting food. It plays a role in toxic clearance hence their responsiveness to Anti- NAFLD compounds [118].

6. Bioavailability and synergy of anti-NAFLD functional foods

Low molecular weight anti-NAFLD compounds such as phenolics, terpenes, phytosterols, small carbohydrates and amino acids might be more bioavailable than high and moderately soluble proteins, polypeptides and large carbohydrate molecules. Nonetheless, large molecules such as proteins and carbohydrates have shown a protective effect against NAFLD. In contrast, substances with low molecular weight might help liver regeneration. Combining one or more anti-NAFLD compounds has been shown to result in improved behavior in several studies. Silybin treatment, in combination with vitamin E and phosphatidylcholine, significantly improved liver enzymes and liver steatosis in NAFLD patients [119]. Another study by Han et al. showed metformin (500 mg orally three times daily) in combination with vitamin E (100 mg) and bicyclol (25 mg) synergistically prevented NAFLD in human subjects by improving liver enzymes and liver histology parameters. The combination of blueberry juice and probiotics 1.5 mL per 100 g (0.07 mg/mL concentration) weight showed protection to hepatocyte mitochondrial function in the HFD induced animals [120].

7. Conclusion and future prospects

Even though there is no FDA approved anti-NAFLD compound, industrial and academic researchers are still investigating for naturally occurring bioactive(s) for potential and safe anti-NAFLD compounds. Prior consideration is necessary for designing anti-NAFLD functional foods, especially for complex diseases such as insulin resistance, chronic liver diseases, etc. Food bioactive such as curcumin, proanthocyanidins that have shown hepatoprotective and anti-NAFLD properties, are the right candidates for incorporating into functional foods. Also, research support is highly essential for accessing the anti-NAFLD properties of other food components. Anti-NAFLD functional foods could be a low-cost strategy to prevent obesity-related complications. NAFLD inhibitors are recognized as one of the targets for obesity therapy [121]. The development of post-genomic functional foods may need to focus on molecular targets such as NAFLD factors that drive the early stages of chronic disease onset/progression. A comprehensive and successful work in functional foods will involve knowledge of ethnobotany, chemotaxonomy, transgenic plants or animal animals (as bioactive compound factories) and interdisciplinary approaches involving foods, nutrition scientists, and biomedical scientists for design. In this context, researchers must have in-depth knowledge in the field of ethnobotany and chemotaxonomy. To determine the efficacy of functional food, the post-genomic wave of functional foods would need to span the entire spectrum, from primary to clinical trials.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ACC	Acetyl-CoA carboxylase
AKR1B10	Aldo-keto reductase family 1 B10
ALT	Alanine transaminase
AMPK	5′ adenosine monophosphate-activated protein kinase
AP-1	Activator Protein 1
AST	Aspartate transaminase
ATF6	Activating Transcription Factor 6
ChREBP	Carbohydrate-responsive element-binding protein
DGAT2	Diacylglycerol O-acyltransferase 2
DPA	Docosapentaenoic acid
EGCG	Epigallocatechin-3-gallate
EPA	Eicosapentaenoic acid
FABPs	Fatty-acid-binding proteins
FXR	Farnesoid X receptor
GGT	Gamma-glutamyl transferase
GSP	Grape seed proanthocyanidins
HFD	High Fat Diet

IRS-1	Insulin receptor substrate 1
IRS-2	Insulin receptor substrate 2
JNK	c-Jun N-terminal kinase/
LDL	Low-density lipoprotein
LPS	Lipopolysaccharides
LPSF/GQ-02	Benzylidene thiazolidinedione
MCD	Methionine and choline-deficient
NAFLD	Non-Alcoholic fatty liver disease
NAFL	Non-Alcoholic fatty liver
NASH	Non-Alcoholic steatohepatitis
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor erythroid 2-related factor 2
PEMT	Phosphatidylethanolamine N-methyltransferase
PGC-1α	Pparg coactivator 1 alpha
PGE2	Prostaglandin E2
PNPLA3	Patatin-like Phospholipase 3
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SCD1	Stearoyl-Coenzyme A desaturase-1
SIRT	Sirtuins
SNPs	Single Nucleotide Polymorphisms
SOD1	Superoxide dismutase 1
SREBP-1c	Sterol regulatory element-binding protein-1c
TG	Triglyceride
TGF-β	Transforming growth factor-β
TM6SF2	Transmembrane 6 superfamily member 2
TNF-α	Tumor Necrosis Factor- α
TXA2	Thromboxane A2
TZD	Thiazolidinediones
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein

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Chapter 11

Role of Functional Food in Treating and Preventing Cardiovascular Diseases

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Abstract

Cardiovascular diseases (CVDs) are still a major cause of mortality worldwide and are a serious health problem. Various factors that contribute toward CVDs include hypertension, tobacco use, physical inactivity, diabetes mellitus, obesity and overweight, alcohol, dietary factors and psychosocial aspects such as stress, anxiety and depression. Nutraceuticals and diet are very important for prevention of CVDs. The potential of nutraceuticals or functional food in mitigating risk of CVDs is discussed. Functional food with health related properties include fruit and vegetable, fish, legumes, nuts, soya protein, coffee, tea, chocolate, animal based functional food. In addition, some diet plans have shown the potential of reducing the incidence of CVDs. This includes the Mediterranean, Dietary Approaches to Stop Hypertension (DASH), Okinawan and vegetarian diets. This chapter examines the risk factors of CVDs, including hypertension, tobacco usage, physical inactivity, diabetes mellitus, overweight and obesity. The chapter also brings to the fore, functional foods with properties related to health and effect of dietary patterns in the treatment and prevention of CVDs.

Keywords: non-communicable disease, cardiovascular diseases, nutraceutical, functional foods, dietary patterns

1. Introduction

Globally, non-communicable diseases (NCDs) account for 73% of all death with cardiovascular diseases (CVDs) and ischemic heart disease (IHD) as the main contributors of cardiovascular mortality in 2017 [1]. Cardiovascular, respiratory as well as associated disorders (CVRDs) are predominant subgroup of NCDs and are major causes of morbidity and mortality in developing and developed countries. In 2012, it was estimated that 55.9 million people died around the world and NCDs accounted for 37.9 million of those deaths. Specifically, CVRDs led to 23.9 million deaths [2]. **Table 1** shows the impact of CVRDs on the total deaths in different countries based on the income group. Cardiovascular diseases killed 17.5 million; respiratory diseases led to 4.0 million deaths; diabetes mellitus to 1.5 million and diseases related to kidney accounted for 864 000 deaths, respectively [4]. In addition, NCDs account for about 35% (around 2.6 million) of all deaths in sub-Saharan Africa and this makes NCDs the second most common cause of death after a combination of communicable, maternal, neonatal, and nutritional related diseases [5]. Researchers

Indicator	Low income	Lower middle income	Upper middle income	High income	Total (world)
Population	850000	2510000	2430000	1290000	7060000
Total deaths	7450	19900	16900	11700	55900
CVRD deaths	1540	7780	9080	5530	23900
CVRD deaths	21%	39%	54%	47%	43%
CVRD death by cause					
CVDs	999	5220	6860	4440	17500
DM	135	549	559	254	1497
RD	309	1630	1460	645	4040
Kidney diseases	99	378	197	190	864

Note: CVRD = Cardiovascular, respiratory, and related disorder, CVDs = Cardiovascular diseases, DM = Diabetes mellitus, RD = Respiratory disorder. [2, 3].

Table 1.
Impact of CVRDs on the total deaths in different countries based on the income group in 2012. Thousands, unless otherwise noted.

estimate that more than three-quarters of deaths will be due to NCDs by the end of 2030 and more deaths in developing countries will be attributed to CVDs alone than contagious diseases such as malaria, tuberculosis and HIV/AIDS [6].

Cardiovascular diseases are complex and composite diseases that are characterised by high serum lipids and triglycerides, cholesterol, elevated plasma fibrinogen and agglomeration factors with increased production of platelet as well as disturbance in metabolism of glucose [7, 8]. They are a broad category of diseases involving the heart and blood vessels causing coronary artery diseases such as angina which can lead to heart attack, heart failure, hypertensive heart diseases, stroke, and many other problems. [9]. Cardiovascular diseases are still the leading cause of mortality globally leading to 12.3 million and 17.6 million deaths in 1990 and 2016, respectively [10–12]. Stroke and coronary artery disease result in of 80% and 75% CVD deaths in male and females, respectively [12].

Various epidemiological studies have demonstrated that diet habits and healthy life style might prevent chronic diseases such as CVDs but poor habits aggravate these diseases [8, 13]. Individuals that consume large amount of fruits, vegetables and sea food are less vulnerable to CVDs incidence [14]. The role of dietary factors such as sodium and saturated fats known to increase the risk of CVDs has been substantially explored [15]. The perception that food does not only furnish fundamental nutrition but can also play a role in preventing diseases and assure good health and life is now gaining attention. High intake of food that is calorie dense, poor nutrition, highly processed and easy to absorb food can contribute to inflammation of system, low insulin vulnerability as well as a group of metabolic diseases which include obesity, high blood pressure, dyslipidemia, and diabetes mellitus [16]. Food that furnish a health benefits apart from basic nutrition such as reducing high blood total cholesterol as well as low-density lipoprotein cholesterol are called functional foods.

2. Risk factors for cardiovascular diseases

The traditional risk factors for CVDs (**Table 2**) have been extensively researched and the dietary factor is important since it leads to high risk factors for CVDs such

Non-modifiable	Metabolic	Lifestyle	Novel
Family history	Diabetes mellitus	Diet	Oxidative stress
Advancing age	Obesity	Smoking	High homeostatic factors
Family history	Hypertension	Physical activity	Small dense low density lipoprotein-C
	Hyperlipidemia		High lipoprotein level
	Metabolic syndrome		High homocysteine level
			High inflammatory marker

[17, 18].

Table 2.
Risk factors of cardiovascular diseases.

as hypertension and dyslipidemia. However, the dietary factor phenomenon has not been fully investigated [3]. A 2011 global report indicated that hypertension contributed to 13% of CVDs deaths, tobacco 9%, physical inactivity 6%, diabetes mellitus 6% and obesity 5% of global deaths [19, 20].

2.1 Hypertension

Hypertension is systolic blood pressure values ≥140 mmHg and/or diastolic blood pressure values ≥90 mmHg. The relationship between hypertension and CVDs has been investigated in different studies [21, 22]. Hypertension exhibits an independent interminable relationship with the incidence of various CVDs such as stroke, heart failure and peripheral arterial [23, 24]. Hypertension is commonly without symptoms which silently damage the arteries that furnish the heart, brain, kidneys and other vital organs with blood and produce various structural changes. Various epidemiological, animal and genetic studies have confirmed that excessive intake of sodium increases blood pressure. For example, excessive consumption of sodium (>5 g sodium per day as defined by World Health Organisation) [25] produces a significant rise in hypertension and is associated with the onset of hypertension and its cardiovascular complications [26, 27]. By contrast, low intake of sodium reduces hypertension prevalence and is associated with low cardiovascular morbidity and mortality rate [28]. As a result, a common nutritional plan to minimise the incidence of hypertension includes achieving and maintaining a healthy body weight; consumption of a diet rich in minerals such as calcium, phosphorus, and magnesium as well as moderate consumption of alcoholic beverages and sodium [29].

2.2 Tobacco use

There are more than one billion smokers around the world and in 2013, tobacco usage accounted for more than 6.1 million deaths [18]. This estimation covers vulnerability to passive smoking (second hand smoke) which increases CVDs risk by 25 to 30% and public smoking bans substantially decrease the rate of heart attacks [30]. The most smoked form of tobacco is cigarette. More than 80% of tobacco users live in lower middle income countries and this number is expected to increase in the next decade [31]. Most smokers in lower middle income countries are male but this is not the case in high income countries. Early cessation of smoking contributes to substantial lower incidence of reinfarction within 1 year in patients who have had a heart attack and decreases the possibility of instant cardiac death in

patients with CVDs [32]. There are two CVDs challenges associated with the use of tobacco products. Firstly, the rate of smoking is higher in the poorest populations of the world [33] and the second problem is smoking among girls [34]. The risk of IHD to tobacco smokers is 2–3 times higher than non-smokers, stroke is 1.5 times higher and lung cancer is 12 times higher. These risks are related to age gradient, with younger age group having higher relative risk (5–6 times) and these are similar for men and women [35].

2.3 Physical inactivity

The World Health Oranisation highlighted the fact that one in four adults is not physical active enough across the globe and the challenge of reduced physical activity increases as the income of a country increases [36]. Low physical activity eventually leads to obesity which has gradually increased throughout the past decades both in developing and developed countries. Low rate of atherosclerotic process, including improvement of endothelial dysfunction, low systematic inflammation and stroke that go along with physical activity, could explain the protective role of physical activity on CVDs risk [37]. Numerous epidemiologic studies conducted with non-identical and big populations have demonstrated that physical activity has protective effect on coronary arteries disease. For example, physical activity reduces blood pressure and the prevalence of hypertension by reducing vascular resistance and terminating the action of the sympathetic nervous and the renninangiotensin systems. **Figure 1** shows the possible channels of physical activity that assist in decreasing the likelihood of CVDs. In 41837 women of age group between 55 and 69 years, Folsom et al. [38] study found that hypertension incidence decreased by 10% and 30% in participants with moderate and higher levels of physical activity than those with low levels of physical activity. The biological pathways support the usefulness of physical activity in decreasing the likelihood of stroke including ischemic and hemorrhagic. The possible ramifications on the likelihood of ischemic might be due to the mechanisms that reduce the development of atherosclerotic; while the possible ramifications on the likelihood of hemorrhagic stroke disease might be attributed to low blood pressure as well as additional associated risk factors. Nonetheless, there are conflicting findings from different studies about the relationship of physical activity and the incidence of stroke, with few studies showing relationships or no relationships [39, 40]. These disagreements are likely the result of the type of the study design, population, definition and evaluation of physical activity of the different studies.

2.4 Diabetes mellitus

Diabetes mellitus (DM) is caused by insufficient production of insulin by the pancreas or resistance by end-organ tissues and presents as a high blood glucose. There are three types of DM, namely, type 1, type 2, and gestational diabetes. Type 1 DM is an autoimmune disorder and usually takes place in early childhood and adolescents, gestational DM occurs during the second or third trimester of pregnancy, increases the future risk of those patients to type 2 DM [41–43]. Type 2 DM, the most common form accounts for 90–95% of diagnosed DM and continues to be rapidly growing worldwide and in the USA [44]. Globally, the prevalence of DM is escalating and its incidence in 1985 was 20 million compared to 382 million in 2014 [45]. The current estimation by the International Diabetes Federation expects that 592 million people will have DM by 2035 [46]. China and India have highest number of people with diabetes estimated at 69 million and 109 million, respectively and these numbers are expected to rise to 123 million and 150 million by 2040 [47]. A

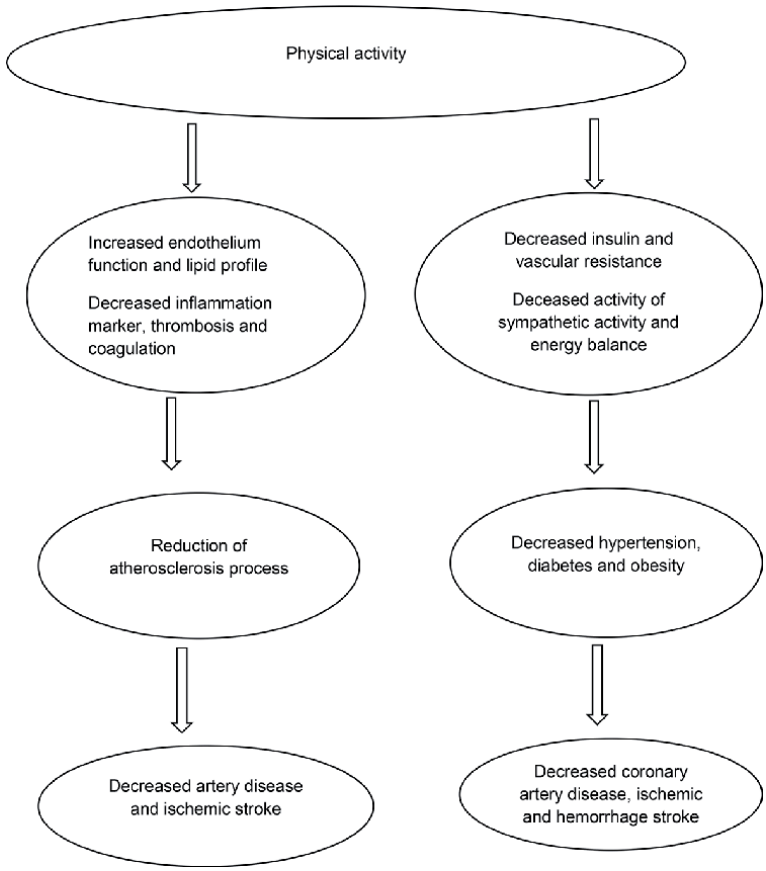

Figure 1.
Possible mechanisms of physical activity that help in decreasing CVDs risk [37].

close association exists between DM and CVDs. The most familiar cause of mortality and morbidity in diabetic patients is CVDs since DM results in complications of both microvascular and macrovascular. Complications such as IHD, ischemic stroke, and amputations because of foot infections are examples of macrovascular. The comparative risk for CVDs morbidity and mortality in adults with DM varies from 1 to 3 in men and from 2 to 5 in women to those with no DM [48]. Many diabetic patients die due to IHD or stroke and both are often registered as the cause of death, not DM [2]. A number of evidences exist that demonstrate that the relationship of type 2 DM and associated cardiovascular risk promote the progressive nature of the vascular damage, leading to atherosclerosis [49]. Cardiovascular deaths account for 44% of death in those with type 1 DM and 52% of deaths in type 2 DM [50]. Debate still remains if the relationship between DM and CVD is associated with diabetes status itself or the risk factors diabetic patients are prone to.

2.5 Obesity and overweight

According to the World Health Organisation data, 39% of the global population above 18 years of age is overweight and of these, 13% are obese. Obesity is an abnormal or excessive fat accumulation in adipose tissue to the degree that health might be compromised [51]. Obesity increases the risk for CVDs since it increases the load of the atherosclerotic plaques, characterised by significant infiltration of macrophage and plaque fluctuation [52]. People who accumulate abnormal body

fat, particularly at the waist, are at higher risk to have stroke and heart disease even if they do not have other risk factors. The incidence of overweight and obesity among adults in low-income countries varies from 4.7–21.0% and from 13.0–42.7% in upper-middle-income countries [53]. Different studies have demonstrated association between obesity and CVDs such as heart failure and cardiac death. Accumulation of abnormal body fat leads to various metabolic changes that increase the common risk factors of CVDs and affects systems modulating inflammation [54]. Recent studies have demonstrated that weight loss in individuals who are overweight and obese reduces the incidence of diabetes and CVDs. The benefit of weight reduction in overweight and obese individuals with or without hypertension is low blood pressure and serum triglycerides, and increased high-density lipoprotein [55, 56].

3. Nutraceuticals and functional foods

Nutraceutical foods, a borderline between food and drugs, are conventional or processed foods that have or added a useful food component which has a health beneficial effect [57, 58]. Nutraceuticals foods provide medicinal and health benefits and these include prevention, management and/or treatment of a disease. Examples of nutraceutical foods are isolated nutrients, dietary supplements, functional foods, medicinal products and processed foods such as cereals, beverages and soups [59, 60]. The interest of nutraceuticals in prevention of CVDs was invigorated after the examinations of a proximate relationship between their consumption, as shown by higher levels of plasma and low CVDs incidence [61, 62]. Japan is the first country that introduced functional foods in the 1980s and is the only country that has distinct regulatory system that approves functional foods [58]. Functional foods contain dietary fibres, polyphenolic compounds, herbs and botanicals and oligosaccharides with their correlating health benefits [63]. It is believed that functional foods use their cardio-protective effects mostly via antioxidant actions which lower blood lipid levels.

The following factors differentiate functional foods from dietary supplements: (1) Functional foods are expected not to only supplement the diet but should also play a role to prevent and/or treat disease(s) and (2) Functional foods are utilised as traditional foods or as exclusive items of a meal or diet [64]. Dietary components play useful roles apart from basic nutrition and this led to the development of nutraceuticals and functional food concept [65]. Functional foods have different mechanism of actions, such as decreasing low density lipoprotein and elevated blood total cholesterols [66].

3.1 Functional foods with health related properties

Different functional foods are beneficial in preventing and treating CVDs (**Table 3**). Dietary fibres of fruit (with pectin) and vegetable, fish oil and oily seeds such as walnut, almond and many others lower the lipid levels in humans and this is attributed to both prevention of fat absorption and termination of synthesis of hepatic cholesterol [81]. A higher consumption of whole grains, bioactive compounds, antioxidants vitamins and folic acid appears to reverse the harmful vascular effects of homocysteine in the heart [82, 83]. A substantial cardiovascular benefit of polyphenolic compounds, vitamins (ascorbic acid, vitamin E), and minerals such as selenium and magnesium in food is thought to be the ability of these components to scavenge free radicals generated during atherogenesis [84, 85].

Functional food	Active component	Mechanism of action	Reference
Fruit and vegetable	Antioxidant vitamins, dietary fibre, carotenoid, polyphenolic compounds	Lower concentrations of the inflammatory mediator C-reactive protein Decrease low density lipoprotein Reduce markers of oxidative stress	[67, 68]
Whole grains	Dietary fibre, minerals, B vitamins and polyphenols	Normal blood pressure and plasma lipids Decrease inflammation	[69, 70]
Legumes and nuts	Mono and polyunsaturated fatty acids, arginine, soluble fibre, polyphenols, folic acid and B vitamins	Lower blood cholesterol Reduce post-prandial vascular reactivity Improved endothelial function Reduce concentrations of blood homocysteine Lower myocardial infarction Positive effect on blood pressure	[71–73]
Fish	Omega 3 fatty acids	Improve endothelial function Lower blood pressure and heart rate Reduce aggregability of platelet Reduce fatal cardiac arrhythmias Anti-inflammatory	[74]
Soy protein	Isoflavonoids, dietary fibre, polyunsaturated fatty acids, vitamins and minerals,	Reduce total cholesterol and low density lipoprotein-C levels Prevent the oxidation of low density lipoprotein Lower total serum	[75–77]
Coffee and tea	Diterpenes (kahweol and cafestol)	Myocardial infarction	[78]
Chocolate	Flavonoids	Improve NO-dependent Vasorelaxation Improve flow-mediated dilation in the brachial arteries Reduce ambulatory serum low lipoprotein-C levels and blood pressure Decrease blood cholesterol levels Increase high density lipoprotein-C Decrease oxidised low density lipoprotein	[79, 80]

Table 3.
Different functional foods beneficial in preventing and treating CVDs.

3.1.1 Fruits and vegetables

There is significant amount that low intake of fruit and vegetable is related to higher risk of CVDs while higher intake is related to low risk of CVDs [86, 87]. Fruits and vegetables are rich sources of polyphenolic compounds such as phenolic acid and flavonoids. Different studies associate the intake of polyphenols foods such as fruit and vegetable with low risk of CVDs [88, 89]. In addition, various studies have shown that the properties of flavonoids such as antioxidants and anti-inflammatory might also improve functions of vascular system [90, 91]. Fruits and vegetables reduce

the risk of CVDs by decreasing vulnerability of low density lipoprotein particles to oxidation [92]. Different types of bioactive compounds found in fruits and vegetables such as dietary fibre, carotenoids, ascorbic acid and minerals such as magnesium and potassium act collaboratively to nurture a comprehensive beneficial effect.

3.1.2 Whole grains, legumes and nuts

Whole grains are more important in terms of nutrition since they have phytochemicals that could work synergistically to decrease the risk of CVDs [93]. Moreover, whole grains are also a rich source of dietary fibre, vitamin B complex and minerals. The preventative effect of whole grains on the risk of CVDs is attributed to their influence on insulin vulnerability, blood pressure and inflammation which is associated with the excessive consumption of antioxidant nutrients available in the germ of whole grains [83, 94, 95]. Legumes are rich source of protein, soluble fibre, micronutrients such as folate and polyphenols [96]. Different bioactive components such as protein, dietary fibre and phytosterols are attributed to the cholesterol-lowering effect of legumes [97]. Nuts are rich sources of mono and polyunsaturated fatty acids, arginine, soluble fibre and various antioxidant polyphenols and these active components contribute to cholesterol lowering effects [71].

3.1.3 Fish

High consumption of fish and fish oil supplements contribute to low incidence of CVDs [98]. Fish is a rich source of omega 3 fatty acids such as docosahexaenoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3). They are available in oily fish such as salmon, tuna, herring and fish oil. Consumption of fish is associated with low risk for myocardial infarction, which is associated with useful influence of DHA and EPA on plaque fluctuation and modulation of endothelial function [99]. Fish oil supplements have beneficial influence on blood pressure and lipid profile [100, 101]. Moreover, DHA and EPA reduce low density lipoprotein oxidative sensitivity in postmenopause women which might assist in reducing the risk of CVDs [102].

3.1.4 Soy protein

Soy products are a rich source of polyunsaturated fatty acids, dietary fibre, micronutrients, low saturated fat content and isoflavones [103]. In addition, the protein content of soybean ranges from 35%–40% having all essential amino acids making soy protein comparable to protein of animal source and it is also cholesterol free [104]. Isoflavones prevent the oxidation of low density lipoprotein and reduces the risk of atherosclerosis [76]. Studies done in China among women and Japan demonstrated that a daily consumption of more than 6 g of soy reduces low density lipoprotein-C, total cholesterol, ischaemic and cerebrovascular incidence than consumption of less than 0.5 g [105, 106].

3.1.5 Coffee, tea and chocolate

Coffee and tea, the most popularly consumed beverages after water, are the chief source of caffeine. Diterpenes such as kahweol and cafestol are suspected to be behind the cardio-protective effect of coffee. Consumption of coffee might potentially decrease the incidence of myocardial infarction, but evidence in this regard is not conclusive [107, 108]. Although results are not consistent, consumption of green tea seems to protect against CVDs [109]. High intake of tea and flavonoids

contribute to the primary prevention of IHD and reduced risk of CVDs mortality [109, 110]. Cocoa is the main ingredient in chocolate manufacturing; it is a rich source of flavonoid and it has been lately evaluated for its plausible role in preventing CVDs [111]. The protective effect of chocolate is attributed to the decrease of blood cholesterols levels, substantial increase of high density lipoprotein including marked decrease of oxidised low density lipoprotein [112].

3.1.6 Animal based functional food

Meat and dairy products are major source of fat in the diet, particularly saturated fatty acids (SFA) which is the leading cause of total cholesterol and CVDs. Meat contains a lot of fat with more than 40% in saturated form, therefore, its quantity and quality has been changed to create new meat products of functional properties. Three meat reformulation methods were proposed in order to develop the functional meat products, namely, low total fat, low total cholesterol intake and modification of fatty acid profile [113]. Dairy products are related to numerous negative health effects because of earlier observations associated SFA content, which might result into increased low-density lipoprotein levels, which in turn increases the risk of CVDs [114]. Dairy products have high SFA and their consumption has long been implicated in contributing to the development of CVDs [115]. Therefore, the consumption of low-fat or non-fat dairy products has been recommended to reduce the risk of CVDs development. Nevertheless, studies tend to show that intake of whole-fat dairy has a favourable effect on health of cardiovascular system and might be more beneficial than intake of low-fat dairy, especially in connection with inflammatory markers. Recent several meta-analyses have demonstrated that low-fat dairy products and whole milk are associated with lower risk of hypertension [116–118]. The presence of calcium, vitamin D as well as other bioactive components such as peptides in dairy products is related to lower blood pressure irrespective of the fat content [119, 120]. High intake of fermented dairy products is associated with low density lipoprotein, low risk of hypertension and CVDs [121, 122]. Consumption of cheese is associated with a low risk of stroke and CVDs [123]. Moreover, consumption of yoghurt is also related to lower risk of CVDs [124]. The presence of bioactive lipids and peptides that have anti-inflammatory characteristics might have contributed to these effects as well as calcium in cheese which might reduce the intake of SFA, thereby decreasing the risk of high cholesterols levels [114]. Moulded cheeses such as Camembert and Roquefort have cardioprotective effects because of the presence of bioactive molecules such as andrastins A–D and roquefortine [125]. Additionally, the cardioprotective effects of fermented dairy products may also be induced by the intake of bacterial metabolites and probiotics. Probiotics reach the gastrointestinal tract while still alive and they can apply their effects directly. Intake of probiotic by supplementation or consumption of fermented dairy products is related to possible health benefits of cardiovascular which include positive effects on blood pressure and hyperlipidaemia [126].

3.2 Dietary pattern and cardiovascular diseases

Various studies have associated the dietary components such as dietary fibre, saturated and trans fats at nutrient level; fruits, vegetables and high fat processed meat at food level [127–129] with changes in prevalence of CVDs. Some diet plans have shown the potential of reducing the incidence of CVDs (**Table 4**). In recent years, various dietary patterns have been recommended for modification in numerous health outcomes apart from the normal dietary guidelines. For example, the Mediterranean, Dietary Approaches to Stop Hypertension (DASH), *Okinawan*

Dietary pattern	High intake	Moderate intake	Low or no intake	Protective effect	References
Mediterranean	Fruits, vegetables, cereals, beans, nuts, seeds, and olive oil	Wine, poultry and fish	Red meat and dairy products	Decrease inflammatory markers	[130–132]
DASH	Fish, fruit, vegetables, whole grains, and nuts	N/A	Dairy products, red meat, sweets, and sugar-containing beverages.	Low blood pressure, anti-inflammatory	[133–135]
Okinawan	Sweet potatoes, and green leafy vegetables	Fish and alcohol	Meat and dairy products	Reduce oxidative stress	[81, 136]
Vegetarian	Fruits, vegetables, legumes and nuts,	N/A	Fish, meat, eggs and dairy products	Anti-inflammatory Low blood pressure and blood cholesterol	[81]

Table 4.
Protective effect of different dietary patterns on cardiovascular diseases.

and vegetarian diets are being promoted as healthy option. Mediterranean diet has gained traction and various reports have shown low incidence of CVDs among populations consuming this diet [137]. Plant foods and olive oil contain high antioxidant content which contributes to the health of the vascular system. The DASH diet restricts saturated fat, red meat, sweets, and beverages with sugar. Regular physical exercise, high intake of dietary potassium, moderate alcohol consumption and low salt intake represent corroborative-based approaches to decrease blood pressure by DASH diet [81]. *Okinawan* diet restricts calorie, there is high intake of vegetables and legumes, moderate consumption of sea foods and alcohol as well as low consumption of meat and dairy products [138]. This is useful for the health of cardiovascular system because *Okinawan* diet is nutrient-dense, antioxidant-rich and has a low-glycaemic-load [139]. Vegetarian diet excludes meat, poultry, or fish and may or may not include dairy and eggs. Vegetarian dietary pattern is recognised for its health promoting compounds because it is rich in dietary fibre, antioxidants, bioactive compounds, plant protein and lower saturated than non-vegetarian dietary patterns [140].

4. Conclusion

Overall, this book chapter highlighted the risk factors of CVDs, functional food with health properties and the influence of dietary in the treatment and prevention of CVDs. Generally there is an increasing trend of using functional food in treating and preventing CVDs. Therefore, there is no doubt that functional food can exert a significant effect on maintenance of human health. Continual consumption of different types of functional foods such as fruit and vegetable, whole grains, nuts, legumes, tea, coffee, chocolate, fish, functional meat and fermented dairy products

may help consumers and patients to lower the risk of CVDs. Consumption of full-fat dairy products contributes to higher intakes of important nutrients such as vitamin D and vitamin K. However, fermented dairy products are preferential for ideal intake of nutrients and possible CVDs health benefits. Dietary patterns such as Mediterranean, DASH, *Okinawan* and vegetarian should be promoted since they are associated with low risk of CVDs.

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Chapter 12

Functional and Therapeutic Potential of γ-Oryzanol

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Abstract

This chapter summarizes the entire literature available on the nutritional value and diverse therapeutic potentials Gamma-oryzanol, a nutraceutical obtained from rice brain oil, composed of a mixture of γ - oryzanol, a mixture of ferulic acid esters of phytosterols and triterpenoids, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate. In brief, the review covers the aspects such as the antioxidant mechanisms, effects on immune system, lipid disorders, diabetes, obesity and inflammation with the details of preclinical experiments, models and observations. Among the other highlights are the hepatoprotective, neuroprotective role in various neurological disorders such as Alzheimer's, anxiety, Parkinson's disease and wound healing effects. An overview of the sources, chemistry, physicochemical properties, pharmacokinetics and toxicity studies are also included.

Keywords: Gamma oryzanol, rice bran oil, pharmacological activities, nutraceutical, organoprotective

1. Introduction

Rice is a staple food in many countries around the world. Milling of rice gives an 8% byproduct, rice bran [1]. Crude rice bran oil is obtained by different methods. According to Godber and Xu the most efficient way to extract rice bran oil is superficial fluid extraction technology [2]. Rice bran oil contains the naturally occurring nutritive and antioxidant phytochemical, γ- oryzanol [1]. It is one of the phytochemicals present in high amounts in rice bran [3].

The molecular formula of γ- oryzanol is $C_{40}H_{58}O_4$ and its melting point is 137.5-138.5°C. It is a white crystalline powder. γ- oryzanol is insoluble in water. However, it is slightly soluble in diethyl ether and n-heptane. Previously, γ-oryzanol was thought to be a single compound [1]. It was later discovered that γ- oryzanol is a mixture of phytosterylferulates of ferulic acid esterified with phytosterols including sterols (campesterol, sitosterol, and stigmasterol) and triterpene alcohols (cycloartenol and 24- methylenecycloartenol) [3]. The structure of gamma oryzanol is shown in **Figure 1**.

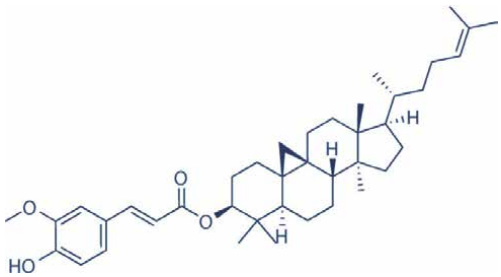

Figure 1.
Structure of gamma Oryzanol.

2. Pharmacokinetics and toxicity of γ- oryzanol

An experiment carried out by Fujiwara *et al*. revealed that γ-oryzanol is readily absorbed by the intestine and is found in high concentrations in the plasma after 1 hour of oral administration [4]. In 2016, Seol-Hee, *et al.* conducted a toxicity study using male and female Sprague–Dawley rats, 1000 and 2000 mg/kg body weight per day were administered to 5 weeks old rats for 90 days. It was observed that the rate of absorption of γ- oryzanol was low and most of it was excreted. The study concluded that there were no adverse effects on administration of 1000 and 2000 mg/kg body weight per day of γ- oryzanol [5].

3. Therapeutic potentials of γ- oryzanol

In the recent times, many researchers have conducted both *in-vitro* and *in-vivo* experiments to explore the bioactivities of γ- oryzanol. Some of these investigations have revealed promising role of γ- oryzanol in certain medical conditions. The pharmacological properties of γ- oryzanol have been summarized in **Table 1** and depicted in graphical abstract, **Figure 2**.

3.1 Antioxidant activity

A compound that halts the effect of free radicals towards cells is referred to as an antioxidant. Free radicals are unstable molecules generated by a reaction to environmental and caused by other factors. Antioxidants can be natural or artificial in nature. Degeneration of lipids by means of oxidation is referred to as lipid peroxidation. In general, free radicals tend to "steal" electrons from lipids in cell membranes, ultimately leading to cell damage which activates the free radical chain mechanism.

The antioxidant activity of γ- oryzanol was assessed against hydroxyl radicals generated by Fenton reaction, and superoxide radicals generated by auto oxidation of $FeCl_2$. The free radical scavenging efficacy of γ - oryzanol was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay while the lipid peroxidation preventive activity was screened by 2,2-azobis(2,4-dimethylvaleronitrile) AMVN assay. Finally, the antioxidant potential of γ- oryzanol within oil samples was analyzed by conductometry in accelerated oxidation conditions. IT, the time taken to achieve sharp increase of conductivity was measured in oils in the presence and absence of γ- oryzanol butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) at different concentrations in a study conducted by Juliano *et al.* [6].

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
1.	Antioxidant activity	Hydroxyl radicals generated by Fenton reaction	1.65 μM 16.5 μM	No interaction with hydroxyl radicals No interference in Fenton reaction	Juliano et al. [6]
		Superoxide radicals generated by autooxidation of $FeCl_2$	10 μM	Did not scavenge superoxide radicals	
		DPPH assay	10,20,30,40,120,240 μM	Dose-dependent DPPH scavenging but weaker than activity. A-tocopherol dose-dependent reduced rate of peroxidation	
		AMVN initiated lipid peroxidation	50 & 100 μM	50 μM of γ- oryzanol more effeicient than 10 μM α-tocopherol	
		Conductometric evaluation of anti-oxidant activity in oils	2.5-20 mmol/kg oil	Progressive ↑ of AI values of all samples max. Effect at 10 mmol	
2.	Antihypercholeterolemic activity		p.o. 0.5%		Wilson et al. [7]
		Non-purified hypercholesterolemic diet induced lipid disorder (HCD-10% coconut oil +0.1% cholesterol for 2 weeks) in golden Syrian hamsters.	Plasma lipids	RBO↓plasma TC, LDL&VLDL(64% &70%); ORY(70%&77%); ferulic acid & ORY (13%-24%) RBO↑ HDL-C conc. (10%-20%) RBO(53%)&ORY(65%) diets↓ plasma TG conc.	
			Plasma vitamin E	No significant difference exhibited on plasma α &γ - tocopherol	
			Plasma lipid hydroperoxides	ORY(73%)↓ plasma lipid hydroperoxides by 46%)	
			Aortic cholesterol	RBO (73%) & ORY (46%) diet ↓ aortic cholesterol ester accumulation	
			Fecal neutral sterol content	ORY excreted significantly more coprostenol(127%) &(120%)	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro*/ *in vivo*)	Parameters of γ- oryzanol	Results	Reference
3.	Anti-diabetic activity	Wistar rats Streptozotocin & nicotineamide induced T2D	Streptozotocin – 45 mg/ kg + nicotineamide 200 mg/kg γ- ORY (5.25 gm)		Cheng *et al*. [8]
			Plasma insulin conc.	AUC for insulin was ↓ by ORY	
			Liver TG&TC	TG ↓ & TC ×	
			Fecal cholesterol		
			Weight gain & food intake	×	
			Plasma glucose	×	
			Insulin sensitivity		
			Plasma TG & NEFA conc.	ORY & PO ↓ TG conc. & NEFA ↓	
			Plasma cholesterol	ORY ↓ LDL-C conc. While ↑ HDL-C. TC/ HDL-C ratio ↓	
			Fecal neutral sterol& bile acid contents	Significant ↑	
4.	Effect on male gonads		33 mg/ mw p.o./ day for 30 days		Escobar *et al*. [9]
	Testicular degeneration induced by scrotenone al insulation in rams		Testicular steroidogenic activity	×	
			Sperm membrane integrity	The semen integrity was affected	
			Sperm morphology & motility	↑ in sperm motility	
			Semen	×	
			Oxidative stress	↓ in ROS by 26%	
			Serum testosterone	×	
			Testicular morphology	Morphology of testes was affected	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
5.	APAP induced liver injury				Shu *et al.* [10]
	Liver injury induced by acetaminophen		Cell viability and toxicity in HL7702 hepatocytes	↑ cell viability, ameliorated toxicity	
			Expression of Nrf2 i-NOS and COX_2	↑ Downregulated	
			Levels of proinflammatory factors – TNF-α, IL-1B, IL-6, NO	↓	
			Serum ALT, AST& LDH levels	↓	
			Activity of caspase −3, −8 and − 9	↓ dose dependent	
			BCl_2 and Bax protenone ein analysis	↓ ↑	
			Histopathology and immunohistochemistry.	ORY attenuated intra tissue hemorrhage & inflammatory cells infiltration	
				ORY modulated AMPK/GSK3β/Nrf2 and NFkB signaling pathways	
6.	Et-OH induced liver injury				Chotimarkorn and Ushio [11]
			Serum AST & ALT	↓	
			Hepatic lipid peroxidation	↓	
			TBARS	↓	
			Glutathione	↓	
			SOD	↑	
7.	CCl_4 induced liver injury				Gomes *et al.* [12]

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro*/ *in vivo*)	Parameters of γ- oryzanol	Results	Reference
			Hepatic function parameters	γ-ORY supplementation & treatment with silymarin stopped ↑ in AST, ALT, ALP, GGT and LDH activities & ↓ the levels of bilirubin	
			Oxidative parameters		
			TBARS	γ-ORY supplementation & treatment with silymarin ↓ lipid peroxidation	
			NPSH	γ-ORY supplementation & treatment with silymarin ↑ NPSH levels	
			AA	Treatment with silymarin ↑ AA levels	
			Inflammatory parameters		
			TNF-α, IL-1β, IL-6, TGF- β1& IFN-γ levels.	γ-ORY supplementation & treatment with silymarin reversed the ↑ in TNF-α, IL-1β, IL-6, TGF- β1& IFN-γ levels	
			MCP- 1	↓ by treatment with silymarin	
			MPO activity	↓ by γ-ORY supplementation and treatment with silymarin	
			NO levels	×	
			Apoptopic parameters	Caspase 3 and 9 activity ↓ **γ**- ORY supplementation and treatment with silymarin	
9.	Effect on hypoadiponectinemia		Serum adiponectin levels	↑ adiponectin levels	Nagasaka *et al.* [13]
10.	Effect on Immune system				Fujimoto *et al.* [14]
			Release of β-hexoaminidase by anti-DNP IgE sensitized RBL-2H3 cells post DNP-HSA stimulation.	↓ in dose dependent manner	
			PCA reaction test and the IgE detection test by ELISA	× mast cell degranulation	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
			Electrophoretic mobility	×	
			NFκB activity	CAF↓	
11.	Anti-inflammatory activity				Rao *et al.* [15]
			Reactive oxygen species	RBO-N generated lower levels of O2 and NO by 51 and 45%	
			Eicosanoids	**γ** –ORY ↓ secretion of pro-inflammatory eicosanoids by macrophages	
			Cytokines	↓ secretion of pro-inflammatory ↑ secretion of anti-inflammatory	
			Lysosomal enzymes	RBO fed group secreted lower levels of collagenase, elastase and hyaluronidase by 42, 43 and 55%	
12.	Anti-parkinsonism activity				Araujo *et al*., [16]
			Dopamine conc. by HPLC	↑ by ORY	
			Survival rate	↑	
			Locomotor	ORY abolished locomotor deficit caused by rotenone	
			AchE activity	Effect of rotenone on AchE activity was overcome by ORY	
			Cell viability	↓ by ORY	
			Mitochondrial viability	↓ by ORY	
			Resazurin reduction assay	↑	
			Antioxidant defenses MDA, SOD, CAT & GST	↓ inhibition of MDA, SOD, CAT & GST.	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro*/ *in vivo*)	Parameters of γ- oryzanol	Results	Reference
13.	Anti-anxiety activity				Akter *et al*. [17]
			Food intake and body weight	↓ in food intake and blocked the stress-induced reduction of body weight gain.	
			CRST (OFT)	γ- ORY ↓anxiety like behavior	
			CRST (EPM)	γ- ORY showed ↓ in anxiety	
			Serum corticosterone	×	
			BMA	Examination of amygdala, hippocampus and cerebral cortex showed that γ- ORY has anxiolytic effect	
14.	Anti-Alzheimer activity				Jha and Panchal [18]
			Alamar blue assay	↑cell survival at 100 nM, 1 μM, 100 μM	
			Total arm entry	ORY ↑ total arm entry	
			Correct arm entry	ORY ↑ correct arm entry	
			Reference memory error	↓ %RME by ORY	
			Memory score	↑ with DONO & ORY	
			MDA	↓ by ORY	
			GSH	↑ by ORY	
			CAT	↑ by ORY	
			BMA	ORY ↑ BMA	
			Brain AchE	↓ AchE activity	
		CRP		DONO & ORY ↓ CRP	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
15.	Anti-obesity activity		0.5 w/w		Francisqueti *et al*. [19]
			Caloric Ingestion	×	
			Inflammatory parameters	↓	
			Renal function parameters	γ- ORY restored renal function in HSF/ HSF + γ- ORY group	
			Redox state parameters	γ-ORY ↑ hydrophilic antioxidant protection, catalase, and superoxide dismutase levels in HSF/ HSF + γ- ORY group	
			Plasma adiponectin levels	↓	
			Protein expression	γ- ORY ↑ expression of Adipo-R2 and PPAR-α	
16.	Wound healing				Aldalaen *et al.* [20]
			Re-epithelization	Early epithelization by day 7	
			Histopathology	Fibroblasts, slight neo-angiogenesis, new capillaries and collagen formation on 5th day	
			Wound diameter	Rapid onset 50% reduction of wound diameters was between 7 and 10 days Complete closure of the wound was achieved after 14 days	

Table 1.
Schematic representation of therapeutic potential of γ- oryzanol.

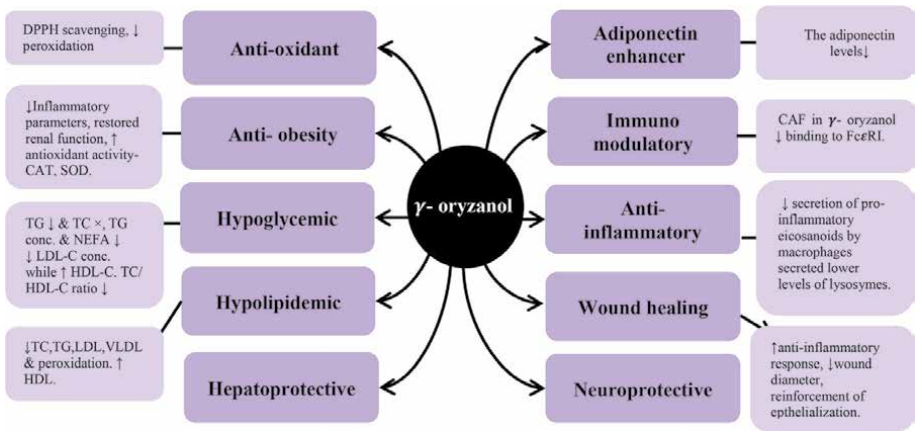

Figure 2.
Effect of γ- oryzanol on diseases.

Gamma oryzanol was unable to react with hydroxyl radicals it was therefore unable to interrupt the reaction with pnitrosodimethylaniline (PNDA). The rate of reduction of nitroblue tetrazolium (NBT) was unaffected by γ— oryzanol addition. It revealed that in the experimental conditions the compound was unable to scavenge superoxide radicals. Although weaker than alpha tocopherol, γ - oryzanol demonstrated dose dependent DPPH scavenging activity. Whether γ- oryzanol was pre-existing in the suspension or added exogenously as ethanolic solution, it was ineffective in inhibiting lipid peroxidation. In the light of the above findings, it was confirmed that γ-oryzanol is unable to compartmentalize into liposomes. During the evaluation avocado and castor oils were found to be resistant to heat induced lipid peroxidation. Particularly, castor oil proved to be impossible to evaluate in terms of antioxidant activity. In contrast rosa mosqueta oil and grape seed oil were very sensitive to oxidation. The remaining oils displayed average sensitivity to lipid peroxidation. An increase in γ- oryzanol concentration showed an increase in AI values of all the samples. AI is the antioxidative index calculated by ITs induction period of oil with the addition of antioxidant and ITo induction period of oil alone.

Free radical scavenging action of γ- oryzanol as well as its preventative nature against lipoperoxidation offer it as a viable contender for natural use as an antioxidant. γ- oryzanol offers a dose-dependent increase in induction time (of maximum capacity) while simultaneously lending protection from lipid peroxidation brought about by means of heat and O_2 exposition. This particular trait was most notable in cases of oils rich with polyunsaturated fatty acids (Rosa mosqueta, linoleic acid, grape seed oil).

Although its individual use as an antioxidant proved to be unimpressive, the use of another natural antioxidant along with γ- oryzanol can lead to enhancement of antioxidant property. The array of benefits offered in terms of pharmaceutical, cosmetic and food use "of rice bran oil which is rich in Gamma-oryzanol" suggest that γ- oryzanol can be studied further as an antioxidant component in complex lipophilic formulations such as ointments/emulsions or as an excipient for topical use.

3.2 Anti-hypercholesteremic activity

Multiple studies conducted among human beings and animals have shown that oils which constitute saturated fatty acids raise serum total cholesterol (TC) levels as well as low density lipoprotein levels. Wilson *et al*. conducted a study in Golden

Syrian Hamsters by feeding a non-purified hypercholesterolemia diet which comprised of 10% coconut oil and 0.1% cholesterol for a duration of 2 weeks, and separated into 4 groups of 12 in accordance to plasma cholesterol concentrations [7]. Blood samples were withdrawn at the 2 and 8 week marks from food deprived hamsters. At the 10 week mark (time of sacrifice) the aortic tissue was collected by administration of anesthesia. The fecal samples, were obtained towards the last 3 days of their exposure. Following procurement, the fecal samples were freeze dried and grinded prior to observation/analysis.

All the hamsters survived the complete course of the experiment. Hamsters that were fed rice bran oil (RBO) γ - oryzanol diets and hamsters that were fed coconut oil, ferulic acid diets displayed no discernable difference in terms of plasma triglyceride (TG) plasma γ tocopherol and alpha tocopherol concentrations. Although insignificant, hamsters administered RBO diet exhibited higher plasma lipid hydroperoxides (LPH) concentrations in contrast to hamsters administered ferulic acid and γ - oryzanol diets. In spite of having increased vitamin E concentrations and laminating high levels of cholesterol from their body through feces, the Coconut oil fed hamsters were found to have higher levels of aortic TC and free cholesterol in contrast to hamsters fed RBO, Ferulic acid and γ- oryzanol diets. The ratio of aortic free cholesterol to the ratio of esters were higher in RBO fed hamsters in contrast to hamsters fed the coconut oil and ferulic acid diets. RBOs contain multiple components, first being plant sterols and γ- oryzanol (unsaponifiable component) which contribute greatly to cholesterol lowering. The other component of RBOs i.e., tocotrienols assist in inhibition of cholesterol synthesis.

It was observed that both γ- oryzanol and ferulic acid both in concentrations of 0.5% each, lower plasma total cholesterol and non-high-density lipoprotein cholesterol (HDL-C) when compared to control hamsters. Increased excretion of cholesterol and its metabolic products could be the major mechanism utilized by RBOs in lowering of blood cholesterol levels. Although hamsters fed RBO, γ- oryzanol and ferulic acid visibly lowered cholesterol accumulation, of the 3, RBO and γ- oryzanol displayed a more significant decrease in ester accumulation in comparison with control. The experiment and its observations imply that at uniform dietary levels γ-oryzanol has better impact on both increase of plasma HDL-C and decrease of plasma non HDL-C in when compared to ferulic acid.

3.3 Anti-diabetic activity

An individual with type 2 Diabetes mellitus is likely to experience an increased rate of mortality as the result of cardiovascular diseases. Studies conducted at random within controlled environment have suggested that lipid lowering substances significantly reduce risk of cardiovascular diseases.

γ- oryzanol induces hypolipidemic action as well as influences reduction of aortic fatty streak formation. Palm oil was observed to significantly reduce plasma cholesterol as well as trigger growth of aortic cholesterol in relation to coconut oil within hamsters. Palm oil also tends to reduce serum lipids within healthy individuals as well as oxidative stress in rats.

The study conducted by Cheng *et al.* evaluated the impact of an effective component in RBO and $\gamma-$ oryzanol on insulin resistance and lipid metabolism within rats induced with type 2 Diabetes and treated with palm oil [8]. Diabetes was induced in Wistar rats by means of intraperitoneal injection consisting of streptozotocin, 15 minutes followed by another injection of nicotinamide. The rats are divided in three groups of 8, first group being the control, the second, Palm oil group (PO) and third group was treated with Palm Oil and γ- oryzanol (POO). After administration of diet for 5 weeks the diabetic rats were withheld from

consumption of food overnight (12 hours) and anesthetized by ether. The rats were then sacrificed by exsanguination from abdominal aorta. The plasma was then isolated by means of collection and centrifugation of blood. Plasma glucose level, triglycerides, HDL-C, LDL-C, non-esterified fatty acid (NEFA) concentration were evaluated by spectrophotometric means.

The diet had no impact on weight gain and neither did it display any side effects (diarrhea/death). No rats were dead as a result of T2DM induced by means of injection. The LDL-C concentration increased in PO groups instead of control. LDL-C in POO groups decreased when compared to PO groups. HDL-C increased in POO groups more than that of PO groups. Total cholesterol TC/HDL-C ratio was lower in POO groups than the other groups. Triglyceride concentrations were observed to increase in PO groups whereas TG concentrations decreased in POO group compared to PO group. At the end of week 5, fecal neutral sterol and bile acid content was notably higher in POO groups in contrast to control and PO groups.

The results gathered imply that PO could impair lipid metabolism in T2DM rats and γ - oryzanol a predominant component of RBO stabilizes irregular lipid status. Animals treated with $\gamma-$ oryzanol also displayed a 25% reduction in cholesterol absorption in comparison to control group. Secretion of acid and neutral sterols was notably increased in RBO administered animals. The AUC value of insulin in POO group observed prominent reduction compared to PO group. The result dictates that γ - oryzanol has tendency to increase sensitivity towards insulin in T2DM rats.

Increase of TG in plasma and liver increases output of glucose while decreasing clearance of insulin thereby promoting gluconeogenesis ultimately resulting in hyperinsulinemia and insulin resistance. Hypotriglyceridemic effect of γ- oryzanol positively impacted insulin resistance in T2DM rats. In summary, the plasma LDL-C, TG and hepatic TG all showed a decrease in concentration. The AUCs for glucose and insulin decreased in minimal concentrations within rats. Addition of γ-oryzanol to PO group minimized the negative impact of PO on lipid metabolism within T2DM rats.

3.4 Effect on male gonads

Testicular degeneration is a condition prevalent in males of domestic species; it is characterized by reduced fertility as a result of many animals being withheld in unfavorable atmospheric conditions. An increase in temperature levels promotes testicular cellular metabolism which in turn is not met with an increase in oxygen levels, thereby resulting in tissue hypoxia.

Escobar *et al.* conducted an experiment consisting of 8 rams with an average age of 10 months and weight of 35 kg bound in a surrounding with a mean temperature of 26°*C* to attain insulation [9]. The animals were administered a 10% solution of γ-oryzanol within soybean oil. The animals were divided into two groups. The first being control group that was only administered soybean oil (33 mg/ body weight) orally per day for a month. The second or the test group was administered 10% solution of γ - oryzanol in soy bean oil orally. Semen samples were collected weekly by an electroejaculator for 11 weeks and analyzed.

In case of testicular consistency and plasma levels of testosterone, there was no apparent difference between the two treatment groups. After the completion of experimental phase the animals were orchidectomized and samples were utilized to evaluate oxidative stress. The test group was observed to have a significant decline in reactive oxygen species (ROS) levels within their testes (by about 26%) when compared to the control group. In general, between week 5 and week 11, more defects were identified within the sperm of the test group as opposed to the control

group. In case of sperm motility, the largest difference was observed in week 1, with the test group displaying increased motility.

It was observed that during week 2, the test group displayed a decrease in lipid peroxidation (TBARS) levels whereas control group displayed an increase. Simultaneously there was a decrease in total anti-oxidant potential (FRAP) levels in the control group. The group receiving γ - oryzanol experienced a decrease in TRAP levels and an increase in the ROS levels in weeks 3 and 9. During weeks 10 and 11, there was an increase in FRAP and TBARS levels respectively in both groups.

The study did help in making the effects of heat stress on the testes and semen of the rams evident as well as reported changes that occurred throughout the duration of the experiment. Though partial protection within oxidative parameters of semen and testes were achieved by administration of γ- oryzanol, the experiment did not assist in improving the negative impact of heat stress among the other parameters. In fact, the administration of γ- oryzanol resulted in an increase in morphological abnormalities in ram on the whole.

3.5 Hepatoprotective activity

3.5.1 Acetaminophen induced hepatic injury

Liver injury because of drug abuse is termed as hepatotoxicity. Acetaminophen (APAP) which is used as an anti-pyretic as well as an analgesic when overdosed can cause acute liver injury, furthermore can lead to liver failure. Natural compounds extracted from food substances such as rice bran oil used as a source of γ- oryzanol are utilized for treatment of autonomic dysfunction and menopause syndrome. γ - oryzanol is shown to have modulatory effects on metabolic syndrome, while inhibiting oxidative stress and delaying cell aging (senescence).

Shu *et al.* performed experiment in male Kunming mice, aged 6-8 weeks. For assessment of hepatoprotective activity, 40 mice divided into 4 groups of 10 each [21]. First group served as normal, while the second received 300 mg/kg of APAP intraperitoneally, the third group was administered the same dose of APAP combined with 7 mg/kgγ -Oryzanol orally daily for a week, lastly the fourth group was administered the same dose of APAP with twice the dose of γ -oryzanol given in the third group.

γ- Oryzanol showed an undetectable cytotoxic effect on HL-7702. The viability of HL-7702 cells was decreased by APAP. Oryzanol was able to inhibit activation of Caspase-3 by APAP which leads to cell apoptosis. The intracellular accumulation of ROS plays an important role in APAP hepatotoxicity. Oryzanol decreased ROS levels in HL-7702 cells and indicated that oryzanol is capable of reversing APAP induced hepatotoxicity. Nrf2 is a crucial part of signaling pathway in anti-oxidative effect. Oryzanol aided the nuclear translocation of Nrf2, increased mRNA levels and downstream protein levels of Nrf2 like H0-1, NQ01, GCL and GCLM. Key upstream signals AMPK and GSK3B regulate Nrf2 activity, oryzanol upregulated the phosphorylation of both AMPK and GSK3B.

AMPK phosphorylation is one of the essential preceding steps in the nuclear translocation of Nrf2 and AMPK depends on phosphorylation of its substrate GSK3B. To confirm the action through AMPK/GSK3B, the test drug was challenged with the inhibitor of AMPK by compound Compund C (CC). It was observed that CC revoked oryzanol mediated phosphorylation of GSK3B eventually, obstructing the transcription of Nrf2 responsive gene. As a net effect, CC abolished the protective effect of oryzanol in APAP model. This established the fact that activation of AMPK accounts for oryzanol mediated upregulation of Nrf2 in its hepatoprotective action.

Histoarchitecture of liver remained unchanged after treatment with γ- oryzanol. AMPK/GSK3B/Nrf2 cascade can be activated by γ- oryzanol without hepatotoxicity. The liver index and serum levels of ALT, AST and LDH increased due to APAP treatment. γ- Oryzanol was able to reduce these parameters on pretreatment. APAP led to loss of hepatocyte architecture, intra-tissue hemorrhage and infiltration of inflammatory cells which were prevented by γ- oryzanol.

The number of apoptotic cells in liver increased when exposed to APAP in TUNEL and Hoechst 33258 staining assay and these were reversed by γ- oryzanol preadministration. The paracetamol intoxication increased hepatic activities of Caspase −3, −8 and − 9. A dose dependent decrease in caspases was observed with the treatment of γ - oryzanol in mice liver of APAP. Bcl-2 is an anti-apoptotic protein while Bax is a pro-apoptotic protein. Acetaminophen treatment leads to upregulation of Bax levels and downregulation of Bcl-2 levels. The effect on Bax and Bcl-2 levels was inverted by γ - oryzanol.

Exposure of liver to APAP led to increase in MDA and decrease of GSH, total superoxide dismutase (T-SOD), and total antioxidant capacity (T-AOC). These were enhanced by γ- oryzanol. Intrahepatic inflammation is a significant part of hepatotoxicity of APAP. Intrahepatic inflammatory contents- TNF-α, IL-1β, IL-6, and NO significantly increased by APAP. The inflammatory markers were restricted by γ - oryzanol. Acetaminophen increased nuclear translocation of p65 of NFκB in the liver. COX-2 and iNOS levels increased after paracetamol intoxication which in turn were suppressed by γ - oryzanol.

3.5.2 Ethanol induced liver toxicity

Ethanol consumption leads to liver injury by inducing hepatotoxicity, oxidative stress and a decrease in antioxidant levels. A therapeutic approach for treating ethanol induced hepatotoxicity is fairly sought after since the liver is among the most essential organs for metabolism of chemical compounds to obtain energy, as well as for detoxification. Trans-ferulic acid and γ - oryzanol exhibit certain physiological activities such as inhibition of tumor promotion, reduction of serum cholesterol levels, as well as antioxidant properties in several models.

Chotimarkorn and Ushio conducted a study to evaluate the effect of γ- oryzanol on ethanol induced liver injury in male C57BL mice. The investigation was carried out by administering γ- oryzanol in ethanol at the dose of 5.0 g/kg, p.o. for 30 days [11]. The experiment consisted of six groups, each group containing 15 mice. Group 1 served as a normal control and received distilled water (5.0 g/kg); group 2, negative control received ethanol (5.0 g/kg); test groups 3 and 4 were treated with trans-ferulic acid and γ- oryzanol respectively at the concentration of 0.025 mmol with ethanol (5.0 g/kg). The positive control groups 5 and 6 received trans-ferulic acid and γ- oryzanol respectively at the dose of 0.025 mmol alone. At the end of the treatment period animals were sacrificed, livers were removed and homogenated for the estimation of AST, ALT, GSH, protein, SOD, TBARS and lipid hydroperoxide by fluorescent imaging. Coadministration of trans-ferulic acid or γ- oryzanol with ethanol exhibited potent inhibition of ethanol stimulated lipid peroxidation or oxidative stress in liver. High increase in 3- PeDPPO in ethanol treated C57BL mice liver reflected high levels of lipid peroxidation. Low intensities of 3-PeDPPO was observed in γ - oryzanol treated group indicating low levels of lipid peroxidation. A significant decrease in lipid peroxide level in hepatic tissue of ferulic acid or γ- oryzanol treated mice was observed. Similarly, a significant decrease in TBARS level was seen. This demonstrated antioxidant effect of γ- oryzanol. However, the mechanism is unclear.

Gamma oryzanol or trans-ferulic acid maintain GSH levels. The co-administration significantly rose levels of GSH and SOD activity. A similar increase in SOD activity in macrophage cell line RAW 264.7 cells is reported [22]. Abnormally high level of serum aspartate and alanine transaminases in ethanol treated mice was reduced by trans-ferulic acid and γ- oryzanol. In the earlier studies, γ-oryzanol has exhibited antioxidant properties in *in-vitro* model systems namely – in cholesterol oxidation by 2,2′-azobis 2-methylpropionamidine, porcine retinal homogenate oxidation accelerated by ferric ion, pyrogallol autooxidation and pharmaceutical oils [12, 23–25]. In short, γ- oryzanol showed high hepatoprotective effect by preserving the livers from chemically induced injury.

Administration of daily dose of ethanol to mice resulted in visible increase in serum enzymes AST and ALT with reference to normal control, trans-ferulic acid, γ - oryzanol, co-administration of trans ferulic acid and γ - oryzanol with ethanol for 30 days. Co-administration of Trans-ferulic acid/γ - oryzanol to mice with ethanol for 30 days showed potent inhibition of ethanol stimulated lipid peroxidation and oxidative stress in the liver.

Trans-ferulic acid and γ - oryzanol reduced AST and ALT activities of ethanol. The observed significant decrease in the activity of these enzymes suggests that trans-ferulic acid and γ - oryzanol protects against liver injury resulting from the toxic effect of daily dose of ethanol. Furthermore, Trans-ferulic acid and γ - oryzanol treatment improved the antioxidative response of the liver defense system. Mechanisms for activation or induction of SOD were investigated. The study demonstrated that oral administration of trans-ferulic and γ - oryzanol exerted a protective action on liver injury induced by chronic ethanol ingestion.

3.5.3 CCl_4 induced liver damage

An organism when exposed to chemical agents or undergoes an infection initiates a process of hepatic fibrosis which ultimately leads to chronic liver damage. Carbon tetrachloride is utilized as a hepatotoxin to induce hepatic fibrosis in rodents. Gomes *et al*. conducted a study in adult male Swiss mice [12]. Twenty-four hours after administration of CCl_4 animals were anesthetized by sodium phenobarbital. Blood was collected and assessed for markers of hepatic damage. Liver sections were then numerically graded to assess histological features for degree of acute hepatic injury. Enzyme activities and bilirubin levels in plasma were measured. Caspase 3 and Caspase 9 activities were measured using a Caspase Glo- assay kit. Bradford method was employed to measure protein concentration using Bovine serum albumin as a standard. The data of experimental and control groups were compared.

Liver tissue from mice exposed to CCl_4 compared to the control group revealed extensive injury, vascular congestion and hepatic fibrosis. γ- oryzanol supplementation reduced hepatic fibrosis and reduced degree of liver damage (injuries). No histological changes were noted. Results revealed that CCl_4 exposure increased liver peroxidation. Two-way ANOVA of caspase 3 and caspase 9 revealed a significant interaction with the treatment of γ - oryzanol and silymarin.

Hepatic fibrosis induced by means of CCl_4 is brought about by oxidative, inflammatory and apoptopoic alterations. γ- oryzanol supplementation led to intermittent suppression of pathological alterations in the model of hepatic fibrosis. Damage caused in the liver by CCl_4 was principally shown as hepatic fibrosis brought about by single administration. The findings indicated that γ- oryzanol prevented hepatotoxicity and consequently protected against CCl_4 induced hepatic fibrosis. Lipid peroxidation is one of the primary causes of CCl_4 induced hepatic fibrosis. The findings reinforced antioxidant potential of γ- oryzanol

supplementation. It is implied that one of the mechanisms involved in the hepatoprotective effect of γ- oryzanol is the regulation of oxidative stress. Anti-inflammatory compounds have the potential to serve as therapeutic agents for various diseases, including, but not limited to, hepatic fibrosis. In the study conducted, it was observed that hepatic inflammatory reactions induced by CCl_4 could be suppressed by γ- oryzanol. The study demonstrated that CCl_4 administration produced a worsening effect by caspases 3 and 9 in the liver of mice, indicating that the apoptopic process had intensified. Several studies have shown that hepatic fibrosis induced by CCl_4 can lead to apoptopic pathway *in-vitro* and *in-vivo* [26, 27].

The author suggested that γ - oryzanol supplementation prevents CCl_4 induced hepatic fibrosis by modulating activity of caspases and that antioxidant action of γ-oryzanol may be at least partially associated with normalization of apoptopic process. Study demonstrates that γ- oryzanol supplementation was able to prevent CCl_4 induced hepatic fibrosis in mice by preventing oxidative, inflammatory and apoptopoic modifications.

The hepatoprotective role of γ- oryzanol in various models of liver injury are shown in **Figure 3**.

3.6 Effect on hypoadiponectinemia

The decrease in plasma adiponectin level is integrally involved in the development of insulin resistance and the resulting type 2 diabetes. In adipocyte and myotube model via nuclear factor kB (NF-kB) transcription factor pathways, palmitate, a major fatty acid observed in meat fat such as beef tallow, was known to cause insulin resistance [10].

In a study conducted by Nagasaka *et al.* (2011), serum adiponectin levels in mice were reduced from 48 to 96 h by oral administration of Beef Tallow and palmitate to approximately half the initial levels. Then γ-oryzanol, a major bioactive ingredient in rice bran, was administered and its effects on hypoadiponectinemia were examined [13].

Single dose of 0.5 ml beef tallow (beef tallow group, n = 5), 0.5 ml maize oil (control group, n = 5) or palmitate (0.17 mg/ml maize oil, n = 5) were administered orally to C57BL6j male mice aged 7 weeks and weighing between 18 and 21 g. Separately, 0.5 ml of beef tallow and maize oil containing 0.025 mmol oryzanol were also given. Blood samples were obtained from the caudal vein from 0 to 120 h per 24 h after administration. In order to estimate the total amount of adiponectin monomer secreted from Adipocyte, serum adiponectin levels were estimated. The

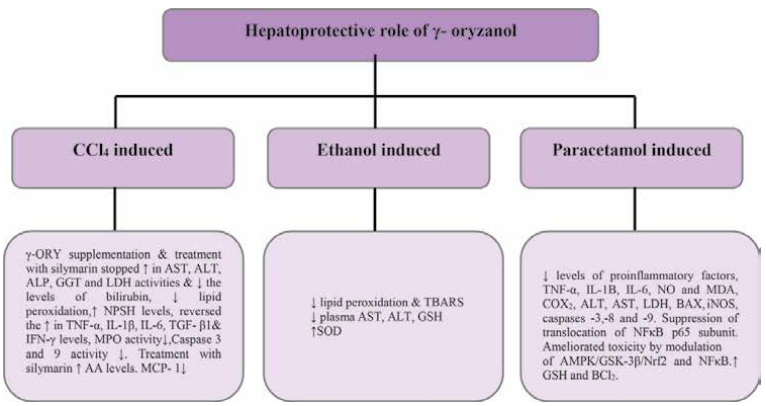

Figure 3.
Hepatoprotective role of γ- oryzanol in various models of liver injury.

immunoreactivity was observed and digitally acquired using an Odyssey Infrared imaging system. Signal intensity for adiponectin was evaluated by Image-J.

In the beef tallow group, the level of adiponectin was significantly suppressed from 48 to 72 h similarly palmitate significantly suppressed serum adiponectin levels from 48 to 96 h after administration. The administration of oryzanol dissolved in corn oil increased adiponectin level from 48 to 72 h gradually and then got the level back almost to the initial level at 120 h. Oryzanol supplementation to beef tallow increased significantly adiponectin levels at 96 h compared to the control group and successfully recovered the hypoadiponectinemia induced by the beef tallow administration. Adiponectin levels at 120 h reverted almost to the initial level, suggesting that effects of the single doses of beef tallow and oryzanol should disappear within 120 h probably due to metabolism or secretion. The rise in the secretion of adiponectin is considered a priority for the development of drugs and the treatment of metabolic diseases associated with obesity.

3.7 Impact on immune system

Fujimoto *et al.* conducted a study on cycloartenyl ferulate (CAF), a major component of rice bran derived γ -oryzanol as an anti-allergic agent in passive cutaneous anaphylaxis reaction and mast cell degranulation and its effect on IgE [14]. Gamma oryzanol was extracted from domestic Japanese rice using flash chromatography on silica gel. Passive cutaneous anaphylaxis (PCA) reaction carried out as the allergic model in Sprague Dawley rats was used in the study. Mast cell degranulation was estimated by the release of β- hexosaminidase. Light and heavy chains of anti-DNP or anti-TNP were identified by using SDS polyacrylamide gel electrophoresis. The concentrations of the compounds are given in **Table 2**.

The major component of *γ*- oryzanol was discovered to be CAF, having >90% compounds affiliated to CAF. Anti-allergic reaction of *γ*- oryzanol and CAF were found to be alike. The effect of CAF on RBL-2H3 mast cell degranulation was studied to verify that the effect of CAF on PCA reaction. Following DNP-HSA stimulation, anti-DNP IgE sensitized RBL-2H3 cells generated *β*- hexosaminidase. Anti-DNP IgE incubated with CAF was added to RBL-2H3 cells. The degranulation triggered by successive stimulation of DNP-HSA was inhibited in a concentration-dependent manner by CAF. The effect of 24-methylene cycloartanyl ferulate, cyclobranyl ferulate, and *β*- sitosteryl ferulate on mast cell degranulation on affiliation to CAF were studied by purifying the compounds. The results showed that, Cyclobranyl ferulate was more potent that cycloartanyl ferulate in inhibiting degranulation while 24 – methylene cycloartanyl ferulate and *β*-sitosteryl ferulate were found to be less effective.

It was found that CAF significantly inhibited mast cell degranulation. Binding of IgE to mast cells led to the failure of CAF to inhibit the degranulation. The researchers also found that CAF failed to inhibit degranulation once IgE binds

Compound	Concentrations
Cycloartenyl ferulate	28.2%
24 – methylene cycloartanyl ferulate	22.4%
Campesteryl ferulate	17.8%
β- sitosteryl ferulate	12.3%
Cyclobranyl ferulate	<1%

Table 2.
The predominant ferulates present in γ- oryzanol.

to mast cells. This is suggestive of some effect of CAF on the ability of IgE bind with FcϵRI.

The concentration of IgE were measured by ELISA. Anti- TNP IgE on incubation for an hour with γ- oryzanol or CAF decreased IgE concentration in a dose dependent manner. The effect was also found to be dependent on incubation time. It was also observed that ELISA failed to detect IgE when incubated with CAF.

To confirm whether CAF acted by sequestration of IgE from anti-IgE antibody or IgE configuration change SDS-PAGE analysis was performed. The amount of IgE in the supernatants decreased when IgE was incubated with CAF suggesting CAF's sequestering role on IgE which makes it undetectable in ELISA. The study demonstrated that CAF found in γ - oryzanol encapsulates IgE and prevents it from binding to FcϵRI. Thereby, attenuating allergic reaction. The report of Nagasaka et al. supports the immune response of CAF as they found this molecule inhibits NFκb activity preventing the late delayed phase of allergic inflammation [22].

3.8 Anti-inflammatory activity

Serum lipid levels and pro-inflammatory mediators which are prime factors for cardiovascular diseases are greatly influenced by dietary oils. The study of Rao *et al.* investigated the effect of minor constituent of rice bran oil (RBO), γ- oryzanol on secretion of pro-inflammatory mediators by peritoneal macrophages of male Wistar rats [28]. 2 mL of fresh medium Roswell Park Memorial Institute (RPMI)-1640 was added to the macrophages and incubated with LPS. ELISA was used to study the cytokines.

The macrophages from the rats that were fed a diet with rice bran oil with unsaponifiable fraction (RBO-N) gave rise to lower levels of superoxide anion (51%) and nitric oxide (45%) compared to groundnut oil containing unsaponifiable fraction (GNO-N). However, the macrophages from rats fed rice bran oil with minor constituents removed (RBO –MCR) exhibited lower levels of superoxide anion (16%) and nitric oxide (8%) compared to GNO-N fed rats. This suggested that the extraction of unsaponifiable fraction from RBO had compromised potential to reduce the production of reactive oxygen species (ROS) by macrophages.

Lower levels of Prostaglandin E_2 (PGE_2), Thromboxane B_2 (TXB_2), Leukotriene B_4 (LTB_4) and Leukotriene C_4 (LTC_4), were secreted by macrophages from rats that were fed RBO-N diet compared to GNO-N diet fed rats. RBO-MCR diet fed group secreted lower levels of PGE_2 compared to macrophages from rats fed GNO-N. The secretion of TXB_2, LTB_4 and LTC_4 in RBO-MCR and GNO-N or GNO-MCR diet fed rats showed no remarkable differences. This deduced that the removal of unsaponifiables from RBO-N impacted its potential to effect the eicosanoid secretion by macrophages. On the contrary, rats fed RBO-N diet showed an enhanced secretion of 6-keto PGF_{1a} by 36% compared to rats given GNO-N diet.

A decrease in levels of pro-inflammatory cytokines like TNF-α (by 65%) and IL-6 (by 40%) was observed in the macrophages of rats fed with RBO diet in contrast to rats fed with GNO diet. TNF- α and IL-6 were secreted in lower levels by macrophages from rats that were fed an unsaponifiable removed RBO diet compared to rats fed with GNO diet. Pro-inflammatory response in hosts was also influenced by lysozyme enzymes secreted by macrophages. Lower levels of collagenase, elastase and hyaluronidase by 42%, 43% and 55% respectively were secreted by macrophages of rats fed with RBO diet compared to rats fed with GNO diet. GNO-MCR diet fed rats secreted collagenase, elastase and hyaluronidase in similar levels compared to GNO diet fed rats.

Pro-inflammatory compounds were secreted in lower levels by macrophages of rats fed RBO diet as compared to that observed from rats fed GNO. Reactive oxygen

species, lysosomal enzymes, eicosanoids, cytokines and matrix metalloproteases are over produced by macrophages when activation of NF-kB induces the pro-inflammatory signaling pathway [29]. The authors demonstrated how macrophages in rats fed RBO containing γ- oryzanol secrete less IL-1β in contrast to rats fed hydrogenated fat [30]. Expression of adiponectins was up regulated by RBO and down regulated expression of Toll like receptors (TLR-2 and TLR-4). The secretion of inflammatory compounds was lowered suggesting that the removal of unsaponifiables from RBO led to a decrease in the potential of RBO.

3.9 Neuroprotective role

The various neuropharmacological actions of **γ**-oryzanol are represented in **Figure 4.**

3.9.1 Anti-parkinsonian activity

Gamma oryzanol has shown potential in reduction of tumor growth and plasma cholesterol levels [31]. Ferulic acid a constituent of γ-oryzanol has protective function towards Alzheimer's, Parkinson's disease and stroke [32]. Parkinson's disease can be induced in Drosophila and rodents models using a chemical, rotenone [33]. Rotenoids consists of a toxic agent called rotenone [34]. Rotenone acts as an inhibitor with high affinity towards mitochondrial NADH dehydrogenase (complex I) [35]. It is suggested that dopaminergic cell death is caused by rotenone. It also causes increase in free radicals and oxidative stress in mitochondria [36].

Drosophila melanogaster is used as a genetic tool for studying biological problems because of its similarities with mammals. They have similar biological, physical and neurological properties and 75% of human disease-causing genes [37]. Several studies on neurodegenerative diseases used Drosophila as a model as it shares genetic similarity with humans in related to Parkinson's disease [38].

Araujo *et al*. performed a study using both genders of *Drosophila melanogaster* of age 1 to 5 days by dividing them into four groups of 50 flies each [16]. Control, oryzanol 25 μM, rotenone 500 μM, and oryzanol 25 μM + rotenone 500 μM. The groups were administered a diet containing rotenone and oryzanol for 7 days. Ethanol and sucrose were used as diluents with rotenone (500 μM) and oryzanol (25 μM) respectively. The dose of oryzanol was decided after conducting an experiment using different doses of oryzanol. Doses of 25 μM, 50 μM and 75 μM were

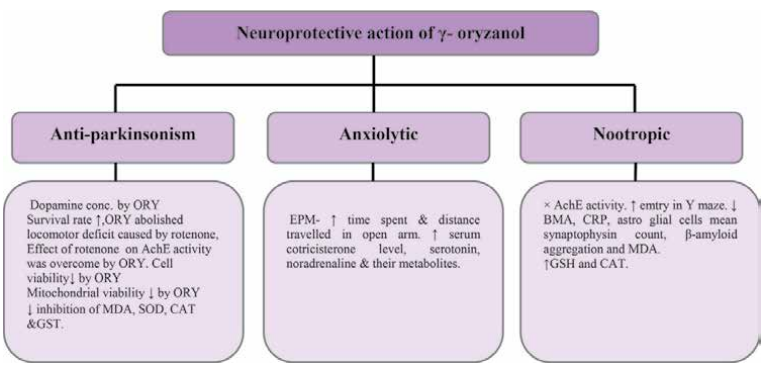

Figure 4.
Neuroprotective action of γ- oryzanol.

checked. After studying mortality and behavioral test negative geotaxia of flies, 25 μM concentration was deemed best for the experiment.

The flies were administered rotenone (500 μM) and oryzanol (25 μM). Two controls were taken one with 1% ethanol and 1% sucrose. Results presented control group with ethanol and sucrose because there was no statistical difference observed in all groups. The diet contained 1% agar w/v, 1% w/w milk powder, 0.08% w/v nipagin, 2% w/v sucrose and 1% yeast w/v beer. During the experimental period, the survival rate of the flies was estimated daily. The time taken to achieve 8 cm height from the base of the glass tube was measured to determine negative geotaxis [39]. Negative geotaxis behavior assay was used to evaluate locomotor activity of the flies [40]. Ice was used to induce anesthesia to 10 flies of both genders. The flies that were unable to climb above the mark were noted. The activity and movement of the fly were estimated by dividing 15 flies with one square cm distance in a covered petri dish [41].

A homogenate of flies was prepared which was later subjected to HPLC to analyze dopamine concentration. The cell viability was estimated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay and the resazurin reduction assay. ELISA (enzyme linked immunosorbent assay) was used for incubation of supernatant and fluorescence was noted. The flies were centrifuged to obtain mitochondria. Spectrophotometry was used with Ellmann reagent to evaluate Thiol protein and non-protein content [42]. Followed by evaluation of reactive oxygen species (ROS) and lipid peroxidation. Catalase activity was determined using method of Aebi [43]. Inhibition of quercetin auto oxidation was used to find the superoxide dismutase activity [44]. 1-chloro-2-4- dinitrobenzene (CDNB) was used according to Habig et al. procedure for the estimation of glutathione-s-transferase activity (GST) [45]. Bradford method was used to estimate protein concentration [46].

The mortality was lower in the group that was administered oryzanol, in contrast to the rotenone group. Hence, suggesting its potential to prevent rotenone induced mortality. An adverse effect was observed on the locomotor behavior on flies given rotenone, which was a decrease in climbing rate compared to the control. This was overcome by the treatment of oryzanol. In the open field test, there was a decrease in exploratory activity compared to control group, preventing a locomotor deficit. Dopamine levels in the head dropped by 42% in flies in the rotenone group compared to control group. Oryzanol prevented the drop in dopamine levels induced by rotenone in flies. Group exposed to rotenone showed MTT reduction and in cell viability observed in the fly homogenates, verifying the reduction in cell viability. Rotenone also led to a decrease in MTT reduction in mitochondria. Oryzanol reversed both the effects.

Resazurin reduction test was used to estimate cell viability. Toxicity of rotenone cell level was verified by decrease in cell. A marker of lipid peroxidation, Malondialdehyde (MDA) was estimated. MDA levels and rotenone induced DCFDA (2′,7′-dichlorodihydrofluorescein diacetate) oxidation was reduced by simultaneous exposure to oryzanol and rotenone. Oryzanol inhibited decrease Superoxide dismutase activity (SOD), catalase (CAT) and glutathione S-transferase (GST) caused by rotenone. The amount of protein thiols and non-protein thiols did not change and remained the same in all groups.

Motor function, dopamine levels and activity of enzyme acetylcholinesterase improved with the treatment of oryzanol. In addition, oryzanol strengthened anti-oxidant defenses, oxidative stress, mitochondrial dysfunction protecting from rotenone toxicity. The constituent of oryzanol, ferulic acid esters could be responsible for the neuroprotective role and its anti-oxidant ability. Ferulic acid demonstrated anti-oxidant activity in neuronal cell culture and arrested apoptosis in focal cerebral

ischemic injury showing neuroprotective action. Ferulic acid on long term administration in mice ameliorated memory deficits induced by centrally administered $\beta-$amyloid [47]. Decrease in expression of active caspase-3 in the rat striatum, increase in interlukin-1 an immunoreactive component, levels of endothelial nitric oxide synthase and 3- nitrotyrosine of mouse hippocampus can be mediated by feruilic acid. It also provides neuroprotection against striatal neuronal cells exposed to oxidized low-density lipoproteins [48]. Neuromotor deficits, geotaxia negative tests (climbing) and open field test (rating exploratory capacity) were successfully reversed by oryzanol.

Rotenone is used to induce symptoms of Parkinson's disease. As Parkinson's disease is related to mitochondrial dysfunction because of anomaly in complex I of electron transport chain, similar symptoms can be induced using rotenone [49]. A decrease in anti-oxidant and an increase in iron levels along with oxidative stress on dopaminergic neurons could be carried out by the inhibition of complex I. Dopamine metabolism involves synthesis, storages, release, reuptake and degradation of neurotransmitter [50]. The flies that were treated with rotenone showed a decrease in dopamine levels. Earlier studies verified that dopamine levels in flies reduce upon treatment with rotenone including loss of dopaminergic neurons in brain and reduction in vesicular monoamine transporter (VMAT) [51, 52]. Oryzanol displayed a neuroprotective effect by preventing the dopamine loss. Further, ferulic acid has shown neuroprotective effect via inhibition COX-2 enzyme, which in turn prevents the oxidation of dopamine and prevents accumulation of α- synuclein [53].

Neurodegeneration in Parkinson's disease is also induced by oxidative stress [54]. The increase in MDA, ROS levels and decrease in CAT, SOD and GST during the study confirms that exposure to rotenone causes oxidative stress. Oryzanol was able to reduce oxidative markers thus, confirming its anti-oxidant ability. Biological membranes can be protected from lipid peroxidation, peroxyl and alkoxyl radicals by ferulic acid [55].

Cell viability was reduced when exposed to rotenone and led to increase in mortality of flies. However, oryzanol treatment increased cell viability and reduced mortality. This suggests the presence of bioactive compounds in it that suppress free radicals and contribute to the anti-oxidant defense system. Finally, the study concluded that oryzanol prevented the toxicities caused by rotenone in *Drosophila melanogaster*. Thereby, confirming its neuroprotective role in Parkinson Disease.

3.9.2 Anti-anxiety

It is believed that chronic stress is correlated to structural degeneration and compromised brain function which could be the reason for increased risk of advancement of neuropsychiatric disorders like anxiety, depression and dementia. In an experiment conducted by Akter *et al*. five-week-old ICR male mice were put through restraint stress by a wire mesh bag (3x6x12) [17]. The mice were subjected to 1st phase chronic restraint treatment for 14 days followed by a recovery phase and second phase for another 5 days. Since, most studies employed 0.5% dose of γ - oryzanol and found it to be effective, 0.5% γ - oryzanol was administered to the mice.

Open field and elevated plus maze tests were used as behavioral tests. Mice were sacrificed at ZT 5.5 to 7.5 post 3 h CRST exposure and EPM test. Samples were drawn and serum was separated after centrifugation for 15 min at 3000 rpm and stored at $-80°C$. Brain matrix was used for excision of hippocampus, cerebral cortex and amygdala. Neuroanatomical landmarks from the brain atlas was used to dissect the brain. ELISA was used to determine levels of serum corticosterone.

The neurochemical mechanisms involved in anxiolytic like effects were studied by estimating the levels of centrally acting monoamine neurotransmitters such as noradrenaline and serotonin and their metabolites 5-hydroxyindole acetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), in the various dissected parts of the brain by HPLC-ECD following the behavioral tests.

GORZ treatment did not show any significant changes in the behavioral parameters in both the tests. Daily food intake sharply decreased in the stressed mice group during the early days of CRST. However, there was substantial recovery in the later period. As a result of CRST negative control group significantly lost body weight when compared to the normal control and test group. The treatment of 0.5% γ- oryzanol reduced such effects indicating that the γ- oryzanol treatment prevented the stress induced weight loss.

A mild reversal of CRST induced decrease in time spent in the central zone was observed by the treatment of γ- oryzanol. 0.5% oryzanol treatment significantly increased number of entries in the central zone in CRST while slightly increasing the total distance traveled in both the conditions. These are suggestive of the decreasing effect of γ- oryzanol on anxiety like behavior. In elevated plus maze test, γ- oryzanol significantly increased time spent in open arm and distance traveled in open arm under cold restrained stress. At the same time oryzanol treatment reduced the distance traveled in the closed arm. These observations indicate the anxiolytic effect of γ- oryzanol. Serum corticosterone levels rose significantly in control and γ-oryzanol treated animals in contrast with the unstressed control animals in CRST.

A mild reduction in the levels of neurotransmitters and their metabolites following CRST was observed in the hippocampus and cerebral cortex. CRST led to a decrease in serotonin and 5-HIAA levels with no significant change in amygdala. The slight decrease in serotonin and nor-adrenaline and their metabolites was restored by oryzanol in the hippocampus and cerebral cortex. Under CRST an increase in 5-HIAA, a metabolite of 5-HT was seen in amygdala of mice that received 0.5% of oryzanol. Similarly, an increase in a noradrenaline metabolite (MHPG) was noticed in oryzanol treated group without stress. These facts are implicative of anti-anxiety potential of γ- oryzanol in chronic stress conditions. CRST induces morphological alterations in BBB. It also remarkably decreased body weight (b.w.) by inhibiting food intake through the reduction of mRNA expression of food intake related genes like ghrelin, pro-opiomelanocortin in hypothalamus. Gamma oryzanol is indicated to be a weak modulator of stress response in hippocampus. Its anxiolytic effect could be related to the up regulation of centrally acting monoamines in amygdala. The anti-stress and anti-anxiety effects are unrelated to corticosterone activity. In gist, the observations of elevated plus maze test evidence the anti-anxiety effect of oryzanol. The locomotor activity was found to be unaffected.

3.9.3 Anti-Alzheimer

The neuroprotective and cognitive enhancement effect of γ- oryzanol in Alzheimer's disease was investigated by Jha and Panchal [18]. In the study, the researchers performed *in-vitro* DPPH assay, AchE enzyme activity inhibition assay, cell viability assay on SH-SY 5Y cell line and alamar blue assay. The nootropic activity was assessed using Y and radial arm maze. The brain corticular homogenate was subjected to estimation of biochemical markers such as catalase, glutathione, malondialdehyde, brain mitochondrial ATPase, brain acetylcholinesterase activity and C-reactive protein. The slices of brain were finally subjected to amyloid-β-plaque staining, immunohistochemistry and histopathology. The IC_{50} of γ- oryzanol was found to be 227.03 $\pm$ 17.24 μM in DPPH assay and 34.04 $\pm$ 3.20 μM in AchE

inhibitory assay. *γ*- oryzanol has shown a dose-dependent enzyme inhibition by preventing hydrolysis of ATCI from AchE *in-vitro*. *γ*- oryzanol treatment increased cell survival by 1.07-1.104 folds in the cell viability assay at concentrations 100 nM, 1 μM and 100 μM. In both Y and radial arm maze tests, *γ*- oryzanol raised entry in Y maze, exhibited significant increase in total arm entry and correct arm entry, reduced reference as well as working memory errors. Memory score improved with the treatment of Donepezil and *γ*- oryzanol treated animals. *γ*- oryzanol increased the level of brain mitochondrial ATPase (BMA), catalase (CAT) and glutathione (GSH) while significantly decreasing the malondialdehyde (MDA), acetylcholinesterase activity and C reactive proteins (CRP) thereby exhibiting free radical scavenging action in a dose dependent manner and was found to be more potent than ferulic acid.

The histopathological examination revealed regular morphological and cytological characteristics in normal control group and disorganized cellular and morphological architecture, presence of dead cells, loss of neuronal cells in CA_1, CA_2, CA_3 and DG regions, altered thickness (CA_1 and CA_2), aberrantly scattered CA_3 pyramidal cells, cell arrangement in granular cell layer (GCL), dentate gyrus ectal limb (DGEC), dentate gyrus endal limb (DGEN) and loss of neuronal cells in entorhinal cortex (ERC) in disease control group. There were no such observations in Donepezil and *γ*- oryzanol treated brains. Immunohistochemical analysis showed non-significant but marked reduction in Mean GFAP count, decrease in active astroglial cells and inflammation. *γ*- oryzanol group brain sections improved synaptic connectivity, indicated by increase in the mean synaptophysin count in CA_1, CA_2, CA_3, DG and ERC regions. This highlighted protective effect of *γ*- oryzanol in streptozotocin induce cerebral damage.

The qualitative and quantitative analysis of cortical area of brains observed as bright red fluorescence revealed higher mean amyloid-β count in disease control group which was lowered by both Donepezil and *γ*- oryzanol as it decreased plaque formation. The immunohistochemical parameters (GFAP and synaptophysin) expression and amyloid β-12 was inhibited by *γ*- oryzanol. The overall effects of *γ*-oryzanol were suppression of neuroinflammation and plaque formation (β-amyloid aggregation) while improving synaptic connectivity and neuronal energy catastrophe in cerebral region preventing neuronal loss. *γ* - oryzanol proved to be beneficial and therapeutic candidate in the experimental model of sporadic Alzheimer disease and demonstrated potent anti-oxidative, anti-inflammatory, cognitive enhancing and amyloidogenesis terminating effects. The researchers recommended further studies and exploration of *γ*- oryzanol use in the neurodegenerative disorder.

3.10 Anti-obesity potential

As *γ*- oryzanol proved to have the ability to treat hyperlipidemia, hyperglycemia, hypoadiponectinemia, etc., the nutraceutical was considered to be a good candidate for screening in obesity induced kidney injury by Francisqueti *et al*. [19].

Male Wistar were divided into 2 groups, control group and high sugar-fat diet (HSF) group after 20 weeks, the rats were treated with *γ*- oryzanol. Post treatment, the rats caloric intake, body weight and adiposity index were used to estimate their nutritional profile. Glucose concentration, triglycerides and adiponectin were estimated using glucometer, automatic enzyme analyzer system and enzyme-linked immunosorbent assay (ELISA) respectively. Plasma and urine were used to estimate the renal function. Amount of urea and creatinine in plasma was recorded along with the glomerular filtration rate (GFR). The renal tissue was homogenized and centrifuged. ELISA was used to measure tumor necrosis factor - alpha (TNF – *α*),

interlukin - 6 (IL-6) and monocyte chemoattractant protein - 1 (MCP–1) levels. Protein amount was used to verify the results.

The caloric intake showed no change and HSF showed higher values for all parameters. The renal function of the group that was given HSF and γ- oryzanol presented lower proteinuria and high GFR. Renal tissue of the group that was administered γ- oryzanol showed decrease in inflammatory response unlike the control group. During the study, the group given HSF diet developed obesity, insulin resistance, hypertension, chronic inflammation, dyslipidemia and oxidative stress.

Antioxidant defense is impaired in subjects suffering from renal insufficiency. γ-oryzanol treatment exhibited a rise in antioxidant capacity - superoxide dismutase (SOD) and catalase activity. Abnormal levels of adiponectin are seen in obesity, diabetes, chronic kidney disease, etc. The secretion of adiponectin by adipose tissue has a great impact on kidney disease. In contrast to reduced levels of adiponectin when it comes to obesity, chronic kidney disease shows rise in the same. Therefore, the higher levels of adiponectin related to GFR in the HSF fed group helped to deduce kidney disease. On the contrary, the HSF diet group treated with γ- oryzanol showed reduced adiponectin levels. The expression of PPAR – α increases in tissue with high mitochondrial and $\beta-$oxidation activity. Increased PPAR – α expression suggests metabolic control in an organ, and it controls several factors involved in renal damage [56, 57]. Thus, supporting the study to conclude that HSF diet with oryzanol showed no inflammation in kidney, as well as low levels of TNF-α, IL-6, and MCP-1.

3.11 Wound healing activity

γ – Oryzanol has both anti-inflammatory and antioxidant properties, which makes it a prospect for wound healing. However, due to the large molecular weight and water insolubility, it is incompatible for topical application. Penetration enhancers containing vesicles were formulated using used transcutol and labrosol in a study conducted by Aldalaen *et al.* [20]. Alpha – bisabolol (BISA) is a derivative of essential oil. BISA is an unsaturated sesquiterpene alcohol. Reports indicate that BISA is employed as a nutraceutical compound in treating wounds. Owing to its anti-inflammatory property, BISA was included as co-penetrator to boost the permeation ability of γ - oryzanol. Thus, increasing its antioxidant and anti-inflammatory properties.

The formulation was developed using the thin film hydration technique. γ-oryzanol (25 mg) and the phospholipid Epikuron (200 mg) were dissolved in a mixture of chloroform: methanol (2:1) v/v. This mixture was then subjected to vacuum evaporation at 40°*C* and 150 rpm. The lipidic film was then hydrated with 10 ml phosphate buffer (pH 7.4) comprising of BISA oil (10 μL) and penetration enhancers (transcutol and labrosol). The vesicular dispersion was treated with rotation for 30 mins at 40°*C* and sonication for 1 hr. Finally, storing it at 4°*C*.

The experiment was conducted in Wistar rats. Intraperitoneal (i.p) ketamine HCL (50 mg/kg) and xylazine HCL (20 mg/kg) were used as anesthetics. The animals were subjected to excision wound model. The posterior of the rats were shaved and cleaned with 70% ethanol. Two circular wounds were induced on the dorsal skin of the rats. A biopsy punch of 20 mm in diameter was utilized for the removal of the skin. The Wistar rats were divided into three groups. The wound on the upper side of the dorsal back was treated while the lower side was left untreated which was used as control. The PEV formulation with γ- oryzanol was given to group 1, the PEV formulation with γ -oryzanol and BISA was given to group 2, and group 3 was treated with commercial wound healing product (Healosol). Fifty μ L of each formulation was applied on the upper side of the wounds every day. The rats were photographed, and

the diameter of wounds were measured on the 3,5,7,10,14 and 21 days. The rats were subjected to euthanasia and the tissue formed was cut out leaving 5 mm of skin. The skin samples were then examined histopathological changes.

During the experiment it was observed that the wound treated with PEV formulation including γ -oryzanol and BISA exhibited a better anti-inflammatory response and wound healing capacity. This formulation could enhance the wound healing due to the pharmacological effects of both γ -oryzanol and BISA, and their antioxidant properties. Gamma oryzanol induced morphological abnormalities in the testes of ram indicating some form of reproductive toxicity. But this necessitates careful scrutiny of the effects of γ – oryzanol on testes and ovaries.

4. Conclusion

Among the noteworthy observations of γ – oryzanol were the acetylcholinesterase inhibitory action, decrease in brain mitochondrial ATPase, increase in mean synaptophysin count, and decrease in astroglial cell in an *in-vitro* Alzheimer study. Another significant discovery was AMPK/ GSK3β/Nrf2 and NFκB modulated hepatoprotection in APAP induced liver injury. γ – oryzanol promoted nuclear translocation of Nrf-2, increasing its expression modulated AMPK/GSK3β axis, suppressed nuclear translocation of NFκB p65 subunit, down regulating expression of iNOS and COX-2. The same experiment also proved limiting action on TNF-α, IL-Iβ, IL-6, nitric oxide. Though, above mechanisms have been established there are few bioactivities where the exact mechanisms of action are yet to be confirmed. Therefore, further exhaustive research is required to unveil the mechanisms and to explore the utility of γ – oryzanol in other disease states.

From the bulk of information related to the nutritional value and diverse therapeutic potentials of gamma-oryzanol, it is concluded that this optimistic molecule can be recognized as a nutraceutical and utilized in the management of various diseases. However, the beneficial role of gamma-oryzanol in certain conditions is not fully understood, necessitating further exhaustive studies to establish the mechanism of action.

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Chapter 13

Dietary Patterns for Immunity Support and Systemic Inflammation against Infections: A Narrative Review

Budhi Setiawan and Masfufatun Masfufatun

Abstract

Nutrition has been recognized to play a regulatory role in human immune response and inflammation which may affect the pathogenesis of diseases. Current evidence suggests that the habitual dietary pattern therapeutical approach provides more synergistic beneficial action than the intervention of a single nutrient constituent. Several healthy dietary patterns are essential for the human immunity support against infectious diseases through alleviation of systemic inflammation. Long-term dietary patterns may affect the diversity of intestinal microbiota composition and lead to the decrease of pro-inflammatory cytokines from immune-related cells. Protease that may cause gut barrier breakdown (leaky gut) can be reduced either thus lessen translocation of endogenous bacterial endotoxin such as lipopolysaccharides (LPS) from the gut lumen to the bloodstream. In this review, we discuss the relationship between common healthy food-based dietary patterns with the protection of infectious diseases as a result of improvement in immune function and low-grade inflammatory indices. In contrary to the deleterious impact of the western diet, healthy eating habits (Mediterranean diet, dietary approaches to stop hypertension, plant-based diet, ketogenic diet) are associated with reduced susceptibility to infectious disease by the improvement of certain underlying metabolic comorbidities. Further studies are needed to determine suitable strategic implications of healthy dietary patterns on infectious disease mitigation in a particular context.

Keywords: nutrition, bioactive, dietary pattern, immunity, inflammation, oxidative stress, antioxidant, infectious disease

1. Introduction

The benefit of dietary patterns has appeared as a complementary and alternative approach to the study of the relation of diet and the risk of diseases. In contrast to a single substance or nutrient approach, an evaluation of whole dietary patterns may provide a more complete picture of a combination of foods and nutrients, such as synergistic and antagonist properties of the foods [1]. People do not eat isolated nutrients and rather consume foods that contain a variety of foods with complex interactive combinations of nutrients. Thus, a single active substance approach might be insufficient for considering complex interactions between food bioactive

components in the human study such as vitamin C improves Fe absorption [2]. Often, the high degree of intercorrelation between nutrients (e.g. magnesium and potassium) can be difficult for the evaluation of their separate effects in particular conditions [3]. Additionally, the cumulative effects of several nutrients are more likely detectable compared to the influence of a single substance. In the clinical trial setting, the dietary patterns approach has shown a positive health outcome in degenerative diseases [4]. Nutritional approach as dietary pattern intervention (e.g. Dietary Approaches to Stop Hypertension and Mediterranean Diet) as an integral part of disease management have been studied extensively on metabolic chronic diseases (cardiovascular diseases and diabetes) with beneficial clinical results [5, 6].

To what extend dietary patterns beneficial as an integral part of management and prevention strategy for communicable disease? The dietary pattern approach is more likely not only effective for non-communicable diseases but also infectious diseases [7]. Perhaps, this is a possible explanation that partial nutritional intervention exhibits lower than expected results in infectious diseases study settings. Partial nutritional interventions (macronutrients or micronutrients supplementation) as adjunctive treatment of standard antituberculosis agents among active tuberculosis patients is one of the examples. These nutritional interventions have shown no beneficial effects for main treatment outcomes. Even though the supplementation improves weight gain of the TB patients in some settings [8]. World Health Organization (WHO) has declared officially coronavirus diseases (COVID 19) as a global pandemic on 11 March 2020. Currently, there have been several attempts to recommend nutritional approaches for mitigation strategy the disease [9–14]. The dietary pattern plays important role in this communicable disease due to its severity is affected by a previous underlying disease. Comorbidities such as respiratory system diseases, chronic obstructive pulmonary diseases (COPD), diabetes, hypertension, cardiovascular/cerebrovascular disease have shown significant evidence of associations with the severity and prognosis of COVID-19 [15].

Intestinal dysbiosis (gut microbiota imbalance) recently has been proposed as a significant factor that is associated with several immune-related human diseases including infectious, inflammatory, neoplastic, metabolic, autoimmune diseases [7, 16, 17]. Within the gut lumen itself, the human gut microbiome will provide antigens and signals with the potential to interact with resident and systemic immune cells. The composition of the gut microbiome changes over the life course, in response to dietary components, infection, antibiotic exposure [18]. All of these may result in dysbiosis. During this condition, nutritional changes have been suggested as a suitable approach to restoring a healthy gut microbiota and host homeostasis [19]. Also, it has been proposed dietary patterns such as Mediterranean diet and low-fat diet possess the ability to restore partially microbiota dysbiosis [20]. Despite commonly studied single nutrient supplementation, this narrative review aimed to provide current perspectives on the association between the major dietary patterns and infectious disease susceptibility through immune response and systemic inflammation. Relevant articles (original articles, literature reviews, systematic reviews and meta-analyses articles) that identified major dietary patterns and related keywords (e.g., “infection”, “disease”, “immune system”, “inflammation”, and “gut microbiota”) were searched in Google Scholar, PubMed, MEDLINE, and Cochrane databases from the year 2010 to the year 2020 with exception for one article.

2. The role of bacteria homeostasis in gastrointestinal

Dietary patterns and quantity of food intake have been described to influence the microbiome in the gut [21]. *Bacteriodetes, Firmicutes, Actinobacteria,*

Fusobacteria, Proteobacteria and Verrucomicrobium are predominant phyla of microbiota in the human gastrointestinal tract [22]. Among these phyla, more than 90% of the microbiome colonies in the colon are *Bacteriodetes* (*Bacteroides, Prevotella*) and *Firmicutes* (*Eubacterium, Lactobacillus*) [23]. In Western countries, it has been shown that *Firmicutes* phylum becomes blooming and *Bacteroidetes* phylum population decrease due to prominent animal product consumption [24]. On the other hand, it has been demonstrated that high content fiber in the diet resulted in more *Bacteroidetes* phylum bacteria dominance and an increased amount of concentration of short-chain fatty acids (SCFAs) among children from Africa compared to children of European origin [25]. Short-chain fatty acids are fatty acids with fewer than six carbon atoms (acetate, propionate, and butyrate) derived from intestinal microbial fermentation of dietary fibers and resistant starch [26]. The concentration of short-chain fatty acids in the colon and systemic blood is crucial for immune response regulation. The fermentation of dietary fiber by gut microbes, resulting in the establishment of SCFAs, has been proposed to regulate anti-inflammatory pathways through numerous receptors such as G-protein coupled receptors [27]. Additionally, fermented foods and beverages are found to produce beneficial improvements in intestinal barrier function and permeability [28].

It has been suggested that vegan or vegetarian diets may stimulate intestinal microbiota that promotes anti-inflammatory response and lead to be more varied and steadier microbiota systems [29]. Contrary to this, particular food items such as red meat, gluten in wheat, and alcohol can induce dysbiosis which might cause a heightened pro-inflammatory response triggered by viral infections such as COVID-19 from underlying diet-derived chronic inflammation [21]. This intestinal mucosal chronic inflammation is characterized by the presence of cytokines (TNF-α and IFN-γ) which are produced by macrophages, T-cells and natural killer. Besides cytokines, various proteases are also released into the mucosa that has been reported to cause leaky gut due to degradation of tight junctions [30]. Thus, leaky gut allows translocation of microbial products such as lipopolysaccharides (LPS) from the gut into the blood circulation. This condition may transform the existing state of gut inflammation into chronic systemic inflammation during infections such as HIV [31]. This chronic inflammation may remain undetected as a predisposing risk factor and can develop any time into serious morbidity including infectious diseases [23].

Several studies have shown the association between the change of intestinal microbiomes with infectious diseases. It has been reported that intestinal bacterial diversity significantly decreases inversely associated with the severity in patients of chronic viral hepatitis C compared to healthy individuals. The gut microbiome could be a biological indicator and a novel potentially therapeutical approach to reduce the complications of chronic liver disease [32]. Another study has identified *Lachnospiraceae*, *Ruminococcaceae*, and butyrate-producing anaerobic bacteria can be significantly decreased in diarrhea caused by *Clostridium difficile* infection [33]. A short-term nutritional intervention study has reported the positive effect of the supplement on HIV-associated dysbiosis, which was most apparent among untreated individuals but less so in subjects with anti-retroviral therapy, whose gut microbiota was found more resilient [34].

In contrast to short-term supplement intake, long-term dietary patterns and habitual diet are key factors that influence the composition of the gut microbiota. It reflects the potential for therapeutic dietary approaches to modulate microbiome variety, formation, and stability. Besides diet, the intestinal bacteria are formed by a composition of extrinsic (e.g., lifestyle and medication) and intrinsic (e.g., host genetics, immune and metabolic regulations) factors [35]. Changes in dietary patterns following the western diet, along with modifications in dietary components,

result in significant changes in the intestinal microbial configuration and function. As an example, changing from a low-fat, high-fiber diet to a high-fat, high-protein, low-fiber diet leads to reduced α-diversity (intra-individual gut microbiota richness), increased β-diversity (inter-individual gut microbiota diversity) and deteriorated richness or even the extermination of *Prevotella* and *Treponema* species, with lower butyrate levels [36].

3. Western-style diet effect

From a current perspective, the western diet is defined as a modern diet that is primarily characterized by high consumption of red and processed meat, sugar-sweetened beverages, with a lower intake of cheese, wine, beer, cream, tea, vegetables and high-fiber foods [37]. This dietary pattern combined with a sedentary lifestyle can induce chronic systemic metabolic inflammation, termed as metainflammation. Systemic inflammation generates common prevalent modern non-communicable disease [38]. Western diet-induced obesity can result in gut dysbiosis then change lean adipocytes to obese adipocytes. **Figure 1** describes deleterious impact of the alteration to macrophages and adipocytes that lead to metabolic syndrome and diabetes. The western diet is closely associated to several degenerative or metabolic conditions such as obesity [40], metabolic syndrome [41], diabetes [42], cancer [43], hypertension [44], cardiovascular diseases [45], chronic kidney disease [46], and Alzheimer's disease [47].

Western diet pattern may also increase the risk of communicable diseases. It has been shown that women with the western diet might have a higher risk of Human Papilloma Virus infection compared to the Mediterranean-like diet [48]. People with obesity due to the western diet pattern may have a higher level of inflammatory cytokines, immunologic tolerance to inflammatory cytokines, reduced leukocyte number and function, and less control of infection [49]. Subsequently, obesity becomes a risk factor for increased morbidity and mortality of COVID-19 [50–53].

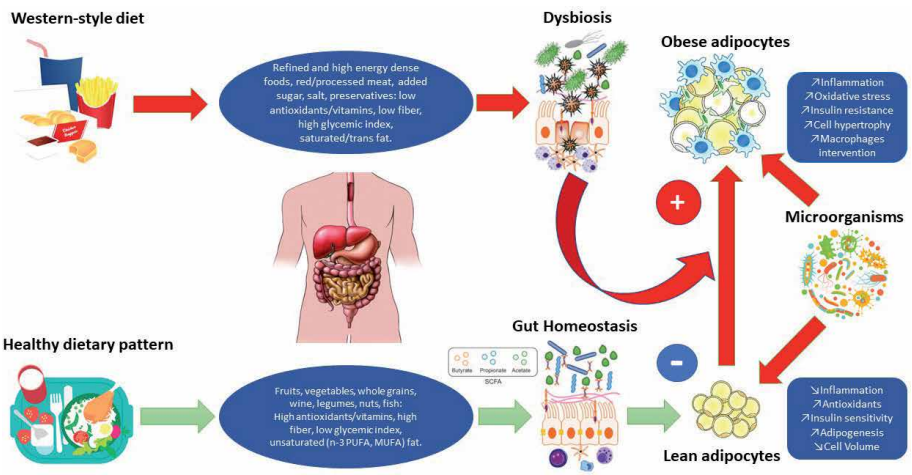

Figure 1.
Unhealthy dietary pattern (western-style diet) along with sedentary life style and genetic predisposition may lead to dysbiosis and contribute toward obesity. Obesity may induce changes to adipocytes and macrophages lead to abnormal inflammation response, decrease insulin sensitivity, and low antioxidant capacity that may finally induce systemic inflammation, oxidative stress, and insulin resistance [39]. On the other hand, healthy dietary pattern provides fiber derived short chain fatty acids (SCFAs) that regulate intestinal barrier and immune system through protein G-coupled receptors signaling. Preservation of gut homeostasis may prevent leaky gut thus reduce inflammation during infection by microorganisms through several mechanisms.

Body mass index (BMI) has been proposed as a prognostic score since higher BMI (BMI ≥30 kg/m^2) more likely results in poor outcome in COVID-19 patients [50, 53]. Body mass index ≥30 kg/m^2 is more likely associated with lower oxygen saturation of blood by weakened ventilation at the base of the lungs. Furthermore, systemic low-grade inflammation due to obesity may occur, such as higher levels of pro-inflammatory cytokines may result in compromised immunity [54]. Therefore, obese COVID-19 patients should receive special attention for their treatment.

Similarly, other non-communicable diseases strongly associated with western diet patterns are also comorbidities for COVID-19. Some comorbidities have been extensively investigated such as diabetes, hypertension, cardiovascular diseases, cerebrovascular diseases, chronic respiratory system diseases, chronic kidney disease [54–57]. Since SARS CoV-2 invades the host cell via ACE-2 receptor on the surface of the cell, certain comorbidities have a strong association with ACE-2 receptor upregulation and impaired immune response that may give rise to susceptibility for viral invasion into the host cells [54]. The infection of SARS-CoV-2 among individuals with these comorbidities can be harmful and might end up with acute respiratory distress syndrome, multiple organ failure, shock, arrhythmias, heart failure, renal failure, and, eventually, mortality [58].

4. Healthy dietary patterns

4.1 Mediterranean diet

Mediterranean diet is a dietary pattern traditionally applied by people who live near Mediterranean Sea, particularly in the region where olive trees are cultivated. It describes the frequent consumption of vegetables, fruits, legumes, nuts, and olive oil as a primary fat source. Additionally, this eating habit is characterized by moderate consumption of fish, poultry, dairy products, wine, and limited intake of red meat [59]. The dietary pattern is one of the most frequently studied for the protective effect of non-communicable diseases such as cardiovascular disease and coronary heart disease [60–62], diabetes [63–65], cognitive disorders [66–68], and malignancies [69–71]. It might be due to the effect of reduced inflammation through changes of C-reactive protein level [72], myeloperoxidase and 8-hydroxy-2-deoxyguanosine [73], white blood cell count and fibrinogen [74], methylation in inflammation-related genes [75]. Additionally, the diet habit also demonstrates modulation of the gut microbiome that results in a reduction of metabolic endotoxemia and subsequently lower systemic inflammation [73, 76, 77].

More likely these positive effects of Mediterranean may produce also protection for infectious diseases. Among communicable diseases, this dietary approach has been proposed for COVID-19 to reduce the mortality rate through the suppression of cytokines [78]. The Mediterranean diet might describe a possible dietary approach to mitigate both short - and long - term complications related to COVID-19 infection. It may decrease the severity and improve mortality and improve the overall well-being of affected populations [79]. **Table 1** shows several studies related to beneficial effects of Mediterranean diet among individuals with diseases caused by virus. A viral infection disease such as chronic hepatitis C and B viruses has shown an inverse correlation to the Mediterranean diet adherence [81]. Cervical cancer that caused by human papillomavirus also has described the same tendency in relation to this dietary pattern and opposite results can be caused by unhealthy diet habit [83]. Mediterranean diet has been found to be beneficial on metabolic indices in human immunodeficiency virus (HIV) patients with the highly active antiretroviral therapy [80]. Perhaps, parasitic infections could be also mitigated by the Mediterranean diet

Author and year	Dietary pattern	Study design (n) and assessment	Population	Outcomes
Tsiodras, S., et al. 2009 [80]	Mediterranean dietary pattern	Cross-sectional study (n = 227). Food frequency questionnaire (FFQ), and Mediterranean Diet Score (MedDietScore).	Human immunodeficiency virus (HIV) positive adult patients with the highly active antiretroviral therapy (HAART) in Israel.	Adherence to a Mediterranean dietary pattern was favorably related to cardiovascular risk factors such as insulin resistance, high density lipoprotein level, and circulating triglyceride level.
Turati, F., et al. 2014, [81]	Traditional Mediterranean diet.	Case–control study (n = 518 *vs.* n = 772). MedDietScore.	Adult hepatocellular carcinoma (HCC) patients, chronic infection with hepatitis B and/or C viruses in Italy and Greece	Adherence to the Mediterranean diet demonstrates protective effect against HCC. Potential benefits from adhering to the dietary pattern for individuals with chronic infection of hepatitis viruses.
Policarpo, S., et al. 2017 [82]	Mediterranean diet	Cross-sectional (n = 571). MedDietScore	Adult HIV positive adults in Portugal	A higher adherence was associated to individuals with a BMI $\geq$ 25 kg/m^2, to subjects with metabolic syndrome and to patients with moderate to high cardiovascular risk.
Barchitta, M., et al., 2018 [83]	Mediterranean diet, prudent dietary pattern, western style diet	Cross-sectional study (n = 539). FFQ and MedDietScore.	Adult women with high-risk human papillomavirus (hrHPV) infection and the risk of high-grade cervical intraepithelial neoplasia (CIN2+) in Italy	Inverse association of Mediterranean-like dietary patterns with hrHPV infection and cervical cancer. The outcomes discourage unhealthy eating habits.

Table 1.
General characteristics of studies examining the role of Mediterranean diet on mitigation of communicable diseases caused by virus.

via the positive effects of omega-3 and omega-9 from olive oil and fish intake. These fatty acids modulate intracellular pathways and transcription factor activation, as well as metabolic and immune regulatory effects [84].

Polyphenols, monounsaturated and polyunsaturated fatty acids, or fiber are more likely bioactive ingredients of the Mediterranean diet [85]. Olive oil is one of the main components of the Mediterranean Diet which has a high profile of fatty acids and phenolics. Oleic acid, a predominant monosaturated fatty acid component in olive oil that can prevent inflammation and insulin resistance induced by palmitic acid in skeletal muscle, adipose tissue, pancreas, and liver. This preventing effect might be due to a reduction in palmitic acid-mediated adenosine monophosphate-activated protein kinase activity which is similar to Metformin [86].

Several minor constituents of Olive oil demonstrate possible synergic effects to counter inflammation. Oleocanthal is known as a minor ingredient of olive oil with significant anti-inflammatory properties that may have therapeutic potential [87]. It has been shown that Oleocanthal has a natural anti-inflammatory property by inhibition of cyclooxygenase enzymes [88]. Hydroxytyrosol is another salient minor phenolic compound of Olive oil that exhibits potential nutraceutical through immunomodulatory and nutrigenomic mechanisms [89].

The immune system is closely correlated with inflammatory processes and oxidative stress [90]. The precise mechanism of oxidative stress during infection is not fully understood, but free radicals have played an important role to defend against microorganism's invasion [34]. Persistent oxidative stress may happen during chronic viral infections and has been associated with a weakened immune system due to long-lasting inflammation activation [90]. The anti-inflammatory effect of the Mediterranean diet may stop a vicious circle in which chronic oxidative stress and inflammation feed each other. Therefore, the Mediterranean diet might prevent further consequences such as impaired immune response. Additionally, The Mediterranean diet which rich in unsaturated fats and fiber may reduce the circulating level of endotoxin from gastrointestinal bacteria that has been proposed as a cause of inflammation [76]. In other words, The Mediterranean dietary patterns possess the ability to restore the gut to normal microbiota homeostasis through its anti-inflammatory effect [91]. Since gut microbial communities are involved in the modulation of the host innate and adaptive immune response so that this approach will play an important role in future therapeutic development for major global infectious diseases [92].

4.2 Dietary approaches to stop hypertension (DASH)

The Dietary Approach to Stop Hypertension (DASH) was designed as a non-pharmacological treatment for lowering blood pressure among adults. The dietary pattern consists of a higher intake of fruits, vegetables, whole grains, fish, poultry but less consumption of saturated fats, meat, and sugar. As result, DASH diet is high in calcium, potassium, magnesium, fiber, and protein. It is not a restricted sodium diet but its effect can be improved by less sodium consumption [93]. Adoption of this dietary pattern is effective not only in reducing blood pressure but also results in lower body weight so that it might be suitable for bodyweight management in overweight or obese individuals [94, 95]. Besides, the diet has shown an association with a lower incidence of cardiovascular diseases, coronary heart disease, stroke, and diabetes. The evidence of cardiometabolic beneficial effects is not only among diabetes patients but also people without diabetes [96]. It has been demonstrated that DASH can control glucose level, infant birth weight and decrease gestational preeclampsia risk among pregnant women [97, 98]. DASH approach is also associated with lower mortality from different cancer types [99, 100]. It means this dietary pattern is suggested as an effective treatment approach for various non-communicable diseases with long-lasting effect [101].

Since hypertension, diabetes and cardiovascular diseases are well-known comorbidities for viral infection COVID-19 [15, 102, 103], so that DASH might be helpful to mitigate the severity and fatality of the disease. The foods in the dietary pattern are rich in bioactive compounds that exhibit potent modulation of pro-inflammatory pathways and may support the immune response to reduce the morbidity and mortality of an infectious disease [104]. Furthermore, the improvement of the antioxidant defense of the body and decrease oxidative stress can be achieved by adherence to DASH diet. These effects might be due to lower malondialdehyde and glutathione levels mechanism [105]. It is suggested that oxidative stress plays a dual role during infections. Reactive species (e.g., nicotinamide adenine dinucleotide

phosphate oxidase, myeloperoxidase, and nitric oxide synthase) can induce cell apoptosis or destroy invading microorganisms as a defense mechanism. However, they can also cause tissue injury and resulting inflammation [106]. It has been proposed that the immune system plays important role in the etiology of hypertension. The DASH may promote the expansion of protective microbes that release gut metabolites such as short-chain fatty acids which are protective for the immune system and blood pressure [107]. Eventually, a healthy eating habit supports the immune system that protects against the invasion of microorganisms or viruses and produces antibodies to eradicate pathogens.

4.3 Plant-based diet

The plant-based diet generally consists of two dietary patterns: vegetarian diets and vegan diets. Vegetarian diets are characterized by reduced or eliminated animal products intake but may include dairy products and/or eggs, while vegan diets contain only plant foods. Both vegetarian and vegan are dietary patterns that emphasize the consumption of vegetables, fruits, grains, legumes, and nuts [108]. Several potential beneficial effects of a plant-based diet are ameliorating insulin resistance, including preservation of healthy body weight, higher intake in fiber and phytonutrients, promoting food-microbiome interactions. The adoption of a plant diet decreases levels of advanced glycation end products, saturated fat, heme iron, and nitrosamines [109]. Several studies have shown plant-based diet efficacy for the prevention and treatment of diabetes [109–112]. The plant-based diet has also demonstrated a significant positive impact on cardiovascular diseases, coronary heart diseases, hypertension, and hypercholesterolemia [113–118].

In contrast to the western diet which consists of red meat, wheat and alcohol consumption, the plant-based diet has suggested having the ability to maintain symbiosis and prevent dysbiosis of the microbiome and results in lower morbidity and mortality during an infection such as COVID 19 [23]. The implementation of plant-based diets could improve the diversity of nutrients for the host by the gut microbiome. The undigested plant cell walls components are not absorbed by gastro intestinal tract and lead to microbiota-derived nutrients such as peptides and lipids. These subtances can promote the development and function of the host immune system [119]. There might be situations in which immune cells of the gut-associated lymphoid tissue come into direct contact with nutrients or gut microbiome, such as in the circumstance of increased epithelial permeability (leaky gut) ocurred in both acute and chronic gut inflammation [120]. Numerous plant-based biologically active compounds exhibit antibacterial [121], antifungal [122], and antiviral activity [123]. Moreover, China and India predominantly rely on plant-based medications under different domain names like Chinese Traditional Medicines and Ayurveda but the plant-based therapeutical approach remains largely unexplored [123]. Therefore, a more likely plant-based dietary pattern has protective effects against infection due to its anti-inflammatory and immune response modulation properties that come from plant bioactive molecules. Plant-based food has been recommended also as a nutritional approach treatment for COVID-19. The dietary pattern improves the gut beneficial bacteria and rich in plant bioactive compounds, vitamins C, D, E, magnesium, and zinc [124].

4.4 Ketogenic diet

Ketogenic diet (KD) is a dietary pattern that promotes a low carbohydrate intake (usually to <50 g/day), adequate proportions of protein and higher percentages of fat [125]. Since the diet provides lower carbohydrates intake, glucose reserves

become insufficient both for Krebs cycle and for the central nervous system (CNS). Thus, after several days of carbohydrate restriction, the CNS is forced to find an alternative source of energy. This alternative source of energy is ketones and there are two types of ketone bodies produced in the liver: acetoacetate and β-hydroxybutyrate [125]. Ketosis is a physiological mechanism and it reflects the breakdown of fats in order to compensate for a low level of glucose. The ketogenic diet originally was introduced for epilepsy treatment and the current evidence suggests that KD could help children with drug-resistant epilepsy [126]. Despite its therapeutic application for a neurological disorder such as Alzheimer, malignant glioma and adult epilepsy have shown potential benefits [127], but this may lead to further lowering of consumed essential nutrients by elderly persons with neurodegenerative diseases [128]. Recent findings suggest that even though not all types of cancers give a positive response but KD as a adjuvant treatment, it may give beneficial effect for body composition and quality of life among cancer patients [129]. However, controversies remain on the implementation of the KD especially for diabetes and obesity since the risks, benefits, and applicability of the diet to avoid unnecessary harm and costs to patients [130]. Additionally, the improvements in some cardiovascular risk factors (obesity, type 2 diabetes and high-density lipoprotein (HDL) cholesterol level) are usually not long-lasting and the development of insulin resistance might occur [131]. The international ketogenic diet study group has suggested that constant nutritional monitoring is needed for ketogenic diet therapy to ensure its effectiveness and to reduce potential adverse effects [132]. Perhaps, the sequential method in a biphasic combination of two dietary patterns such as ketogenic diet and Mediterranean diet may provide an effective strategy against obesity-related inflammation with higher compliance of consumers [133].

On the other hand, the short-term ketogenic diet therapy has been applied for COVID-19 patients in order to perform a rapid reduction of comorbidities. These comorbidities (obesity, type 2 diabetes and hypertension) are well known as modifiable risk factors for COVID-19 patients [134]. The rationale behind this approach is the induction of ketosis may reduce hyperglycemia and eucaloric ketogenic diet could affect macrophage phenotype M1 limiting cytokine storm syndrome. Furthermore, SARS-CoV-2 replication could be inhibited by the antiglycolytic action of eucaloric ketogenic diet [135]. It has been suggested that therapies that increase levels of (R)-beta-hydroxybutyrate, such as the ketogenic diet or consuming exogenous ketones, should restore altered energy metabolism and redox state in patients with COVID-19. This approach is marked at the molecular level by reduced energy metabolism, modulate redox state, decreased oxidative stress, and cell death lead to blunt cytokine storms caused by Human SARS-CoV-2 infection [136].

Social distancing, quarantine, and isolation for prevention of COVID-19 spread may lead to a sedentary lifestyle, one of the factors for overfat that can affect negatively immune function [137]. The pandemic may aggravate depression due to social distancing and isolation, and thus unhealthy eating habits are used to compensate [138]. These Societal interventions against the COVID-19 pandemic might induce a sequence of psychobiological mechanisms that stimulate obesity incidence and raise the risk of comorbidities [139]. Perhaps, an alternative combination of dietary patterns between the ketogenic diet and a low-calorie diet may provide a safe, rapid and long-lasting approach for body weight as well as fat mass reduction. Very-low-calorie ketogenic (VLCK) diets are a dietary pattern that imitates fasting by limiting carbohydrates and fat intake with a relative increase in protein consumption [140]. Very-low-calorie ketogenic diet is able to reduce body weight especially in a relatively short time at the expense of fat mass and visceral mass; muscle mass and strength were preserved [141]. This effect can be long-lasting up to one year among patients that lose more than 10% of their initial weight without any impact

on the muscle mass [142]. It has been shown that the VLCK diet also induced more weight reduction compared to the low-calorie diet until 24 months follow-up and decreased the individual burden of disease among obese patients [143]. The modified ketogenic dietary patterns may exhibit a more suitable and safer solution for a longer effect to mitigate obesity-linked comorbidities.

5. The health implication aspects of dietary patterns

Several factors can be differentially contributed to the implication of major dietary patterns such as meal-specific patterns which are identified as one of these factors [144] besides dietary composition [145]. An unhealthy meal pattern may have an association with dietary quality and diversity and it has been shown that lower dietary diversity scores increase the probability of metabolic syndrome [146]. A low dietary diversity score might be predisposed to nutrients deficiency such as iron deficiency anemia among adolescent girls [147]. Nutrients deficiency is considered a significant factor for infection susceptibility due to immune response impairment if left untreated in some settings [148, 149]. Furthermore, age category might affect the dietary pattern preference for example adults, their common dietary pattern is a western diet-like style and it may increase the risk of metabolic syndrome, obesity, hypertension and cardiovascular disease [150, 151]. Older people are more likely to consume fruits and vegetables and less likely to consume red meat, whole milk, and other fatty foods compared to younger people. However, older individuals tend to consume less calorie intake and a reduction in the quantity of food due to a decrease in physical activity as well as muscle mass [152]. Diet alone may not be sufficient to prevent micronutrient deficiency during aging and this situation can compromise immune function and increase infection risk [153]. Food insecurity is also an essential factor that associated with the unhealthy dietary pattern and it may refer to the limited ability to acquire nutritious food in socially acceptable ways [154]. Therefore, food insecurity may affect negatively infectious diseases susceptibility such as viral suppression of HIV/AIDS [155], COVID 19 spread [156, 157] TB treatment failure and mortality [158], and Malaria [159].

6. Conclusions

Healthy dietary patterns might be protective against inflammation triggered by oxidative stress which is an important determinant of chronic diseases. The proposed mechanisms include preservation of gut microbiome homeostasis and integrity of the epithelial lining of the gastrointestinal tract. These conditions could alleviate lipopolysaccharide-induced inflammatory response and endotoxemia due to leaky gut. Additionally, short-chain fatty acids from fermented dietary fiber as common component of the dietary patterns exhibit anti-inflammatory properties. Therefore, healthy dietary patterns may improve metabolic indices, certain medical conditions and pre-existing comorbidities in infectious disease. In a nutshell, the healthy dietary pattern might be suggested as an alternative for prevention or an integral part of infectious disease management that can be adjusted to local settings.

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Conflict of interest

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Chapter 14

Disease Modifying Potential of Functional Foods for Neurodegenerative Disorders: Status Update on Regulatory Compliance

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Abstract

Progressive loss of functional neurons is typically characterized as neurodegeneration. This is particularly pronounced during aging and results in debilitating conditions such as Parkinson's disease and Alzheimer's disease. Symptoms appear typically after 70–80% neuronal loss, resulting in irreversible damage. Several drugs have been clinically approved but they only alleviate symptoms and additionally lead to undesirable side effects. Hence there is a dire need for drugs and/or supplements which address this lacuna. Functional foods are known to offer health benefits beyond their attributed nutritional values. Unlike dietary supplements which are made from foods or food-like substances with enriched nutritional value, functional foods are foods that are modified for greater nutritional value. Conceptually, as an expansion of dietary supplements, functional foods are known to be neuroprotective. Here we discuss functional foods which can potentially be used as adjunctive therapy, with a note on the regulatory compliance.

Keywords: Neurodegenerative diseases, neuroprotection, functional foods, adjunctive therapy, regulatory compliance

1. Introduction

Since 1900 the global average life expectancy has more than doubled and is now 70 years. This is expected to further increase over the coming years. This can be mainly attributed to better healthcare systems, access to timely medical and emergency services, lifestyle changes, decrease in infant mortality, and to a large extent higher income. A corollary to this is an increase in the geriatric population, which brings with it several age-related health concerns. Not surprisingly, diseases of the aging brain are also rampant. The brain is the most privileged organ. Yet, what appears to be an advantage could also classify as a drawback as early detection of brain disorders always poses to be challenging. Age associated progressive deterioration of the neuronal structures typically results in a group of conditions called

Sl No	Physiological Effect	Food source	Phytochemical/ Bioactive compound	Regulatory compliance (RC)	References
1	Multiomic alterations	1. Green tea 2. Turmeric	1. Butyrate, flavonoids, and genistein 2. Caffeine and theanine 3. Curcumin	F, K, P, R, HH	[3, 4]
2	Epigenetic changes	1. Soy products 2. Broccoli 3. Brazilian nuts 4. Green tea 5. Garlic 6. Grapes 7. Cruciferous vegetables 8. Blueberry, fish, olive oil, diet rich in vegetables	1. Genistein 2. Sulforaphane 3. Selenium 4. Catechins epigallocatechin-3-gallate 5. Resveratrol Dihydrocaffeic acid, malvidin-3′-O-glucoside 6. Folic acid, isothiocyanates, docosohexanoic acids 7. Vitamin B12, choline, folate, methionine, betaine, biotin, pantothenic acid, curcumin	C, D, B, E, F, K, L, M, O, P, S, W, AA, GG, HH	[5–13]
3	Mitochondrial health enhancing	1. Red meat, dairy products, avocado, chicken, fish, beans 2. Organ meat, muscle meat, fatty fish, spinach, cauliflower, broccoli, orange, strawberries, sesame seeds and pistachios. 3. Protein rich meats such as beef, turkey, pork and limited in chicken 4. cherries, berries, eggs, milk, fish, nuts	1. L-carnitine 2. Co-enzyme Q # 3. Carnosine 4. Green tea catechins 5. Melatonin	C, D, G, H, K, O, P, T, U, R, X, Z, HH	[14–18]
4	Anti-inflammatory function	1. Turmeric, Berries, kale, grapes, spinach, bell peppers, cocoa, broccoli 2. Soybeans 3. Peanut oil, corn, peas and beans 4. Olive oil 5. Mint	1. Flavonols 2. Flavans 3. Palmitoyl ethanolamide 4. Hydroxy-benzoic acid 5. Patchouli alcohol	A, I, K, P, S, U, AA, EE, HH	[19–26]
5	Antioxidant function	1. Tea, grapes, lentils, cocoa, apples, apricots, cherries 2. Flax seeds # 3. Walnuts	1. Flavanols 2. Alpha-linolenic acid 3. Flavonoids and Alpha-linolenic acid	D, G, J, K, P, S, EE	[27–32]

Sl No	Physiological Effect	Food source	Phytochemical/ Bioactive compound	Regulatory compliance (RC)	References
6	Improved cognitive function	1. Grape seeds, soy products 2. Barley grass 3. Fermented foods 4. *Ginkgo biloba*	1. Isoflavones 2. Saponarin 3. Ginkgolides	B, G, K, N, P, Q, V, FF	[33–38]
7	Neuroprotective role	1. Grape fruit, lemons, oranges 2. Olives, plums, chickpeas, herbs and spices 3. Coconut 4. Mushroom	1. Flavonoid glycoside 2. Tannins/Proanthocyanidin 3. Phenolic acids 4. Polysaccharides	D, K, P, S, V, Y, AA, BB, DD, FF, HH	[27, 39–48]
8	Mitigation of neuroinflammation and microglial activation	1. Peaches, 2. Blackberries, 3. Black grapes 4. Strawberries	Flavanols	B, D, G, K, P, V, EE	[49–51]
9	Free radical scavenging property	1. Grape skin 2. Peanuts 3. Red wine 4. Cranberries	Trihydroxy-stilbenes	B, D, K, P, S, V, CC, EE, FF, HH	[52–54]
10	Suppression of oxidative stress	1. Celery 2. Fresh parsley 3. Olives 4. Oregano 5. Peppers 6. Rosemary	Flavones	D, K, P, S, V, CC, EE	[55–57]

A: *Belgium-Federal Agency for the Safety of the Food Chain (FASFC);* **B**: *Canadian Food Inspection Agency (CFIA);* **C**: *Center for Food Safety and Applied Nutrition (CFSAN);* **D:** *China-National People's Congress (NPC);* **E**: *Council of Europe (Flavouring substances and natural sources of flavouring);* **F:** *Dietary Supplement Information Expert Committee (DSIEC);* **G**: *European Food Safety Authority (EFSA);* **H:** *European Medicine Agency (EMA);* **I**: *Europe-Center for Food Safety and Applied Nutrition (CFSAN);* **J**:*Flax Council of Canada (FCC);* **K**: *Food and Drug Administration, USA (USFDA);* **L**: *Food for Special Dietary Use (FSDU);* **M:***Food Safety and Standards Regulations (FSSR);* **N**: *Food Standards Agency, UK (FSA);* **O**: *Food Standards Australia New Zealand (FSANZ);* **P**: *FSSAI- Food Safety and Standards Authority of India;* **Q;** *Hong Kong-Centre for Food Safety (CFS);* **R**: *International Alliance of Dietary/Food Supplement Associations (IADSA);* **S**: *Japan- Ministry of Health, Labour and Welfare (MHLW);* **T**: *Korean Food and Drug Administration (KFDA);* **U:** *Malaysia-Food Safety and Quality Division (FSQD);* **V:** *Malaysia-Ministry of Health;* **W:** *Medicines and Healthcare products Regulatory Agency, UK (MHRA);* **X:** *Natural health product (NHP) in Canada;* **Y:** *Nepal-Department of Food Technology and Quality Control (DFTQC);* **Z**: *NIH- Office of Dietary Supplements (NIH-ODS);* **AA**: *Norway-Norwegian Ministry of Agriculture and Food (NMAF);* **BB**: *Philippines-Food and Drug Administration (PFDA);* **CC**: *Portugal-Economic and Food Safety Authority (EFSA);* **DD**: *South Korea-Ministry of Food and Drug Safety (MFDS);* **EE**: *Taiwan FDA (TFDA);* **FF**: *UK-Department for Environment, Food, & Rural Affairs (DEFRA);* **GG**: *UK-Food Standards Agency (FSA);* **HH**: *United States Department of Agriculture (USDA).*[#]*Not approved by FDA.*

Table 1.
Table depicting a comprehensive list of the various physiological effects exerted by functional foods, their dietary sources, and status of their regulatory compliance.

neurodegenerative disorders; Alzheimer's Disease (AD) and Parkinson's Disease (PD) being classical examples. These debilitating disorders negatively impact neuronal functions in a progressive manner. Although age is the predominant risk factor, environmental influences play a significant role. Pharmacological and surgical approaches are being rampantly used as therapy. While these strategies address the clinical symptoms and provide relief to the patients with minimal side effects, they fail to prevent disease progression. Further, there could be additional risk involved due to chronic drug administration. Owing to these serious lacunae, there is a dire need to investigate novel approaches which are disease modifying, neurorestorative, possibly curative, and have minimal side effects. Adopting a nutritional approach is being increasingly considered for its protective function and has already proven effective in several patients. Special diets and a dietary plan have been investigated for their neuroprotective roles. Increasing the nutrient value and quality of the diet and adherence to the dietary plan are being emphasized for long term benefits.

Foods that we consume have three functions: primary, secondary, and sometimes tertiary functions. While the primary function would be to meet the body's energy requirement, the secondary function is attributed to the flavor, smell, appearance, texture, etc. In addition, certain foods have been known to modulate human physiology and hence prevent disease. This function is sometimes regarded as the tertiary attribute and includes anti-carcinogenic, anti-inflammatory, antioxidant, anti-mutagenic, and anti-ageing, to list a few. Foods that possess such attributes are classified as 'functional foods'. Functional foods are known to offer health benefits beyond their traditional nutritive values. Physiologically, they help reduce the risk of contracting chronic diseases. These are conventional foods which can be consumed like a normal diet, but are fortified with a specific or cocktail of well-balanced health promoting nutrients. Good examples of such fortified foods are iron-fortified cereal, iodized salt, vitamin D-fortified milk etc. Functional foods contain the required amounts of antioxidants, carbohydrates, vitamins, proteins, fats, and other components so as to boost the immune system and improve cell survival. Functional foods overlap with nutraceuticals, medical foods, probiotics, designer foods, pharmafoods, and vitafoods. The health benefits and physiological effects attributed to functional foods and nutrients such as polyphenols are also relevant in the context of brain health but are relatively under investigated. Consumption of foods containing natural antioxidants such as legumes, fruits, herbs, whole grains, and vegetables, or processed foods enriched for natural antioxidants such as vitamin C and E, omega 3-fatty acids, carotenoids, polyphenols, stilbenes, etc. can provide the desired protection against neurodegenerative and metabolic disorders [1, 2]. Here, we discuss the various biological effects modulated by functional foods in the context of neurodegeneration, with a note on the regulatory guidelines and regulatory status of such functional foods (**Table 1**).

2. 'Omic' and physiological effects exerted by functional foods

2.1 Multiomics

Advancing technology has made possible the study of entire gene sets or protein complements as one unit resulting in the 'omics'-era. High throughput investigations allow access to huge amount of data and aid in understanding biology in an interactive and holistic manner. Diet offers many health promoting and disease preventing factors which target not just one gene/protein/cell type/pathway, but can affect multiple entities resulting in a cumulative outcome. The influence of bioactive food components on the gene expression is termed as 'nutrigenomics' and the response of gene variants to a particular nutrient is called 'nutrigenetics'. Nutrients

from the diet can interact with the genetic material by functioning as co-factors or substrates for processes that regulate DNA metabolism and gene expression. Early life exposure to nutrients and environmental factors both during the prenatal and postnatal period has a significant influence on gene expression, cellular plasticity, and susceptibility to various adult diseases [58].

The first and most widely employed omics technology is transcriptomics which is highly efficient and provides high-throughput data. It provides a snapshot into the mRNA complement of the tissue at a particular time. It allows us to understand the influence of bioactive dietary compounds on gene sets and biological processes. Microarray technology offers the possibility of understanding the change in gene expression patterns after exposure to a particular nutrient. Using a wide range of bioinformatic tools one can build interaction networks and pathway for the observed gene profiles [59, 60]. An accumulation of large - scale nutritranscriptomic microarray data has necessitated the need for integrated web-based databases which have been built on open-source platforms and ensure efficient organization, storage, and analysis of the humungous data [61].

The proteome represents the protein complement of the genome that is expressed at a particular time in response to a particular stimulus and is more complex, dynamic, and subject to spatio-temporal changes. While the genome is constant, the expression of the gene is largely dependent on several parameters; alternative splicing and post translational modifications included. Protein identification technologies have largely evolved from simple gel-based techniques to mass spectrometry and multiplexed immunoassays which are further assisted by powerful bioinformatic tools. Nutritional proteomics hence allows us to examine the effect of food components on protein expression and also offers a platform for biomarker identification in relation to dietary interventions. *In vitro* and *in vivo* studies have revealed the impact of dietary component such as butyrate, flavonoids, and genistein on the proteome [62–66]. One study identified thirteen candidate proteins as potential biomarkers of neuroprotection in response to grape seed extract supplementation [67].

Metabolomics is the newest addition to the omics family in nutritional research. Metabolome is the complete set of metabolites synthesized as a result of the genome-environment interactions under a given environmental condition. Similar to the other omics approaches advanced techniques such as mass spectrometry and Nuclear Magnetic Resonance (NMR) in addition to powerful bioinformatic tools enables us to detect the biochemical changes in response to nutritional interventions and also understand the impact of genetically modified foods on food safety [68].

Studies regarding the effect of functional foods with metabolomic effects related to neurodegenerative disorders are limited. Green tea and curcumin are among those that have been reported to exert metabolome changes. Use of green tea polyphenols on aging rats resulted in metabolomic alterations. Dysregulation of lipid metabolism was moderated by consumption of caffeine and theanine enriched green tea [69]. NMR and MS based metabolomics showed the effect of curcumin on hyperlipidaemia mice induced by high-fat diet [70].

2.2 Epigenetics and functional foods

Unlike what was popularly believed a few years ago, somatic heritable states need not necessarily depend on the DNA sequence alone. There could be environmental influence resulting in DNA methylation, histone modifications, and chromatin remodelling. Both external and internal factors could bring about such epigenetic changes which can in turn control the gene expression pattern. These changes are also heritable and hence provide a framework for the quest

of etiological factors governing several diseases including neurodegenerative diseases. It is aptly said 'you are what you eat'. Nutrition and the bioactive food components can influence the epigenetic mechanisms and bring about changes at the transcriptional level. Vitamin B12, choline, folate, methionine, betaine all of which are constituents of the diet, mediate 1-carbon metabolisms involved in DNA and histone methylation. Biotin is a substrate for histone biotinylation; niacin and pantothenic acid facilitate histone ADP-ribosylation, acetylation, and deacetylation processes [71–73]. Several phytochemicals like tea catechins, resveratrol, sulforaphane, curcumin, etc. affect epigenetics [74, 75].

Natural products from tea, garlic, soy products, herbs, grapes, and cruciferous vegetables have epigenetic targets. Selenium in Brazil nuts, sulforaphane in broccoli, epigallocatechin-3-gallate in green tea, resveratrol in grapes, and genistein in soy beans have been shown to be dietary inhibitors of DNA methyltransferases [76, 77]. While epigenomic alterations owing to dietary interventions are well documented in carcinogenesis, their effects in neurodegenerative diseases are limited. Vitamin B12, folic acid, dietary polyphenols, isothiocyanates, docosohexanoic acids, olive oil, blueberries, fish, and a diet rich in vegetables have been suggested to modify the epigenetics of AD [78]. Dihydrocaffeic acid from grape juice and malvidin-3′-O-glucoside from grape seed extract, reduced proinflammatory cytokines via down-regulation of DNA methyltransferase 1 and upregulation of histone deacetylase 2 respectively, hence attenuating depression-like behaviour in mice [79].

2.3 Neuroinflammation and functional foods

Inflammatory processes localized to the central nervous system (CNS) are categorized as neuroinflammation. It can be regarded as a double edged sword as it is deleterious but at the same time triggers the repair and recovery mechanisms. Glia, the CNS specific immune cells play a major role in this process. A cross talk between the CNS and the inflammatory cells mediates an inflammatory response involving the production of mediators such as cytokines, chemokines, reactive oxygen species (ROS), and second messengers, by the latter. Recruitment of immune cells, edema, tissue damage, and possibly cell death are some events that follow inflammation. The positive responses of neuroinflammation can involve immune surveillance, injury induced remodelling, immune preconditioning, development, memory, and learning; all of these leading to tissue repair, neuroprotection, and enhanced plasticity [80]. Hence, even though neuroinflammation is one of the most evident consequence of neurodegeneration, therapeutic strategies could be evolved so as to potentiate the positive effects of inflammation and concomitantly mitigate the negative effects.

Neuroinflammation in the context of AD and dementia were mitigated by functional foods. Mediterranean diet such as fruits, vegetables, whole grains, nuts, and legumes; moderate intake of fish, poultry, and alcohol; and low intake of red and processed meat improved cognitive function in elderly, probably due to the anti-inflammatory mechanisms [81]. Dietary components categorized as functional foods encompassing carrots, tomatoes, cranberry, grape seeds, papaya, pomegranate, curcumin, ginger, green tea, PUFA, dark chocolates, and cocoa have shown antioxidant and anti-inflammatory properties [82].

2.4 Mitochondrial health and functional foods

Mitochondria are the seat of cellular energy metabolism and several key housekeeping activities. Neuronal functions are highly energy demanding and the presence of healthy mitochondria is indispensable. Several evidences suggest a link between dysfunctional mitochondria and neurodegenerative diseases. Mitochondrial

glucose metabolism, oxphos enzyme activities, mitochondrial dynamics, motility, fusion and fission have all been implicated in neurodegeneration. TCA enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, were impaired in post-mortem AD brain and fibroblasts from AD patients [83]. Mitochondrial electron transport chain complexes I, III, and IV showed reduced activities in platelets and lymphocytes and post-mortem brains from AD patients [84, 85]. Additionally, aberrant expression of Drp1, a protein associated with mitochondrial dynamics was reported in AD brains, AD mouse models and APP cell lines [86]. Likewise, deficiency in mitochondrial complex I in the SNpc of the brain, lymphocytes, platelets, and muscle from PD has been well established [87]. Several genes related to genetic forms of PD such as phosphatase and tensin homolog-induced putative kinase 1 (PINK1), DJ-1, α-synuclein, parkin, and leucine-rich repeat kinase 2 (LRRK2) are associated with mitochondria. Parkin and PINK1, implicated in mitochondrial quality control, mitophagy, and mitochondrial dynamics are reported to be abnormal during PD [88, 89]. Knockout mice for mitochondrial transcription factor A (*Tfam*), associated with mitochondrial copy number, maintenance and transcription of mitochondrial DNA, survive to adulthood and display Parkinsonian phenotype such as intracellular inclusions within dopaminergic neurons, dopaminergic cell degeneration, and loss of striatal dopamine [90, 91]. Considering the significant role of mitochondrial health in neuronal function, use of functional foods that protect and/or preserve mitochondrial health and in turn its function is a promising approach. L-carnitine and co-enzyme Q10 play a key role in mitochondrial biogenesis and health. L-carnitine exhibits antioxidant function within the mitochondria by scavenging ROS and prevents inflammation. It drives ATP production, lowers cholesterol, and helps build lean muscle. Acetyl L-carnitine enhances mitochondrial health [92]. Carnitine exerts neuroprotection by acting on the NrF2 inhibitor, keap1, and activating the phase II antioxidant system [93]. Unites States and Japan are marketing L-carnitine and carnosine fortified foods as energy boosting and health promoting products. Green tea catechins have antioxidant function and improve cellular energy production and mitochondrial health [94]. Melatonin has antioxidant properties and is neuroprotective [95].

2.5 Oxidative stress and functional foods

Increased reactive oxygen species (ROS) can potentially harm cellular macromolecules such as lipids, proteins, and nucleic acids. While moderate production of ROS is essential for normal cellular function, excess production is countered by a battery of antioxidants within the cell. A normal cell maintains this homeostasis but if this balance is disturbed a cell experiences oxidative stress. Mitochondria are the primary site of ROS production and also its principal target. Increased ROS beyond the threshold and/or a failure of the antioxidant defence contributes to mitochondrial dysfunction, cellular damage, and oxidative stress contributing to a series of events resulting in neurodegeneration. The dopaminergic neurons associated with PD are particularly vulnerable to oxidative stress due to their increased iron content, which catalyzes the Fenton reaction leading to the generation of superoxide. Dietary intervention can effectively manage oxidative stress [96–98].

Polyphenols in fruits, vegetables, cereals, dry legumes, chocolate, and tea have antioxidant potential and prevent neurodegenerative diseases. Flavonoids from fruits and vegetables such as spinach, pepper, asparagus walnut, sunflower seeds, and chia seeds have the highest antioxidant capacity [99]. Additionally, α-lipoic acid, anthocyanin, *Ginkgo biloba*, garlic, black cumin, and green tea prevent neurodegenerative disorders through antioxidant mechanisms [100]. Black raspberries, rich in the antioxidants anthocyanin and ellagitannin, have successfully been

used in clinical trials and are reported to reduce the risk of cancer [101]. Preclinical research demonstrated the use of berries in preventing neurodegeneration owing to their anthocyanin, caffeic acid, catechin, quercetin, kaempferol, and tannin content. They have been shown to reduce oxidative stress, have anti-inflammatory function, alter levels of brain–derived neurotrophic factor, and enhance memory and cognitive function [42]. However, their clinical uses have not been reported.

2.6 Gut microbiota, neurodegeneration and functional foods

Recent research points to the microbiota-gut-brain-axis as a novel contributing factor for neurodegeneration and mood disorders, by directly affecting the neuroimmune, neuroendocrine, and direct neural pathways such as the vagus nerve. Microbial metabolites which include but not limited to bioactive constituents, neurotransmitters, and epigenetic regulators cross the blood–brain-barrier (BBB) exerting physiological effects. A dysbiosis in this microbiota causes dysregulated gut-brain signalling resulting in oxidative stress, neuroinflammation, immune disturbances, and metabolic imbalance [102, 103]. Indeed, altered gut microbiota and metabolites like butyrate and amyloid are associated with neurodegeneration [104]. The gut of AD patients was shown to have decreased levels of Bacteroidaceae, Veillonellaceae, and Lachnospiraceae family members, which contain several key butyrate producers, but increased abundance of microbes from Ruminococcaceae, Enterococcaceae, and Lactobacillaceae. Pathogenic gut residents such as *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Salmonella enterica*, *Salmonella typhimurium*, and *Staphylococcus aureus*, are known to produce amyloid proteins, implicated in AD [105]. Stools from patients with Amytrophic Lateral Sclerosis (ALS), had increased content of potential inflammatory pathogens such as *Escherichia coli* and members of Ruminococcaceae and Enterobacteriaceae family [106, 107]. PD gut showed reduction in the relative abundance of *Prevotella*, which may be associated with reduced metabolism of high-fiber foods, mucin production, gut barrier function, and small chain fatty acid (SCFA) levels [108–110]. Reduced levels of butyrate producers such as *Blautia*, *Coprococcus*, *Faecalibacterium,* and *Roseburia* species in PD stools suggests increased intestinal permeability and inflammation. These observations support emerging evidences of neuroinflammation in neurodegenerative conditions probably induced by peripheral circulating inflammatory products [111].

Considering the role of gut microbiome dysbiosis in neurodegeneration, strategies for modulation of gut microbiota are under investigations. The use of probiotics, synbiotics, and prebiotics either as isolates or herbal supplements appear to be promising avenues. Prebiotics are particularly carbohydrates that are selectively fermented by gut microbes and modify the microenvironment favourably to the microbes and in turn offer health benefits to the host [112, 113]. SCFA such as butyrate, of microbial origin are known to modulate histone acetylation pattern and inflammatory responses via altered gene expression, induction of T-regulatory cells, BDNF expression, and function as signalling molecules between the gut-brain axis [114, 115]. Probiotics, contrary to prebiotics, are a cocktail of live intestinal bacteria and yeasts which are consumed to improve gut health. They improve the permeability of the intestinal wall and the BBB. A randomized, placebo controlled clinical trial using pre and probiotics helped improve PD associated bowel dysfunction [116]. In the peripheral blood mononuclear cells (PBMC) of PD subjects given probiotics the expression of pro-inflammatory cytokines was reduced. Reduced oxidative stress was noted in probiotic treated PD subjects as indicated by a decrease in C-reactive protein (CRP), malondialdehyde (MDA), and increased glutathione levels [117]. Likewise, a double blind placebo controlled clinical trial in AD subjects using probiotic

cocktail of *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum* showed improved learning and memory as scored by Mini Mental Score Examination (MMSE) and a reduction in oxidative stress markers such MDA levels [118]. Clinical trials using selenium containing probiotics improved the MMSE score, antioxidant capacity, reduced serum triglycerides and LDL cholesterol, reduced CRP level, and improved insulin sensitivity in AD subjects by altering the gut microbial composition [119]. Probiotic Annurca apple puree minimised plasma lipid profile and trimethylamine-N-oxide levels, hence offering cardioprotection [120]. However, their efficacy in neurodegeneration needs further investigation. Likewise, Danone, a French company markets its dairy products enriched with *Bifidobacterium lactis* as a gut health promoting probiotic product [121]. Yet, no studies exist till date indicating its role in improving brain health. Several studies in *in vivo* models as well as in clinical subjects indicate promising outcome. Nevertheless, the use of pre/probiotics as neuroprotective agents is in its infancy as many unanswered questions remain. Their therapeutic use is constrained by the absence of (1) strong translation of preclinical studies, (2) thorough elucidation of pertinent mechanisms, and (3) larger cohort studies.

2.7 Calcium as functional food

Calcium forms a major macronutrient which serves multiple functions in our body. Some of them are bone and skeleton formation, coagulation, enzyme functions, nerve conduction, and cardiovascular functions. The source of calcium in our body is solely through the diet hence malabsorption or inadequate intake could lead to myriad of altered functions in our body. On account of the key physiological functions of Calcium, the use of Calcium/Vitamin D supplementation is rampant particularly in the elderly and post-menopausal women. Occasionally, regular consumption of calcium causes nausea, constipation, and indigestion. Hence, consumption of calcium fortified foods is gaining popularity. Examples of calcium fortified foods include milk and related products like cheese, yoghurt, and probiotics [122]. Several studies have demonstrated the effect of calcium fortified milk and milk products with or without Vitamin D on osteoporosis in the elderly [123]. Non-dairy food products rich in calcium are seeds (poppy, sesame, celery and chia seeds), edible bones of sardines and salmon, beans and lentils, almonds, and vegetables (broccoli, kale, spinach, okra and greens). Although green leafy vegetables are rich sources of calcium, their absorption in the gastrointestinal tract depends on the presence of inhibitory factors, such as oxalates, phytates, and tannins which bind with calcium, rendering it insoluble and therefore unavailable to the body. Therefore, the bioavailability of calcium via functional foods is a key factor for consideration [124].

3. Regulations of functional foods

The health benefits of food have always been a subject of discussion from ancient times. While most initial claims regarding the disease-preventing attributes of foods lack scientific evidence, foods such as green tea have been extensively investigated for their health promoting role. The modern concept of functional foods was born in Asia, and Japan was one of the earliest countries to fund research for the systematic analysis and development of functional foods. Research in the recent past has clarified that food can be designed not just to meet primary functions, but also to adjust the human body's homeostasis so as to regulate health and wellness. This idea of physiologically relevant functional foods led to the

Sl. No	Country	Regulatory Policy	Governing body	Coverage	References
1	Japan	FOSHU	Ministry of Health and Welfare	Dietary fibers, lactic acid bacteria, oligosaccharides, soy proteins, sugar alcohols, peptides, calcium/ iron, polyphenols, glycosides, sterol esters, 4-Aminobutanoic acid	The Nutrition Improvement Law Enforcement Regulations, (1996)
2	Taiwan	Health Food Control Act (HFCA), Regulations for food labeling	The executive Yuan, Department of Health	Foods containing bioactive compounds, foods with specific healthcare abilities, disease preventing, health improving foods	Health Food Control Act, (1999), Food Administration Act. 2007., Yen GC. 2003.
3	Hongkong	No specific regulation on nutrient fortified foods but referenced from the Codex Alimentarius, which issued a general principle for the 'Addition of Essential Nutrients to Foods' in 1987 and subsequently amended the principles in 1989 and 1991	Director of health, Health and Welfare Bureau	Dietary supplements, nutraceuticals, designed foods, functional foods, and natural health products	Regulatory ordinances: 1. Pharmacy and Poisons Ordinance (PPO; Cap. 138) 2. Chinese Medicine Ordinance (CMO; Cap. 549) 3. Public Health and Municipal Service Ordinance (PHMSO; Cap.132) 4. Undesirable Medical Advertisements Ordinance (UMAO; Cap. 231)
4	India	Food Safety and Standards Authority of India (FSSAI)	Ministry of Health & Family Welfare, Government of India	Dairy products, Fats, oils and its emulsions, Fruits and vegetable products, Sweets & confectionery Sweetening agents including honey Salt, spices, condiments and related products Beverages, s Other food product and ingredients Proprietary food Irradiation of food Fortification of staple foods i.e. vegetable oil, milk, salt, rice and wheat flour/maida	Food Safety and Standards Act, 2006

Sl. No	Country	Regulatory Policy	Governing body	Coverage	References
5	Sri Lanka	Drug Regulatory Authority (DRA) or Food Administration Unit		Dietary items based on the traditional wisdom of their ancestors as treatment for certain illness	Food and Nutrition Policy of Sri Lanka (2004–2010)
6	China	State Food and Drug Administration (SFDA)	The Chinese Food Composition Tables (FCT)	Enhancing the immune function, lowering blood cholesterol and sugar, lowering blood pressure, improving sleep and assisting in memory improvement. Chinese foods and traditional Chinese medicines	Approved by SFDA from July 2003 till July 2004
7	USA	Food and Drug Administration	US government	Conventional foods, supplemented foods, and natural health products	The Federal Food, Drug, and Cosmetic Act (FFDCA)
8	Canada	Natural Health Products Directorate	Canadian and US government	Conventional foods, supplemented foods, and natural health products	Food and Drugs Act, 2005
9	South Korea	Korea Food and Drug Administration (KFDA), The Korea Health Functional Food Act (HFFA), Food Sanitation Act (FSA)	1. Food headquarters 2. Nutrition and Functional food headquarters: both under the Korean Ministry of Health and Family Welfare (MHW)	Phenols (green tea, aloe extracts, Co Q10, soya isoflavones), terpenes (ginseng, red ginseng, chlorella, spirulina), fatty acid and lipids (omega-3-fatty acids, linolenic acid, lecithin, squalene, polysterol, lutein), sugars and carbohydrates (gaur gum, glucomannan, soybean fiber, wheat fiber, barley fiber, corn bran), fermented microorganisms (probiotics, red yeast rice), amino acids and proteins (soy proteins), aloe gel, chitosan etc.	The Presidential Decree issued in 2003, Ministerial Ordinance issued in 2004

Table 2.
Table depicts a list of the countries along with their functional food regulatory acts.

formulation of the Japanese regulatory systems. The label and labelling information such as the nutrient content or health claims, accompanying the product would be a primary determinant of its regulatory status. Thus, if the label claims that the

product is intended for use in diagnosis, cure, mitigation, treatment, or prevention of a disease, it would be regulated as a 'drug'. However, if the claim relates to any alteration of the structure and function of the body, with no specific reference to a disease it would be regulated as a dietary supplement [125].

One of the earliest regulatory guidelines was established by the Ministry of Health and Welfare in Japan under a policy termed 'Food for Specified Health Use (FOSHU), under which health claims for few selected functional foods was made legal (The Nutrition Improvement Law Enforcement Regulations, (1996)). The repertoire of functional foods has now expanded to include over 800 FOSHU products [92]. Following the Japanese example several countries have scripted regulatory guidelines for the health enhancing claims of functional foods and nutraceuticals. Nevertheless, regulations for functional foods have not been well established in most countries. Also, the legislations widely vary from country to country.

In the Indian context, the Food Safety and Standards Authority of India (FSSAI) under the Ministry of Health and Family Welfare, Government of India, has framed the guidelines and regulations for health supplements, nutraceuticals, foods for special dietary use, foods for special medicinal purpose, functional foods and, novel foods. The quality requirements and general monograph for such foods are defined in the Indian Pharmacopoeia, which provides guidelines on the usage of food coloring agents, flavours, quantity of nutrients to be added as per Indian Council of Medical Research (ICMR) recommended daily allowance. Interestingly, FSSAI clarifies that mere foods such as vegetables, fruits, cereals, legumes, spices, and other plant or botanicals with minimal processing such as cleaning, de-husking, de-weeding, sorting, drying, or powdering, cannot be considered as 'health supplement', 'nutraceutical', 'food with special dietary use', or 'food for special medical use'. However, the formulation of articles of food must be based on the principles of medicine of nutrition and must be supported by validated scientific data, wherever applicable. More importantly, the label and or leaflet must specify details including the specific use, intended target consumers, the physiological or disease conditions which they address, recommended duration of usage etc. [126].

A list of the countries along with their regulatory acts has been provided in **Table 2**.

4. Conclusion

Age related neurodegenerative disorders pose a serious healthcare challenge to the medical fraternity worldwide. This is a cause for concern, particularly because symptoms are evident largely during advanced stages of the disease. Presently available monotherapy and pharmacotherapy only provide symptomatic relief. Hence there is an urgent need for improved therapeutic approaches. Also, currently available pharmaceuticals are not free from adverse effects. Therefore, the world is now embracing natural sources as health promoting and disease modifying agents. Towards this objective, the use of nutraceuticals and functional foods is on the rise (**Figure 1**). While several scientific reports prove their neuroprotective functions, extensive clinical validations are lacking. Further, while some functional foods and foods for medical purposes have been evaluated by preclinical and clinical studies, the regulatory guidelines for their labelling and use are still evolving. Increasing awareness among consumers has brought the functional food market to the forefront. It is hence inevitable for authorities to formulate regulatory guidelines with respect to their labelling and usage. While some countries worldwide have put forth

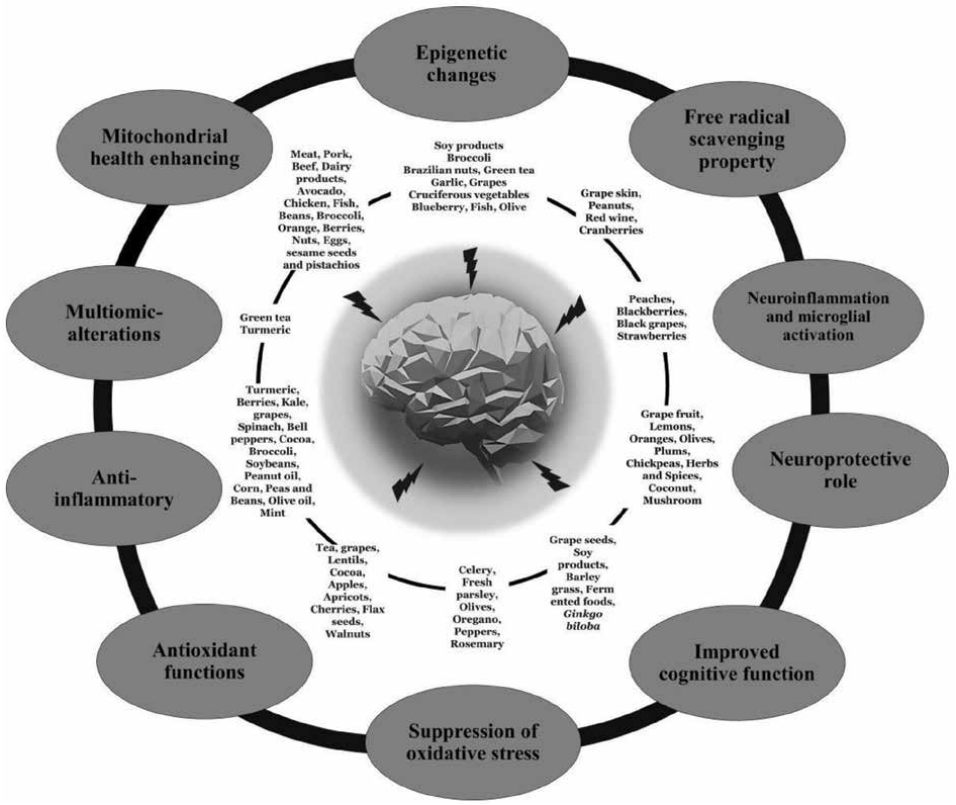

Figure 1.
Neurodegeneration, Physiological Effects, and Functional foods. Image indicates the various physiological dysregulations observed during neurodegeneration (outer circle) and an illustration of functional foods which positively modulate these effects (inner circle).

legal guidelines, many more are lagging. In addition, a unified set of guidelines across nations is absent.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 15

Therapeutic Potential of Dietary Polyphenols

Amy L. Stockert and Seth Hall

Abstract

The chapter summarizes available research on polyphenols and the potential for polyphenol based therapeutics. Polyphenols have the potential to be used in a multi-target fashion therapeutically. The majority of the polyphenol benefits appear to share positive effects across multiple disease states including inflammatory diseases, diseases of metabolic dysregulation and cancer. The reviewed literature includes human, animal and cell culture based studies. Selected mechanisms within each disease state are highlighted including interleukin inflammatory markers, NF-κB, acetyl-CoA concentration regulation of metabolism, and p-glycoprotein multidrug efflux pump associated with cancer treatment failures. Reviewed studies discuss polyphenols inhibiting transcription factors that control expression on inflammatory factors as well as activating other transcription factors that increase expression of enzymes protective of oxidative damage. Levels of metabolic regulatory enzymes are also affected positively by polyphenol addition through epigenetic modifications. Epigenetic modifications affecting cancer development and progression appear positively affected by polyphenol treatment. Additionally, oxidative damage protection of normal cells can be achieved by polyphenol treatment thus limiting chemotherapeutic damage. Upon review of the available literature, a strong case for the potential use of polyphenols in therapeutic situations stands out. Potential risks included are that the purity and specific concentrations required to achieve therapeutic benefits without potential side effects need to be examined prior to the adoption of therapeutics.

Keywords: polyphenols, therapy, natural products

1. Introduction

Polyphenols are molecules with multiple hydroxyl groups that have been shown to provide numerous health benefits including reduction of inflammation, metabolic control and anti-cancer properties. Polyphenols can be found in a variety of natural sources such as cinnamon, green tea, coffee, vegetables and fruits. Although some evidence exists for the mechanisms of polyphenol molecules that lead to these health benefits, there is still much unknown about how these compounds act to alter metabolism, inflammation levels and cancer pathways. Some research suggests epigenetic modifications that alter expression level of disease state genes explain the benefits, while others identify potential signaling pathway and transcription factor targets affected by polyphenols or their metabolites as the method of control. One difficulty in researching the potential for polyphenol based therapies is the tremendous crossover between pathways that can affect multiple metabolic actions

ranging from dysregulation of metabolism to loss of cell cycle control. On top of the multi-targeted effects from signaling, epigenetic modifications can be detected and identified but not always possible to predict. Although the field of dietary therapeutics provides a much needed alternative to many other treatments, the uncertainty of use in certain cultures make it a challenging research topic but all the more important to tackle.

Some of the most commonly studied polyphenols include the catechin group. Available evidence suggests that some forms have higher activity on some targets but the active forms differ from target to target. For example, it has been suggested that resveratrol, which is a fused ring complex between two catechin molecules, binds to and modulates the allosteric effects of Sirt1 thus playing a role in deacetylation/acetylation of multiple targets [1–3]. The metabolic regulator FoxO1 is a transcription factor that is deacetylated by Sirt1 thus changing metabolic function as well as cell death pathways [4]. Modified catechins known as epigallocatechin gallate (EGCG) are found in high levels in green tea and have been shown to modify the epigenome of MCF7 breast cancer cells [5]. Observed results show multiple types of epigenetic modifications including both methylation of the DNA and acetylation of histones. Compounds found in cinnamon include polyphenol A, a linked dual catechin, which has been studied as a potential insulin mimic compound [5]. Additional compounds found in cinnamon include rutin, an acid labile bond between quercetin and epicatechin that have been predicted to interact with Sirt1 [1]. Additionally cinnamon has been shown to increase expression of both GLUT4 expression and translocation in adipose and muscle, although the responsible component of cinnamon has not been identified with certainty [6, 7].

The collection of evidence suggesting that natural products provide profound therapeutic benefits is growing. Although pharmaceutical industries have developed drugs that are effective at treating symptoms of metabolic dysregulation and inflammation, the majority of these medicines are focused on symptom management and targeted effects. The spectrum of benefits that are possible with polyphenol based therapies offer a potential to treat multiple disease states by targeting the root cause of disease. There is still much to be learned about how the effects of polyphenols can be predicted and if cross reactivity exists that could benefit one disease state but hurt another. However, the likelihood of reciprocal effects of a therapeutic is less likely when one known effect is beneficial on a broad scale than if the polyphenol therapy were aimed at controlling only one target. As many people become concerned about potential side effects of pharmaceuticals, some find comfort in alternative medicine such as polyphenols.

2. Effects of inflammation

Recent literature has demonstrated that a growing number of disease states result from chronic inflammation [8]. Inflammation is the body's response to a variety of stimuli such as viral or bacterial infection, disease state markers, and injury resulting in tissue damage. The body has developed and refined this inflammatory response as an immune response to protect the body from damage from these stimuli. In general, an individual's immune response is elicited only as a result of the stimulus. This specific response may cause an acute inflammatory response of increased inflammatory markers which generally function to protect the body for a short period of time, but then return to normal levels. Acute inflammation, although potentially serious in some cases, can be expected to alleviate over time. In contrast, chronic inflammation is the type of inflammation that is typically associated with disease states or development of disease. Chronic inflammation is an

ongoing, constant and consistent release of inflammatory markers that ultimately result in damage to the tissues. In many cases, chronic inflammation results from obesity, continuous stress or anxiety, diabetes, and poor sleep. Autoimmune conditions can result from chronic inflammation, or can themselves add to the chronic level of inflammatory proteins circulating in the body. Oftentimes this is a whole body inflammatory response rather than an isolated location or injury as seen in acute inflammation.

2.1 Why is inflammation a health issue?

Although it appears to have taken decades to confirm the involvement of inflammation in disease risk, it seems to be a logical expectation. Inflammatory markers in circulation are chemokines that direct the body's processes. They exist simply to allow the body to detect a problem and illicit a response. Inflammation represents one of our most innate protective responses. However, it stands to reason that if the body detects a damage risk, the response will be elevated. This elevated inflammatory marker level itself can result in damage that increases the response further. The inflammatory response becomes chronic and continues in a feed forward loop continually signaling the body to respond. The effects of this endless response cycle result in disease state symptoms that require therapeutic intervention. In a similar fashion, tissue damage resulting from chronic inflammation can result in the development of diseases of metabolic dysfunction, asthma, cardiovascular disease, cancer, arthritis, among others.

It has become more common to exist in a continuous loop of stressors. Some of these stressors include: malnutrition, chronic stress, obesity, inactivity, and toxin exposure. All of these conditions can lead to chronic inflammation and result in further tissue and cell damage. Although a healthy lifestyle can certainly help to alleviate these responses, sometimes the disease state itself makes achieving the ideal healthy lifestyle nearly impossible. Careful attention to the type of foods selected can help decrease the chronic inflammation and break the continuous cycle. Certain foods and spices contain high levels of polyphenols which have been shown to help mediate the inflammatory process. Visceral obesity in particular can increase inflammation and associated markers including reactive oxygen species, interleukins 6 and 8, adiponectin and tumor necrosis factor alpha (TNF-α) [9, 10].

2.2 How can polyphenols limit inflammation?

There are several potential ways in which polyphenols can help to moderate the inflammation levels: these include targets in the signaling pathways, limitation of reactive oxygen species and reactive nitrogen species. Once generated the reaction oxygen or nitrogen species can result in reactive organic molecules as well. These reactive compounds can cause damage to the cells' genetic material, lipids found throughout the body especially in cell membranes and proteins required for cell structure and repair [11, 12].

Polyphenols show considerable potential to regulate oxidative stress and therefore inhibit inflammation [13–15]. Reactive oxygen species (ROS) are generated readily by metabolic reactions and include hydrogen peroxide, superoxide and hydroxyl radicals. These ROS can be particularly troublesome when they interact with nitric oxide in the blood vessels because nitrogen reactive species peroxynitrite is produced. Similarly, ROS and lipids circulating in the arteries can react to form oxidized lipid which can worsen the development of atherosclerosis. Some polyphenols, such as flavonoids, have the ability to scavenge superoxide and peroxynitrite thus inactivating them and preventing the almost certain cell damage [16].

In addition to inactivating ROS and peroxynitrite, some polyphenols also chelate the metal ions required for creation of these reactive species thus inhibiting their activation [17].

Several studies have identified signaling protein and transcription factor targets that can be affected by polyphenols [18–20]. In many cases the polyphenols target multiple proteins within a cascade of responses that can alter not only inflammatory response, but also mediate expression of cell death genes and metabolic function. Although initially the effect on inflammation will be discussed, there is significant overlap in signaling cascades that result in multiple effects.

Available data trends demonstrate that polyphenols are able, in part, to help regulate the expression of nuclease factor kappa beta (NF-κβ), a transcription factor that is expressed during conditions of oxidative stress [18]. NF-κβ binds to DNA when active and functions as a positive transcription factor for a variety of pro-inflammatory cytokines. An increase in inflammation can result in increased levels of TNF-α, interleukin-6 (IL-6) and enzymes inducible nitric oxide synthase and cyclooxygenase 2. Inducible nitric oxidase synthase is responsible for the production of the reactive nitrogen peroxynitirite, and cyclooxygenase 2 catalyzes the production of prostaglandins. The ability of polyphenols to interact with these factors identifies polyphenols as potentially healthful food bioactive that can help fight both the results of inflammation as well as the disease that cause inflammation. Curcumin, a polyphenol found in turmeric root, has been shown to block activation of NF-κβ, thus blocking this pathway and excluding NF-κβ from the nucleus [18]. Similarly, some polyphenols can decrease NF-κβ activity by directly interacting with subunits of the factor [21].

Some polyphenols have been found to activate the transcription factor Nrf2, which when expressed at high levels protects the cells from the damaging effects of reactive oxygen species and inflammatory markers [20, 22]. Nrf2 is a key inducer of protective mechanisms against oxidative stress, leading towards increased production of enzymes such as superoxide dismutase, catalase, and glutathione-s transferases, all of which help to modulate the ROS produced. Polyphenols have been shown to increase nuclear translocation of Nrf2, thus allowing increase transcription of the oxidative stress protective genes [22].

3. Metabolic effects

3.1 Metabolic dysregulation

The loss of proper metabolic regulation results in a variety of disease states including those associated with chronic inflammation mentioned above. Some such diseases include obesity, diabetes, hyperlipidemia, and type 2 diabetes. Patients that develop a dysregulated metabolism also tend to experience chronic inflammation and vice versa. The two health concerns feed off of each other. Metabolic dysregulation historically has been assigned to lifestyle and diet only, but more recently consideration of multigenerational epigenetic effects and environmental contributions have been included as a cause for the beginning of metabolic dysregulation [23–25]. The days of it being dismissed as caused completely by the patient are or should be past. Fetal programming has been adopted as a significant cause of metabolic syndrome in offspring that can lead to the development of obesity and insulin resistance [26–29].

Many epigenetic modifications lead to changes in metabolic function. One such example is acetyl-CoA carboxylase, the enzyme the converts acetyl-CoA into malonyl-CoA for entry into the fatty acid synthesis pathway. Galdieri and Vancura

have demonstrated that this enzyme aids in regulation of histone acetylation. Specifically they have identified that histone acetylation, one major method of epigenetic control, depends on acetyl-Co generated prior to entry into the Kreb's cycle that can be limited in conditions where acetyl-CoA carboxylase is expressed at higher levels. Interestingly, they noted that the acetyl-CoA required to acetylate histones for transcriptional regulation was more readily available when expression of acetyl-Co carboxylase was limited [30].

The importance of this acetyl-CoA – histone acetylation connection is that a global connection between metabolic activity and the transcriptional control of all genes. Any upregulation in the acetyl-CoA carboxylase, which you would expect in individuals with excessive glucose intake due to either limited balanced meal options or overindulgence in high carbohydrate foods, could potentially be inhibiting their ability to acetylate histones. Additional enzymes that contribute to the intercellular concentration of acetyl-CoA, such as ATP citrate lyase have also been identified [31]. ATP citrate lyase converts citrate formed in the first step of the Kreb's cycle to acetyl-CoA. Again this further demonstrates how food intake and availability can be communicated to cells in such a way that allows gene transcription to be silenced or enhanced.

Histone acetylation is catalyzed by histone acetyl transferases (HATs) and responsible for reprogramming gene expression along with histone deacetylases (HDACs). In general acetylation of histones in a specific gene region will increase expression of the gene, while deacetylation (catalyzed by HDACs) decreases gene expression of subsequent genes. Polyphenols have been shown to interact considerably with the HAT and HDAC enzymes and therefore have the potential to assist with re-regulation of metabolism.

3.2 Polyphenol potential for re-regulation

Numerous studies have demonstrated the potential to reverse, at least partially, some of the changes resulting from metabolic dysregulation [32–35]. Reversing or alleviating some of the inflammation associated with conditions such as metabolic syndrome may help to mediate the increased risk of cardiovascular disease with these conditions. The incidences of cardiovascular disease and diabetes are increasing globally and polyphenols offer a natural, inexpensive way to help slow the development of the comorbidities associated with these disease states. In most cases the effectiveness of the polyphenol treatment comes from its effects on insulin resistance and inflammatory reduction [32, 33, 36]. Although there seems to be significant effects on improving health with the use of dietary polyphenols, the more comorbidities a patient suffers from, the more unlikely it will benefit them to the extent necessary. For this reason, it is important that patients hoping to achieve results from polyphenol consumption begin polyphenol therapy at the first sign of metabolic dysregulation or perhaps even better, begin using polyphenols as a preventative measure. Few studies have been conducted that look at polyphenol use as an adjunctive therapy for metabolic conditions, but rather as a potential sole therapy. Similarly, the effectiveness of polyphenol therapy alone show strong ties to specific populations [34].

As discussed previously, availability of acetyl-CoA is controlled by metabolic enzymes and dietary input. Levels of acetyl-CoA also affect histone acetylation which can control transcription of a variety of genes. A proposed link of HAT activity to diabetes exists because of the interaction between the HAT, glucokinase and hepatocyte nuclear factor that relates to a transcriptional change rather than a true epigenetic change [35]. The transcriptional changes come from the increase in acetylation marks present because of the HAT activity that interacts with the

gene promoter for pro-inflammation gene products that depend also on NF-κβ for expression [35]. This example demonstrates yet another link between diseases of metabolic dysregulation and those of inflammation or cancer.

In terms of therapeutic potential of polyphenol for metabolic dysregulation, it seems that enhancing acetylation of histones is not the only benefit of consumption. Polyphenols, particularly those found in cinnamon, improve insulin resistance and improve lipid profile [32, 33, 37, 38]. Some clinical studies have demonstrated reductions of 12.9–52.2 mg/dL in blood glucose levels while others have found less robust and potentially null effects [38–40].

4. Cancer prevention possibilities

Polyphenols likely have many different mechanisms of how they can prevent proliferation and overall survival of cancerous cells. As mentioned in previous sections, there is substantial overlap among mechanisms. Due to this overlap, it is almost certain that multiple mechanisms are involved to provide the cancer prevention properties of polyphenols. It is therefore more convenient to present research based on some of the individual actions of polyphenols such as anti-oxidative properties, pro-oxidant activity, mediation of cellular signaling, and epigenetic modifications [41].

4.1 Antioxidant properties

The structure of polyphenols makes them great antioxidants due to the high availability of hydroxyl groups attached. The more hydroxyl groups present on the molecule, the greater the potential for antioxidant activity [41]. Cancer cells have been shown to increase greater amounts of reactive oxygen species (ROS) than non-cancerous cells. Through various different pathways, ROS have been shown to promote both tumorigenesis and the proliferation through mechanisms such as angiogenesis and the promotion of cell migration [42]. Flavonoids have been shown to lower the amount of ROS by scavenging free radicals, chelating of transition metals that help form further ROS, and regulating oxidative stress-mediated enzyme activity [43]. Research has shown that rats treated with epigallocatechin gallate (EGCG) had increased levels of antioxidant enzymes [44]. Lowering ROS levels results in the prevention of cancerous cells to undergo proliferation or migration.

4.2 Pro-oxidant activity

Effectiveness of cancer treatment can also be improved by modulating oxidative levels in the cells. Oxidative stress can damage cells and cancer cells have an increased capacity to handle oxidative damage. Taking advantage of this increased capacity, cancer cells can somewhat be recognized more specifically. Therapies involving polyphenols generally reduce oxidative damage, but in cancer cells the standard signaling is already modified, thus some polyphenols actually increase oxidative damage to a level in which apoptosis and therapeutic sensitivity increased. Research shows that cancer cells undergo changes to better handle the high levels of ROS in their environment such as generating higher levels of nicotinamide adenine dinucleotide phosphate (NADPH) [45–47]. This better equips cancerous cells to resist the effects of oxidative stress that can lead to apoptosis. However, this resistance is still able to be overcome by increasing the amount of ROS to a level more than the cells can handle.

Many polyphenols have been shown *in vitro* to have pro-oxidant activity by utilizing transition metals already present in biological systems to create more ROS and overcome the natural resistance that cancerous cells possess [48–50]. Vitamin C has been shown in high doses to inhibit tumor growth as well as metastasis without harming non-cancerous cells present. Ascorbate as a standalone treatment has been shown to reduce both tumor growth and weight by 41–53% in Ovcar5, Pan02, and 9 L tumors. It was also shown to reduce the amount of metastases that were present in approximately 30% of 9 L glioblastoma control groups [51]. This shows promise, as a difficulty surrounding cancer treatment is the incidental harm of non-cancerous cells simultaneous to cancerous cells. Potentially utilizing natural polyphenols already present in biological systems may be a way to work around this issue. Another class of polyphenols, hydroxycinnamic acids, has shown the ability to damage DNA molecules in the presence of Cu(II) ions [52]. Further studies are crucial in determining the *in vivo* ability of polyphenols to replicate results shown *in vitro*.

4.3 Mediation of cellular signaling

As mentioned in both the inflammation and metabolism sections, NF-κβ is an important component in the inflammatory nature of cancerous cells. It is believed that it is the primary factor responsible for inducing a variety of cancer molecules such as adhesion molecules, growth factors, angiogenic proteins, cell proliferation proteins and inflammatory cytokines [41]. NF-κβ also increases expression of inhibitors of apoptosis and suppresses the expression of genes involved in cell death [53]. Research has shown that polyphenols have the ability to interfere with NF-κβ's mechanisms specifically involved with cancer. Flavonoids disrupt inhibitors of kappa kinase (IKK), an activator of NF-κβ, as well as binding directly to NF-κB and preventing its binding to DNA [41, 54, 55]. The mediation of polyphenols in these pathways can provide valuable anti-inflammatory benefits that can both prevent the formation of cancerous cells and tumors, as well as removing the suppression of apoptosis that is caused by NF-κβ leading to cell death.

4.4 Epigenetic modifications

Methylation of specific cancer genes has become a key predictor of both markers of cancer and cancer survival. One example of this is the methylation state of the BRCA1 promoter gene in ovarian cancer. Research showed that patients with a higher level of methylation on the gene, had a shorter median for disease free interval. It also showed that facilitating demethylation of the gene results in increased survival time and decreased occurrence rate [41, 56]. DNA methyltransferase (DNMT) is the enzyme responsible for methylation of genes. Polyphenols have been shown to decrease methylation by inhibiting DNMT. *In vitro*, DNMT was inhibited by EGCG at a concentration of 20 μmol/L [57]. The ability for polyphenols to inhibit DNMT and other methyltransferase enzymes makes them of interest for not only cancer prevention, but other disease states that are affected by an increase in methylation of DNA [41]. Research in this area should continue so a greater understanding of the ability for polyphenols to affect methyltransferase enzymes such as DNMT.

4.5 Cancer treatment adjunctive therapy

Cancer therapies are an ever changing area of interest as we better understand ways to induce cancer cell death as well as maintain the health of non-cancerous

cells in the body. Resistance to chemotherapies is also an area of concern making it difficult to achieve appropriate therapy and leading to more aggressive treatments which leads to an increase in harm to non-cancerous cells. One of the ways that cancer cells present resistance is in the increase of the multidrug resistant p-glycoprotein transporter. The p-glycoprotein transporter pumps the drugs out of the therapeutic intracellular location. Curcumin has been shown to suppress the action of the multidrug resistant p-glycoprotein transporter. Sulfasalazine, a specific substrate for the multidrug resistance protein ABCG2, was shown to have an increased Cmax concentration in the presence of a 400 mg/kg dose in mice. The change was 1230 ng/mL in the absence of curcumin and 3350 ng/mL with curcumin present [58]. This would in turn result in an increase in concentration of medication inside the cancer cell and therefore greater efficacy of the therapy making curcumin a good possibility for adjuvant therapy. Another issue arises during chemotherapy in the possible need to increase ROS to produce apoptosis of cancerous cells [59]. These ROS also negatively affect non-cancerous cells so the need to protect these cells is crucial to ensure appropriate chemotherapy can continue. As mentioned above, one of the mechanisms in how polyphenols can prevent cancer is through their antioxidant activity. Through this mechanism, polyphenols can provide valuable adjuvant therapy for patients allowing them to prolong their chemotherapy without increasing negative effects associated with the increase in ROS.

5. Conclusion

The potential for polyphenols to be used therapeutically appears more probable as more research is completed. Additionally, the benefits likely cross into multiple disease states with positive effects for them all. Pro-inflammatory transcription factor NF-κβ is inhibited by binding of polyphenols, thus limiting the expression of harmful inflammatory factors. Similarly, NF-κβ activity can also be blocked by limiting its activation via the signaling pathway. While limiting NF-κβ activity limits inflammation, activating the positive transcription factor Nrf2 increases expression of enzymes protective of oxidative damage. Studies demonstrate that polyphenols activate Nrf2.

From a metabolic perspective, limiting inflammation is also ideal, thus overlapping benefits are observed from the anti-inflammatory effects described when considering metabolic dysfunction. Limitation of NF-κβ activity provides a protective effect against the inflammatory state caused by obesity. Polyphenols have also been shown to improve insulin sensitivity and metabolic regulation by modifying the level of acetyl-coA directly. Levels of acetyl-coA strongly determine the activity of acetyl-coA carboxylase which can regulate metabolic flux. Polyphenols also affect the expression of acetyl-coA carboxylase by altering the activity of histone acetyltransferase (HAT) enzymes. These enzymes along with histone deacetylase enzymes (HDAC) appear sensitive to polyphenol interaction and are responsible for epigenetic reprogramming that alter gene expression level. Modifications at the epigenetic level offer re-regulation of metabolism.

A role in cancer prevention and adjective therapy also emerges as studies show that epigenetic modifications by polyphenols can limit expression of tumor growth proteins while protecting cells from oxidative damage by traditional therapies. Much of this effect is also due to the limited activity of NF-κβ and activation of Nrf2 that was described previously. Limiting inflammatory proteins while also protecting against oxidative damage reduces the risk of DNA damage that could develop into cancer. In addition to the effects on HATs and HDACs, polyphenols have also been shown to affect DNA methyltransferase (DNMT) activity. These enzymes,

responsible for methylation and demethylation of DNA, are inhibited by polyphenols in general and can limit the methylation level at a gene promoter, thus allowing its expression. One such study showed higher levels of methylation at the BRCA1 gene promoter resulting in decreased expression and shorter interval before disease return. Treatment with catechin polyphenols limited the methylation of BRCA1 and increased disease free intervals. Early studies of adjunctive therapies with polyphenols demonstrate a potential for polyphenols mediated transition metal increased oxidative damage to the cancer cells that can overcome the cancerous cells' increased ability to handle oxidative stress and therefore achieve cell death. There is still much to be studied regarding this pro-oxidant effect of transition metal interaction with polyphenols, but the potential to target cancer cells more directly is encouraging.

A summary of the potential for polyphenols to be used to reduce inflammatory markers, particularly the one associated with a most negative outcome, are suppressed with the addition of certain polyphenols. An overlap exists between the suppression of inflammatory factors and the potential for metabolic re-regulation. In addition to the effects on metabolic regulation achieved by decreasing inflammation, polyphenols also offer improved support of metabolic regulation via metabolic enzyme control and transcription factor mediation. Epigenetic effects cross over between metabolic control and anti-cancer potential, demonstrating again the potential for multi-targeted benefits.

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Section 4

Product Based Functional Foods

Chapter 16

Flour-Based Confectionery as Functional Food

Sanja Oručević Žuljević and Asima Akagić

Abstract

Nowadays, the flour-based confectionery industry is facing different challenges in reducing caloric and increasing nutritive values in order to produce healthier products, given that consumption of flour-based confectionery products has been growing steadily worldwide. In addition to wheat flour, these products include sugar and fat, which contribute to high energy value, but have few micronutrients and are mostly poor in nutritional terms. Due to frequency of consumption, they can harm a balanced diet, especially when it comes to children and young people. Flour-based confectionery is highly suitable for enrichment with ingredients that have pronounced functional properties. In this sense, the text offers some possibilities for improving such products through different approaches and presents new trends in developing functional, flour-based confectionery by using different supplements that could decrease caloric value, improve nutritional and non-nutritional values and develop products with pronounced functional properties.

Keywords: flour-based confectionery, nutritive and non-nutritive improvements, reduction of caloric value

1. Introduction

Confectionery products are concentrated food items that belong to the group of energy-rich foods obtained through treatment of carbohydrate raw materials during industrial or handcraft processing. They are mostly characterised by sweet taste, as the main ingredients are sucrose and other sugars. They are consumed on a daily basis depending on individual desires, habits and customs, regardless of the consumer's age, gender or status and it is food that enjoys great popularity among wide range of population.

According to their ingredients, methods of production, and final product, confectioneries can be divided into three main categories: sugar confectionery (candies, caramels and others), chocolate and chocolate products and flour confectionery (biscuits, cookies, crackers, wafers and others). This is just a rough classification of confectionery. Precise classification is not simple because of overlapping categories and new complex products constantly being created. Today we can find a lot of different products as a combination of these three categories.

The chocolate, biscuits and confectionery industries represent a prominent and dynamic food sector in Europe. The latest Statistical Bulletin demonstrates that the chocolate, biscuit & confectionery industry is a major contributor to the European economy. According to data collected from Eurostat, the production in Europe has

increased by 2.3% in 2015, reaching 11,736.975 metric tons. This shows that the passion to create innovative treats and bring pleasure to consumers remains vivid among these sectors [1].

Flour-based products are the most often consumed ones among the confectionery in general and their growth in the market has been boosting per capita consumption around the world.

Due to the popularity and increasing interest in the functional food concept, the relationship between food and health has an increasing impact on food innovation. Nutrition knowledge has been used to improve consumer health which represents the functional food concept in general.

As the role of diet in the prevention of human ailments such as cancer, cardiovascular diseases and obesity has become more evident, many consumers are increasingly seeking functional foods to improve their diets. Consequently, there is a trend to search for natural raw materials rich in dietary fibre and high in antioxidant capacity as functional ingredients for the food industry [2].

Regarding flour-based confectionery, these efforts are especially important, as children and young population consume such products gladly and very often, and sometimes without control. Uncontrolled consumption of high-calorie products in unnecessary amounts, especially confectionery, can harm balanced diet and consequently lead to obesity.

Therefore, confectionery manufacturers are facing two different challenges. New products need to develop with improved health-promoting properties and at the same time they should be tasty and look like conventional confectionaries that are popular and regularly widely consumed.

2. Definition of flour-based confectionery

Confectionary products with flour as the main ingredient include a great number of different products that vary not only in their formula but also in the way they are manufactured. They are generally made of soft wheat flour. Understanding the common terms is sometimes rather difficult because of numerous kinds of such products and their different names. For example, *cookie* is a term used in the USA, defined by a formula high in sugar and shortening and relatively low in water. Similar products made in Europe and the United Kingdom are called *biscuits*. The biscuits made in USA are more accurately defined as chemically leavened bread. In addition, a number of products do not fit to this definition of cookies but are still called cookies mainly because they do not fit elsewhere [3].

Cakes are characterised by a high level of sugar in the formula. The difference between cookies and cakes is that cakes also contain relatively high levels of water. Because the molar sugar concentration is much lower in cakes than in cookies, the starch gelatinises during baking. Therefore, cakes set when baked, giving a light product. The specific structure of the cakes is obtained by incorporating air into the batter in form of small air cells during mixing. As with cookies, the definition of crackers must be quite broad, as there are many types of crackers. In general, crackers contain little or no sugar but moderate to high levels of fat (10–20%), based on flour weight. The doughs generally contain low levels of water (20–30%) [3].

In addition to flour, main ingredients incorporated during flour-based confectionery manufacturing are sugar and fat. The other ingredients such as milk, eggs, salt, aerating agents, emulsifiers, flavour and colour can be included as well. Water is added in different amounts to connect ingredients and to make dough. The water acts as a solvent, too. The main technology operations in flour-based confectionary are: mixing and moulding, baking and cooling. Within these basic operations,

a great number variations and different regimes can exist. This is why we can find a great number of different kinds of products on the market today.

Ingredients play an important role in creating an acceptable product. Whether alone or together, each ingredient contributes an important quality to the final product. Flour, liquid, sugar, leavening agent, eggs, and fat are present in a proportion that, when properly mixed, make a quality product [4]. Thanks to high amount of carbohydrates and fat, flour-based confectionary products are very rich in caloric value. Caloric values for cookies and other similar products range from 1400 to 2300 kJ/100 g [5].

3. Improvement of flour-based confectionery products

All main ingredients in flour-based confectionery products contribute to high energy value. On the other hand, they are low in micronutrients and generally poor in a nutritive sense. Hence, there are two basic trends in confectionary industry: improving nutritive value and at same time, reducing the energy value.

Nutritive value can be improved by: using wholemeal and flour with high extraction rate instead of white wheat flour, and other cereals with evident considerable nutritive value, adding fruit and other high nutritive ingredients.

In addition, the energy value in confectionery products can be reduced by decreasing the sugar content, substituting sugar partially or completely by different low-energy sweeteners and reducing fat in formula or replacing the fat with some suitable substituents.

Flour-based confectionery products are considered as good material for fortification and nutritional and non-nutritional improvement to make a new product with functional properties.

Flour is a basic ingredient and it is supposed to be of optimum quality appropriate for a certain product. Thus, probably the greatest influence on improving the functionality and nutritive value of flour-based confectionery just involves the replacement of flour in recipes with other kinds of flours, such as wholemeal wheat flour and flours obtained from other cereals and non-cereals with evident nutritive and non-nutritive values like oat, barley, rice, soy, buckwheat, flaxseed etc. Whole grains are rich in dietary fibre, trace minerals, antioxidants, and phenolic compounds, which are important for human health because they reduce risks of different diseases like cancer, diabetes, obesity, and cardiovascular diseases [6]. The importance of dietary fibre in the human diet is widely accepted, and over many years, extensive research has been undertaken on the enrichment of food products with fibre [7].

Some pseudo-cereals like buckwheat, amaranth and quinoa are especially interesting not only because of their high nutritive and non-nutritive value but also because of the fact that they do not contain gluten.

In addition, protein fortification of biscuits is of current interest and they can be prepared from composite flours, such as wheat flour fortified with soy, cottonseed, peanut, corn germ flour or mustard flour [7], and with supplementation of health-promoting ingredients like whey protein concentrate and skimmed milk powdered [8].

Fruits have received much attention recently as a source of biologically active substances because of their anti-oxidant, anti-carcinogenic and antimutagenic properties and they had an important relevance for confectionary industry, especially for biscuits, cakes and the other bakery products.

As mentioned earlier, flour-based confectionery belongs to high calorie food, and a trend to reduce the calorie value of those products has gained considerable

importance recently. Reducing or substituting sugar for other sweeteners, reducing fat in recipes and complete elimination of fat could decrease the caloric value in flour-based confectionery in general.

Changing the recipes to improve nutritive and non-nutritive values of products could at the same time lead to decreasing the caloric value and glycaemic index, too. For example, if wholemeal flour is used instead of white flour, the content of fibre in a product will rise, and the starch content will at the same time decrease. So, the content of digestible carbohydrates (4 kcal/g) will be decreased. In addition, using honey in recipes can slightly reduce caloric value and the glycaemic index, as honey is sweeter than sugar and is incorporated in recipes in a lower amount [9].

Nevertheless, microencapsulation should certainly be pointed out as a relatively new technique in improving of flour-based confectionery, as well.

In general, flour-based confectionery is supposed to be of great nutritive and non-nutritive quality, low in caloric value and above all, they should be not just edible, but desirable with attractive sensory properties and texture, and packed in appropriate well-designed packing. All efforts in improving these products are supposed to be followed by sensory evaluations as a kind of final approval to ensure better competitiveness in the market of this kind of food. The texture, flavour and appearance of these products are major attributes that affect their acceptability. In fact, sensory analyses should cover all results of scientific and other researches that investigate improving and promoting food quality.

3.1 Nutritive and non-nutritive improvement of flour-based confectionery

Different cereals, legumes and fruits have been widely recognised as important sources for improvement of flour-based confectionery and provision of functional properties. There are a lot of scientific studies that investigate influences of different plant material on fortification of biscuits, cakes and other bakery products.

3.1.1 Wheat flour replacement and fibre increase

The most common source of dietary fibre in bakery products has been bran from various cereals and non-cereals.

Nevertheless, using only wheat flour of higher extraction rate rather than white flour for biscuit production can have positive effects on the antioxidant activity and general quality of the product due to the incorporation of higher content of external grain components in flour when extraction rate is higher. The flour extraction rate has a strong effect on the physical and chemical quality of flour. Antioxidant capacity of biscuits and total phenol content significantly increased when flour extraction rate was higher and was the highest in the samples produced of flour when extraction rate was up to 90%. The same biscuits had the highest rating for overall sensory impression [10].

A total phenol content and overall sensory evaluation of biscuits made of wheat flour and mixtures with other cereals is presented in **Figure 1**. Wheat flour with the extraction rate of up to 90% was blended with spelt, barley and buckwheat flour with similar extraction rate in different ratio: 25, 50, 75 and 100% of non-wheat flour in mixture [11].

Spelt (*Triticum spelta* L.), an ancient wheat species, was one of the major feed and food grains in ancient Europe [12]. This crop has very modest requirements in terms of environmental conditions and production technology. It is highly resistant to diseases and pests and it has very modest requirement in terms of fertiliser, which makes it very advantageous for organic production. Recent interest in use of spelt for ecologically grown foods has led to resurgence in its cultivation and research regarding the possibility of its utilisation [13–15].

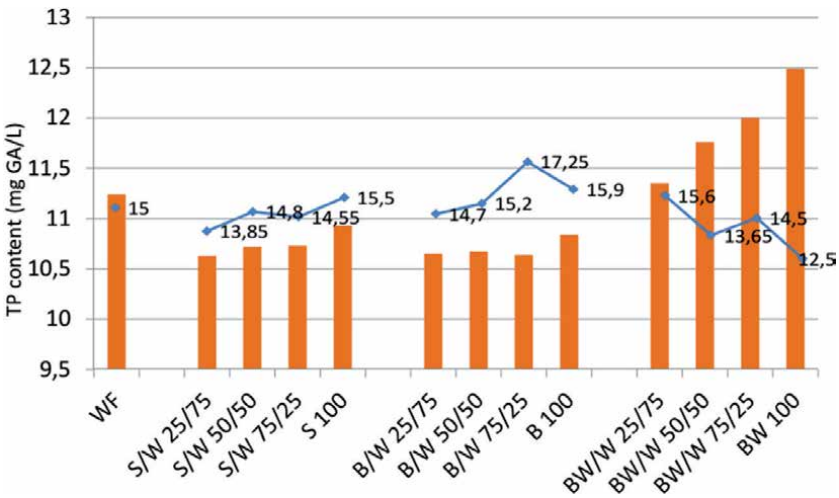

Figure 1.
Total phenolic (TP) content and overall sensory evaluation – Blue line (20 points maximum) of biscuits made of wheat flour (WF) and mixture of WF with non-wheat flour in different ratio (data from the author's private archive). WF – Wheat; S/W – Spelt/wheat; B/W – Barley/wheat; BW/W – Buckwheat/wheat flour; flour blends: WF with spelt, barley and buckwheat flour in 25, 50, 75 and 100% share.

Barley is gaining renewed interest as an ingredient for production of functional foods due to high concentration of bioactive compounds [16–19], especially because of high β-glucans content which ranges up to 9.0 (%, dry weight) [20] and is successfully used in flour-based confectionery such as biscuits and cakes. With its slightly nut-like aroma, it contributes to a unique flavour of these products and improves their nutritive values. Barley flour addition decreased spread ratio and increased antioxidant activity as the proportion of barley flour increased [21, 22].

The highest total phenolic content was recorded in samples made with buckwheat flour, and it was higher as share of buckwheat flour was increased (**Figure 1**). In addition, same samples were baked on two different temperatures (150 and 205 °C) to find out whether the baking temperature had any influence on the biscuit quality. Better physical properties were achieved when the baking temperature was 205 °C rather than 150 °C. Baking on 150 °C for prolonged time causes total phenol content reducing and makes some sensory properties worse. The best score for overall sensory evaluation had biscuit samples made of flour blend wheat/barley with high share of barley flour (75%). Nice and attractive nutty flavours were marked in these samples and they increased with the increase of the share of barley flour [11].

β-glucans content in same biscuits samples with barley flour was increased with the increase of the content of barley flour, and after 6 months of storage, the β-D-glucans content in all samples decreased slightly [23].

Over the last two decades, interest for buckwheat and related products has increased, especially in bakery products. Botanically, buckwheat does not belong in cereals but is studied together with them due to the same manner of utilisation [24]. Buckwheat flour has a huge potential in terms of improving the baking products in respect of considerable content of antioxidants, especially polyphenols and tocopherols. Its polyphenols are represented by phenolic acids and flavonoids, mainly rutin, a proven potent antioxidant. Due to a relatively high content of antioxidants in light and wholegrain buckwheat flours, they are used for substitution of wheat or other cereal flours in bakery, pasta and confectionery formulations in order to create either added value or gluten-free products [25].

However, a high content of buckwheat flour in biscuits deteriorates physical characteristic and sensory attributes (**Figure 1**). Total phenolic content in biscuit samples made with buckwheat flour was significantly higher than in samples made of wheat flour and other composite flours, but overall sensory score decreased with the increase of the content of buckwheat flour in the recipe and it ranged from 15.6 (good sensory quality) in samples with 25% buckwheat flour added to 12.5 (satisfied sensory quality) in biscuits made of buckwheat flour only [11].

In addition to high nutritive and non-nutritive potential of buckwheat, we cannot forget tartary buckwheat (*Fagopyrum tataricum* Moench), which contains up to 100 times the amount of rutin found in common buckwheat [26]. Tartary buckwheat showed about 9 times higher antioxidant activity in comparison to common buckwheat and about 20 times higher total phenol content [27]. The importance of tartary buckwheat is widely recognised, but some disadvantages of its usage in flour-based confectionery should also be taken into account, as particles size and share of bran in tartary buckwheat flour. Besides, in final products with tartary buckwheat the slightly bitter taste can appear [24].

Enrichment of biscuits can be achieved by partial replacement of wheat flour with chia seeds [28]; with defatted maize germ flour [29] and with flaxseed [30–32].

Sesame seeds, as the main ingredients, add a nutty taste and a delicate aroma to tahini halvah. Besides making halvah, sesame seeds are widely used for the preparation of rolls, crackers, biscuits and cakes [33].

Dietary fibres in the bran and germ of cereal grains have been added to biscuit formulations that originally contained almost no dietary fibres in original recipes. Purple rice is a good choice to replace wheat flour because it contains high fibre contents and a range of antioxidants; and has a considerable potential for use in biscuits and related products [34].

Substituting wheat flour with germinated brown rice flour for sugar-snap cookies resulted in increasing the residual moisture content and decreasing the hardness. Cookies with acceptable quality and improved nutrition can be prepared by partial or complete replacement of wheat flour with the heat-moisture treated germinated brown rice flour [35].

Fibres of different cereals (wheat, rice, oat, and barley) caused different changes in biscuit dough behaviour. For a bran level of addition from 10% to 40%, oat and rice bran increased water absorption less than barley and wheat bran. Also, dough development time was higher in the case of wheat and rice bran blends than for doughs with oat and barley brans. Dough stability, which indicates dough strength, decreased significantly in the case of oat and barley blends, whereas the extent of decrease was relatively marginal in the case of wheat and rice bran blends. Extensibility values were greatly reduced by the addition of bran from either of the sources. The sensory quality of biscuits was acceptable at 20% for wheat bran and barley bran and 30% for oat bran only [36].

Jerusalem artichoke powder and cocoa beans shell powder additions in biscuit formulation had considerable effects on the physical–chemical properties of biscuits. The nutritional value increased due to the content of dietary fibre increased [7].

Through the substitution of wheat flour with malted barley bran, it is possible to produce nutritious and consumer acceptable cookies especially at 5% substitution level [37]. One possibility to increase the content of fibres in the diet is to enrich products with pure isolated fibres. Fibre-rich preparations are produced primarily with the use of parts of cereals, fruit and vegetables which are by-products from milling and fruit and vegetable processing [38].

3.1.2 Milk and whey powder in flour-based confectionery improvement

Usually, milk is a popular liquid for use in cake batter. In addition to contributing water, milk adds flavour and nutrients especially protein and contains certain compounds that help produce a velvety texture, a creamy white crumb, and a browner crust. The lactose in the milk participates in the Maillard reaction resulting in a brown crust [4]. Adding of milk derivatives also were reported to help the product to brown during baking and add to its nutritive value.

Whey is an excellent source of proteins which are considered to be high quality proteins that contain all of the amino acids required by humans, as well. Whey proteins were found to contain relatively high amounts of lysine, a dietary essential amino acid, that in sometimes limiting in the diets for humans, particularly those high in cereals. Increasing the levels of supplementation with whey powder resulted in a significant increase in the score of aroma of biscuits. Biscuits made from wheat flour supplemented by 10% whey powder showed the best scores for overall acceptability [39].

The nutritional value of whey proteins depends on the favourable ratio of amino acids, especially essential amino acids. Cysteine, lysine, and tryptophan proteins of whey have a better biological value and the favourable ratio of cysteine/methionine has a better biological availability in the organism when compared to other meat-based or plant-based proteins [40]. Whey proteins, due to their nutritional value and functional properties, can be an acceptable alternative to carbohydrates and fats and they can be a highly valued protein component in gluten-free baked goods.

3.1.3 Fruit and related products in flour-based confectionery

The incorporation of fruit in different forms into recipes for production of flour-based confectionery is a good way to improve not only nutritive and non-nutritive value but also physical and sensory quality of final products.

A number of researches confirmed a possibility to develop different flour-based confectionery with improved functional characteristics using fruit and fruit products and by-products like pineapple powder [41]; banana flour and sesame seeds - cookies made with formulation 20% banana flour and sesame 8% had high antioxidant capacity with good stability in storage time [42]; raspberry pomace [38]; mango kernel and seed to improve protein content [43]; citrus dietary fibre as a potentially functional ingredient in biscuits and other sweet bakery products [44]; white grape pomace for the novel formulation of biscuits as an alternative source of dietary fibres and phenols [2] and blackcurrants and jostaberry powder as good sources of antioxidants and fibres [45].

By-products from fruit processing are pomace, spent fruit or fruit stones. These contain considerable amounts of bioactive components including dietary fibres which are highly desirable for dietary purposes. Using by-product raw materials such as raspberry and blackcurrant seeds in the form of food additives is one of the ways to increase health properties of food and prolong their shelf life [38].

Berries have a good nutritional profile and attractive sour–sweet taste and colour, mainly blue, red or purple. They are typically high in fibre, vitamin C, antioxidant and polyphenols and present a good choice for flour-based confectionery improvement.

Blueberries can contribute both flavour and nutritional value to cereal-based foods, adding fibres, vitamins and minerals, and only low levels of sugar and fats. Most importantly, blueberries are rich in plant polyphenols that can complement cereals, which are rich in fibre but low in total phenolic [46].

Fruits of the forest plant species can be used as basic or complementary raw material for production of a series of very nutritious food products (jam, marmalade,

juices, carbonated soft drinks, distillates) or as ingredients to confectionery and bakery products. Great importance is ascribed to the group of herbaceous and woody plants, among which the most important are: Cornelian cherry (*Cornus mas L.),* woodland strawberry (*Fragaria vesca*) and bilberry (*Vaccinium myrtillus L.*) as excellent sources of phenolic compounds, anthocyanin contents and other bioactive components. These species are very rarely attacked by plant diseases and pests. Apart from features of genetic durability and resistance, fruits have medicinal properties deriving from an abundance of very valuable components. All this resulted in a greater demand for organically grown food, rich with biologically valuable components, and products based on these varieties can certainly meet this demand [47].

Numerous studies have dealt with apple powder or pomace addition in cookie formulation and the influence on final product quality: effect of the addition of commercial apple fibre powder on dough characteristics and the physical and sensory properties of cookies [48]; the nutritional value, sensory evaluation and the hypolipidemic effect of biscuits fortified with apple fruits powder [49]; influence of pineapple, apple and melon by-products on physicochemical and sensory quality [50] and nutrition, rheology of dough and quality of cookies with apple pomace powder [51]. Replacing wheat flour with 15% or 30% of apple pomace resulted in 3–6% of fibres in the finished product [52].

In terms of apple processing autochthonous cultivars are becoming more and more interesting as they are more tolerant to diseases and highly rich in nutritive and non-nutritive compounds such as polyphenols and can improve final products [53].

A potential raw material which can be used for production of food with added nutritional value is tomato pomace, a by-product from tomato processing: a tomato pomace powder [54] and insoluble tomato fibre as alternative source of bioactive components in cookies [55].

3.1.4 Honey and bee products in flour-based confectionery

Honey and other bee products (pollen and propolis) are considered as valuable components for food improvement, widely respected for their high nutritive value and protective characteristics. Besides acting as a sweetener, honey contributes to more intensive aroma, thanks to its unique flavour, and in same time balances and enhances the flavour profiles of other ingredients. Honey also extends the shelf life of bakery foods naturally, and products that contain honey dry out more slowly and have a lesser tendency to crack. It caramelises quicker than sucrose and gives a darker appearance to final products [9].

The honey biscuits made from buckwheat, rye, spelt and wheat flour are products with good quality and long-term shelf-life and meet the parameters of a dietetic food because of low percentage of fat [56].

The applications of bee pollen as a dietary supplement and finding an optimal recipe for biscuits have been studied and according to obtained results, the addition of bee pollen did not affect the fat content in biscuits; it had a statistically significant effect on sugar, protein, ash, fibres, as well as the content of polyphenols and antioxidant potential. Biscuits that had been improved with bee pollen were characterised by higher penetration work and a darker surface when compared to the control ones [57].

3.1.5 Some examples of cakes improvement

It is already known that the particle size of wheat flour can influence the quality of flour-based confectionery. This is particularly important when we talk about cakes. Those products usually include eggs in their formula and are softer than biscuits.

Eggs affect the texture of cakes in several ways. They perform emulsifying, leavening, tenderising and binding functions. They also contribute colour, nutritional value and desirable flavour and they are essential for obtaining the characteristic quality of most cakes. The cakes are relatively high in sugar and shortening. A typical cake formula contains quite a lot of water and depends on air incorporated during mixing for much of its leavening. Due to eggs and the intensive mixing process, the dough/batter for cakes is tenderer and has a higher specific volume than the dough for bread and biscuits.

The differences between dough and batter are reflected in texture: dough consists essentially of flour and liquid as milk or water and is stiff enough to knead or roll, while batter is made of flour, eggs and milk or water and is thin enough to pour or drop from a spoon.

Batter for cake making or sponge foam is a colloidal system composed of liquid and gaseous phases where the air presents the dispersed phase. The air is distributed in the form of very fine bubbles more than 60% in the mixture. That is why the specific weight of the batter ranges from 0.3 to 0.5 g/mL [5].

Due to such fine and foamy structure of the cake batter, the particle sizes of the flour should be very fine and uniform.

White wheat flour is suitable for cake production, while flour with high milling extraction rate does not have an adequate quality for cake making, because of the high presence of bran.

Possible increase in the fibre content of industrial cakes can be achieved by adding fine milling wheat bran of small particle sizes (32–100 μ - 55% and 15–45% below 32 μ). Wheat fibre were added (1, 2, 3, 4 and 5%) in three types of flour (T-400, T-500 and T-850). The type of flour is determined on the basis of the ash content (%) in the flour and is closely related to the milling extraction rate. Wheat flours T-400 (ash content up to 0.45%) and T-500 (ash content 0.46–0.55%) belong to white flours. T-850 flour contains ash in the amount of 0.8 to 0.9% and belongs to semi-white wheat flours.

According to the obtained results excellent sensory properties (shape, structure, chewing, smell and taste) showed samples made of flour T-400 with fine wheat fibre added up to 5% and samples made of flour T-500 up to 4% of wheat fibre [58]. These samples can be treated as *Source of fibre* according to EC Regulation on nutrition and health claims made on food [59].

Cake samples made with just T-850 flour and 1–5% fine wheat fibre added elicited inferior results with significantly smaller diameter and height and greater weight which is not in accordance with the parameters determined by the manufacturer's specification. Acceptable values determined by the manufacturer for this product are: diameter 52–55 mm, height 7–10 mm and weight 4.7–5.0 g [58].

An excellent way to improve nutritional value of cakes is using omega-eggs in formula instead of common eggs which can reduce omega-6 and omega-3 fatty acid ratio in cakes.

Several sources of information suggest that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids of approximately 1, whereas in Western diets the ratio is 15/1–16.7/1. Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared to the diet on which human beings evolved and on which their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular diseases, cancer and inflammatory and autoimmune diseases, whereas increased levels of omega-3 (a low omega-6/omega-3 ratio) exert suppressive effects [60].

Cake samples made with omega-eggs (obtained by enriching the chicken feed with fishmeal or flax) contain 5 times more omega-3 fatty acid approximately 10 times more EPA - C 20:5 (n = 3) and 2.8 times more DHA - C 22:6 (n = 3) than standard samples. Moreover, omega-6/omega-3 fatty acid ratio in samples made with standard eggs was 14.94 and 2.84 in samples made with omega-eggs. Using omega-eggs instead of standard eggs does not affect the specific weight and viscosity of the batter. Differences in basic chemical composition of cake samples produced with standard and omega-eggs are negligible and do not influence the general cakes quality [61].

3.1.6 Gluten-free flour-based confectionery

Gluten proteins are storage proteins in wheat, situated in kernel endosperm. Gluten is responsible for dough development and for forming the viscoelastic properties. In addition, the gluten directly influences the dough's ability to retain gas. The role of gluten in bakery products making is crucial. However, recently we are aware of an increased interest in gluten-free diet because of the increased incidence of celiac disease, gluten intolerance and allergies. However, gluten-free diet for people who have never been diagnosed with celiac disease or gluten intolerance can lead to unbalanced nutritional diet caused by low dietary fibre intake.

The European Union (EU) adopts common rules concerning the composition and labelling of foodstuffs intended for people suffering from intolerance to gluten (coeliac disease). Food labelled *gluten-free* must contain less than 20 mg/kg of gluten in the finished product, and *very low gluten* must contain less than 100 mg/kg of gluten in the finished product [62].

Celiac disease is widespread and is often underdiagnosed. It can affect a variety of genetically susceptible people from the young to the old. Presently, the only treatment for celiac patients is lifelong gluten-free diet. Scientists and technologists continue in their quest to improve the quality of gluten-free products and their main goal is to create a product of a similar standard to the gluten-containing products. Studies are focused on ingredients and processing methods which have been documented to develop or improve the processing characteristics and nutritional properties of gluten-free products [63].

Many studies have investigated the properties of gluten-free biscuits using different types of cereal which do not contain gluten (maize, rice and rice starches); sorghum and pearl millet flours; and pseudo-cereal flours.

Some formulations of gluten-free baked goods include additional starch to increase viscosity and create an appropriate texture of the gluten-free dough or batter and of the finished product. Potato, corn, rice, and tapioca starches are common ingredients in gluten-free baked goods [64]. Pseudo-cereals such as amaranth, buckwheat and quinoa are valuable nutritious ingredients in gluten-free formulations thanks to their high protein quality and abundant quantities of fibre and minerals such as calcium and iron. Buckwheat is especially a good choice for production of these products when it comes to its proteins and high content of valuable bio-components.

The use of buckwheat flour generally has positive results in the finished product, particularly when a hydrocolloid is also present in the formulation [64]. There are several flour blends with buckwheat convenient for preparation of gluten-free biscuits: dark rise flour (50%), corn starch (30%), buckwheat flour (10%) and millet flakes (10%); buckwheat flour 25–75% and bean flour 25–75%; buckwheat flour (130 g), corn starch (60 g) and corn flour (60 g) [25]. Addition of buckwheat flour into the rice flour cookie formulation did not lead to a negative impact on

the sensory quality of the evaluated samples. The addition of 20% buckwheat flour into the cookie formulation achieved the lowest differences in instrumental properties compared to the control sample, and good sensory properties [65]. In products such as crackers, cookies, and biscuits where less of a gluten-like structure is needed to achieve a desirable texture and dough workability, buckwheat alone, with no supplement, may be appropriate [66]. Potato, cassava flour, rice flour, soybean, extruded soy protein, pumpkin powder, taro, sweet potato and potato have been successfully used to produce gluten-free biscuits [67, 68]. In addition, other valuable ingredients can be used for these products: xanthan gum which had significant effects on chemical, physical, textural and sensorial characteristics of gluten-free cookies [69]; rice and coconut flour mixture [70] and maize starch and pea protein added in rice flour as tools to modify the characteristics of gluten-free cookies [71].

According to a number of studies in general, more desirable quality and sensory properties are expressed when the formulation includes a mixture of gluten-free flours and starches rather than a homogeneous formulation [64].

Gluten-free biscuits made of Jerusalem artichoke and corn composite flour showed an increase in protein, fibre, ash and minerals especially Fe, Ca and Mg with the increase in the Jerusalem artichoke concentration [72]. Some similar gluten-free products that included linseed meal, amaranth and buckwheat in their formulation had increased protein, fibre and alpha-Linoleic acid in the composition of the linseed meal [73]. Lupin flour and buckwheat flour could successfully use in gluten free cakes and each of them individually showed a positive effect on quality of products. Samples with buckwheat flour added in amount of 5% showed improved volume index and softness, such as samples with 20% of lupin flour added. In addition, cake samples with lupin had higher content of protein, fat, Ca, Fe, Mn, P and Zn. Buckwheat flour particularly influenced on content increase of K and Mg in cakes. However, high levels of lupin (30–40%) and buckwheat (15–20%) had negative effects on cakes quality [74].

Rice, maize, sorghum and pearl millet had also a good potential for production of gluten-free cookies [75].

According to analysis of gluten-free cookies based on coconut powder as main ingredient it was found that samples were acceptable in relation to sensory evaluation and general quality and concluded that coconut could be used as main bakery ingredient and a successfully alternative to wheat flour [76].

In general, production of gluten-free food means the exclusion of all possible gluten-containing raw materials, selection of an alternate flour source, acceptable sensory characteristic, enhancement of the nutritional quality, product safety and labelling [77].

3.2 Reducing of caloric value in flour-based confectionery

Many calorie-rich dietary components contribute to obesity. However, the contribution of confectionery to obesity in children and adolescents has not been well determined and there is no evidence of positive associations between confectionery consumption and overweight, obesity or other obesity-related outcomes in children and adolescents has been found. The study suggests that, whatever its adverse contribution to other aspects of human health might be, confectionery is not a major driver of obesity [78].

Nevertheless, confectionary has focused for the last couple of decades on the production of low-calorie, high-fibre foods in response to public interest for low-calorie and functional products.

3.2.1 Reducing of sugar in flour-based confectionery

Sugar provides energy for the body (1 gram of sugar provides 4 kcal or 17 kJ). In the form of glucose, sugar serves as an immediate energy source for the brain. Taking too much sugar (including free sugars) may lead to excessive energy intake and increase the risk of getting overweight and obesity and also increase the risk of dental caries.

Sucrose is the main sugar used in flour-based confectionery contributing up to 30–40% of all recipes and it plays an important role in the manufacturing as well as in the final product quality. However, for health reasons, high levels of sucrose are undesirable, making sucrose replacement an important issue today.

The question of how to replace sugar in bakery products by using healthy sugar alternatives has been challenging scientists for a long time. The main problem with reducing the amount of sugar is ensuring the product quality in terms of appearance, sweetness, texture, volume and microbial shelf life. Sugar contributes to browning, crystallisation, control of starch and protein thermal settings, structure, bulk, bodying and viscosity, fermentation, hygroscopicity, humectancy and moisture migration control, as well as to freezing point depression and osmotic pressure control [79].

Sugar replacement strategies should especially focus on mimicking the sucrose functionality concerning morphology, i.e. spread and volume, and texture. Sugar replacements can be classified into two groups: extensive and intensive sweeteners [80].

Extensive sweeteners are bulking ingredients such as polyols, oligosaccharides, dextrins which are often used in a one-to-one replacement of sugar. In terms of their functionality, the extensive sweeteners mainly act as plasticizers/co-solvents, although some such as polyols also have some sweetening function.

On the contrary, intensive sweeteners are mainly used for their sweetening and consequently added in small amounts. For these reasons, it can be safely assumed that they do not contribute to the structure, texture, and morphology of biscuits [80].

Nevertheless, we have to keep in mind that the reduction of calories in flour-based confectionery is particularly difficult if only sugar is replaced. According to Regulation EC [59] different claims can be used based on sugar concentrations: Low sugars; Sugar-free and No added sugars. If sugars are naturally present in the food, the following indication should also appear on the label: 'CONTAINS NATURALLY OCCURRING SUGARS'.

Some polyols can be used to substitute sucrose in cakes and biscuits, especially erythritol and maltitol. They have lower energy value than carbohydrates, namely 2.4 kcal/g, except for erythritol that has no energy value [81].

Mannitol, sorbitol, maltitol, erythritol, isomalt, xylitol and lactitol are considered as food additives and listed as an" E" number in the list of ingredients [82]. In general, the features of polyols are as follows: they are stable during storage at high temperatures; they do not react with other food constituents; they do not participate in Maillard reactions, caramelisation and inversion; they have a lower energy value then sucrose and other natural sugars; they do not raise the level of toxic substances in blood (cholesterol, triglycerides and lipoproteins); they are not toxic and they do not cause tooth decay.

A limiting factor when using polyols in confectionary in general is their relatively low sweetness compared to sucrose, except for xylitol which is of equal sweetness as sucrose. Given that they have a negative heat of solution, some of them cause a cooling effect in the mouth, especially xylitol and erythritol, which can be also considered as a limiting factor when they are used in some confectionery products.

In addition, it is important to note another negative effect of polyols. As they cannot be fermented and are very slowly absorbed, osmotic diarrhoea may occur when consumed in high amounts. The extent of laxative effect depends primarily on the type of polyol. Compared to other polyols, the absorption of erythritol is more efficient and its intake causes very small or no laxative effect [83]. Foods containing more than 10% added polyols have to be labelled with a claim "excessive consumption may produce laxative effects" [82].

Erythritol can be applied successfully in cookies, biscuits and cakes, where it improves stability and shelf-life if about 7% of it is added [84]. Maltitol offers the closest approximation to the properties of sugar. It can substitute sucrose in the quantitative and qualitative sense without damaging the final product texture and exhibiting aftertaste and compared to the other polyols, it has been evaluated as the most suitable sucrose substitute in biscuits and cookies and showed the highest sensory acceptance [81, 85–88].

More recently, there have been many researches dealing with the replacement of sucrose with different polyols such as using maltitol and fructo-oligosaccharides-sucralose as sweeteners and polydextrose as a fat replacer to produce highly acceptable reduced-calorie biscuits by using dairy–multigrain composite flour [89]; sucrose reduction and its replacement by erythritol and maltitol in short dough biscuits where sugar-free and erythritol-containing biscuits were compact, elastic, and resistant to the breaking force compared to the control biscuits and the maltitol-containing biscuits [90]. The use of sorbitol, maltitol, isomalt and erythritol as total sucrose replacers in muffins resulted in muffins with polyols that had lower height than samples with sucrose and this shows that this type of polyols also influenced the texture. At the same time, no differences were found in the sensory acceptance of sucrose, sorbitol and maltitol muffins [91].

A combination of polysaccharides, such as polydextrose, oligofructose or maltodextrin and high-intensive sweeteners, in order to ensure the techno-functional properties of sugar-reduced flour-based confectionery represents relevant perspective in sugar reduction and caloric value decreasing, as well [83].

It is important to recognise that in addition to being important bulking agents such as gluco-polysaccharides (polydextrose, resistant starches and maltodextrins) and fructo-oligosaccharides supporting high-intensity sweeteners, polyols or sugar in formulations, these particular polymeric materials have noticeable physiological benefits that are related to their soluble dietary fibre and potential prebiotic properties [92].

In recent times, stevia and its extracts have become increasingly popular high-intensive sweeteners and their use in confectionery is becoming widespread.

Natural high-intensive sweeteners are steviol glycosides, stevioside and rebaudioside A (Reb-A) found mainly in the leaves of stevia. *Stevia rebaudiana* Bertoni is a plant with great potential as an agricultural crop for the production of a high-potency natural sweetener. Owing to its proximate composition and its content of health-promoting phytochemical constituents, it is also a suitable raw material for the extraction and production of functional food ingredients. *Stevia* is a low-calorie sweetener 300 times sweeter than saccharose. The sweetening compounds, found mainly in the leaves of the plant, are steviol glycosides, with stevioside being the most abundant, followed by rebaudioside A [93].

Nevertheless, if only intensive sweetener is used in cakes or biscuit making instead sucrose or the other natural sugar, side effects on the texture and sensory characteristics of the product may occur. Intensive sweeteners do not have the same bulking ability as sugar, and texture may be altered in some baked products, especially biscuits. Also, the taste may be slightly altered due to the aftertaste of some sweeteners.

Biscuits made of Reb A as sweetener were less acceptable than biscuit made of sucrose or fructose (**Figure 2**). They were lighter in colour than samples made of natural sugars which have a caramelising/browning effect [11].

Reb A biscuits, without natural sugars added, had less caloric value than biscuits made of sucrose or fructose. However, the caloric reduction did not reach 30%, so these products could not be classified as "energy reduced" products [11].

The effect of stevia and steviol glycosides on the flour-based confectionery physical and chemical quality and dough rheology has been widely investigated, and very often in combination with some polyols or bulking agents to mimic the characteristics of sugar and to provide adequate moisture and texture.

The aqueous extract of stevia can be a potential bioactive ingredient in developing functional cookies produced of oatmeal flour [94]; and potential to regulate glycaemic response of muffins by incorporation of 50% stevianna or 50% inulin with no effects on the final product texture [95]. Erythritol and stevia as sugar substitutes influence various rheological characteristics of wheat dough depending on their level and type. The addition of stevia and erythritol, reduces the consistency of the dough and the water absorption [95, 96].

The sucrose replacement by different concentrations, as well as a combination of isomalt, maltodextrin and stevia, was approached to produce sugar-free biscuits and it was found that neither isomalt, maltodextrin nor stevia individually has the potential of producing sugar-free biscuits. The addition of appropriate amounts of isomalt, maltodextrin and stevia (6; 2.5 and 0.06%, respectively) to correct the appearance, texture, and sweetness of sugar-free biscuits can replace sucrose and give products with acceptable properties. Despite the fact that used sugars-replacements do not participate in Maillard reactions, biscuits had an even better golden-brown colour than those manufactured with sucrose [97]. The colour of biscuits made of stevia and maltitol as sweeteners can be improved with the addition of coffee silverskin as a natural colouring and a source of dietary fibre, as well [98].

The market aims at removing the unhealthy ingredients in formulations, particularly sugars, but pays attention to customer satisfaction. Baked goods manufacturers are currently utilising intense as well as high-volume artificial sweeteners as conventional sugar replacements [99].

3.2.2 Reduction of fats in flour-based confectionary

Fats have the highest calorific value of any other ingredients used in flour-based confectionery - 9 Kcal/g or 37 KJ/g, which is more than twice than that of carbohydrates and proteins. This is why the consumers are highly concerned about the fat content in biscuits.

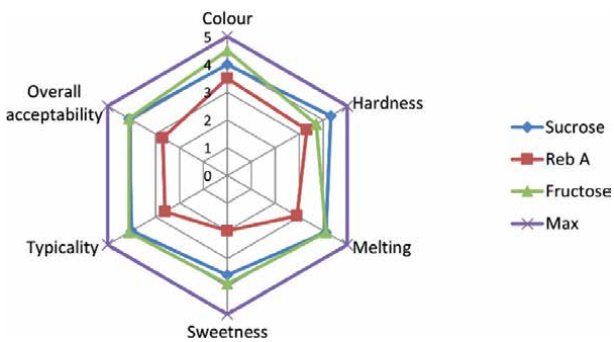

Figure 2.
Sensory evaluation of biscuits made of sucrose, fructose and Reb a (data from the author's private archive).

However, the simple reduction of fat in a biscuit recipe and recipes for other similar products usually results in quality deterioration such as excessive hardness, non-porous and unsatisfactory structure, and poorly expressed taste and aroma. In addition, low fat doughs are tougher, harder and stickier than normal doughs and a number of difficulties appear during manufacturing. In general, the important roles of the amount and type of fat in flour-based confectionery are reflected in an even distribution and possibility of dough aeration, final dough hardness, spread in the oven, texture of the final products, sensory quality like mouthfeel and aroma and shelf life. Odour and aroma are more expressed in samples with higher fat content, as a consequence of liposoluble character of the most aroma components.

According to sensory evaluation of semi-hard industrial biscuits with different commercial vanilla aroma forms added it was found that biscuit samples with 12% fat showed better sensory characteristics for all vanilla aroma forms than samples with 10% fat in a recipe [11].

From a sensory point of view, it is more acceptable to reduce the fat than the sugar content in biscuits manufactured in industrial conditions, at least when products are not perceived as less sweet [100].

Using fats with lower levels of saturates is a good way to increase nutritive value of flour-based confectionery. A general principle in the reduction of saturate levels in fat is seen through an increase in the level of liquid oil in the product, as these liquid oils, such as rapeseed oil or sunflower oil, have the lowest levels of saturated fats. However, an increased risk of oxidation of the fats and the development of rancidity in the biscuit during storage can influence shelf-life of the products [101]. Those products have a relatively higher spread value than the others and had a relatively harder texture. Studies also showed that the cookies containing the oil started to spread earlier and continued to spread for a longer time. However, the quality of these cookies could improve by including 0.5% sodium steroyl lactylate in the formulation [102].

Fats in flour-based confectionery mainly containing a high level of saturates may represent up to 20% or 35% of the product. Trans fats are also usually added to bakery products and are related to the risk of heart diseases, since they increase low-density lipoprotein (LDL) cholesterol levels and reduce those of high-density lipoprotein (HDL) [99]. Reduction of saturated fat, in recipes is perhaps the simplest and preferred way in manufacturing, as it allows decrease in calories using standard ingredients with modifications to an existing, established recipe. It also offers potential for cost savings, as fat can often be one of the most expensive ingredients in a biscuit recipe. Apart from the general quality issues arising from a reduction in overall fat content in biscuit doughs, there is also an impact on the biscuit dough consistency and processing properties [101].

Due to these reasons, a number of different fat substitutes have been investigated to meet the demand for a reduced-fat biscuits and similar products. These fat substitutes can be categorised as protein-based, carbohydrate-based, or fat-based substitutes [103].

Some of carbonate-based fat substituents, alone or in combinations, have been widely used include polydextrose, maltodextrins, tapioca dextrins, potato starch, microcrystalline cellulose and gums such as alginates, xanthan gum, carrageenan, and locust bean gum. Combination of maltodextrin and guar gum resulted of 62.5% the fat reduction in low-fat soft dough biscuits [104].

A good approach to improve cakes is combination of inulin and oligofructose as fat and sugar replacers respectively. Regarding the sensory analysis, a replacement up to 50% of fat and 30% of sugar separately and simultaneously did not significantly change the overall acceptability of the cakes [105].

The consumer study revealed that fat replacement up to 15 g/100 g with inulin or hydroxypropyl methylcellulose (HPMC) provided acceptable biscuits, but higher replacement decreased the overall acceptability [106].

Biscuits prepared with either olive oil or sunflower oil and xanthan gum as fat replacer differed the most from the biscuits made with shortenings. The biscuits formulated with either olive oil or sunflower oil and HPMC as fat replacer had the closest sensory properties to the shortening biscuits while the sunflower oil/HPMC systems seemed to be the most suitable system for obtaining biscuits with a healthier fatty acid profile [107].

A mixture of tapioca dextrin and tapioca starch was used as a shortening replacement in short-dough biscuits. The trained panel results showed that the fat replacer increased the hardness and crumbliness, and that these effects were balanced out by the addition of resistant starch [108].

In general, those substitutions affect the textural properties of the baked products, making them harder, and thus they have lower acceptance levels than control products. Chia seeds (*Salvia hispanica* L.) contain a high content of oil (30%–40%) with a significant amount of omega-3 fatty acids (linolenic acid, 54%–67%), protein (15%–25%) and fibre (18%–30%) which makes them an excellent choice for improving the nutritional profile of bakery products. Many authors have used Chia seed in different forms to reduce fat content and also to improve general nutritive value of flour-based confectionery [99].

The most satisfactory technique used to reduce fat levels in biscuits is to improve the fat functionality by using surface active agents, emulsifiers. Emulsifiers contribute to reduced-fat biscuit recipes in improving texture, volume and dough consistency but the basic function of the emulsifiers is to provide the distribution of fat in the dough. Emulsifiers, principally diacetyl tartaric acid esters of mono- and diglycerides (DATEM) show a fat-sparing effect and its use is not limited to their pure action as emulsifiers. They also influence the properties of baked goods during manufacturing and storage. The fat-sparing effect of DATEM-ester is most effective if the emulsifier is added with the fat [101, 109].

The "ideal" fat substitute does not exist. However, a good solution presents a combination of different ingredients that may or may not belong to any of the classes of fat replacers. This approach may include emulsifiers, fat substitutes or mimetics, fibres, water control ingredients, and/or flavour and bulking agents [103].

3.3 Microencapsulation in flour-based confectionery

In food products, fats and oils, aroma compounds and oleoresins, vitamins, minerals, colourants, and enzymes have been encapsulated and show a promising approach to develop functionally active food products. The advantages of microencapsulation are reflected in its easy application, stabilised active component, enhanced acceptability, protection, controlled release and creating new functional food [110].

Bioactive compounds have been incorporated in microcapsules to stabilise them, to convert them into powders, to alleviate unpleasant tastes or flavours, as well as to improve the bioavailability. This technology enables the covering and protection of bioactive components by completely enveloping them with a physical barrier. This is a way of packaging solids, liquids, or gaseous materials in small capsules that release their contents at controlled rates over prolonged periods and under specific conditions.

As the biscuits or cookies are widely consumed snacks, they are therefore an ideal food for fortification [111]. The incorporation of microencapsulated vitamins

into a food matrix contributes to the improvement of the food nutritional value, reduction of off-flavours, it permits the time-release of nutrients, enhances the stability to high temperature and moisture and reduces each nutrient interaction with other ingredients [112]. Microencapsulation presents a successful tool to raise lycopene stability. Besides, microcapsules of spray-dried lycopene were able to release pigment and colour into cakes samples in a homogenous manner [113].

Studies of improving biscuits with microencapsulated highly susceptible micronutrients including 5-methyltetrahydrofolic acid, which is regarded as one of the most bioactive forms of folate [111] and cookies with β-carotene [114] have shown encouraging results, as well.

Encapsulation is also a favourable method that allows extending the shelf life of specific confectionery products such as cakes.

Some high added value components such as essential oils have significant properties from nutritive and medicinal aspects. Antimicrobial activity is particularly important to use in functional foods making and enable prolonging the shelf life of the final product. Furthermore, their strong and atypical tastes and smells could be successfully camouflaged by encapsulation [115].

An illustrative example of this is the study of using encapsulated thyme (*Thymus vulgaris*) oil as a natural food preservative that can be applied to cakes to promote their shelf lives and avoid synthetic preservative [116].

Due to the numerous possibilities of application and various methods that allow for the most suitable solutions for the incorporation of certain functional ingredients into food matrix, microencapsulation will certainly find its place in the commercial production of flour-based confectionery products [110].

4. Conclusion

Improving the general quality of flour-based confectionery products is a common topic of many recent researches conducted in different fields. Based on the consumers' demand, food scientists and producers are now focusing on developing functional foods.

Given that the production of flour-based confectionery products is one of the most innovative branches of the food industry, as well as the fact that these products are most represented in the diet compared to other confectionery products, the trend of creating food that affects physiological functions and can reduce the risk of various diseases is particularly pronounced.

These products are extremely suitable for improvements and for creating new functional products. Numerous researches show that the addition of dietary fibres from cereals and fruits, as well as by-products produced during fruit processing can improve functional properties while retaining sensory characteristics. Furthermore, enriching these products with raw materials rich in bio-components such as berries and various seeds contributes to an increase in antioxidant activity, while improving sensory properties. Also interesting are researches related to the creation of flour-based confectionery products with probiotic properties.

Particularly important researches are being conducted regarding the substitution of ingredients in biscuits and similar products primarily when it comes to fats and sugars. When talking about the replacement of individual ingredients, it is necessary to take into account complex processes of interaction of water and other components such as fats, starch, proteins and sugar. Fat replacers play a key role in creating products with reduced fat content, i.e. reduced caloric value, and it is important to consider their rheological and physical properties, as well as to adjust process parameters. In the process of creating new flour-based confectionery

products, numerous studies include examining the consistency of the dough, adjusting process regimes, as well as evaluating the appearance and texture of these products.

It is necessary to point out the advantages of microencapsulation in the production of functional flour-based confectionery products as a relatively new technique used to enrich various food products. It allows the incorporation of bio-components that are not naturally found in these products or that can be lost during baking. The microencapsulation allows the retention of thermolabile valuable compounds in the product and their gradual release. In addition, this technique allows the incorporation of some substances that extend the self-life of the product, such as cakes, and at the same time camouflage possible uncharacteristic taste and aroma of added components, which can jeopardise the overall sensory impression of the product if added directly during mixing. It is to be expected that this method, due to its numerous benefits, will be increasingly adopted in the production of flour-based confectionery products. However, its application in practice is still limited due to the possibility of raising product prices, which is an additional challenge for manufacturers.

Some different directions in producing functional flour-based confectionery products are sometimes difficult to implement in practice due to strict consumer demands to select and buy high value nutritive food with desirable sensory properties. However, producers mostly take into account the consumers' awareness of the importance of functional food and their willingness to buy such products. Anyway, continuous education of consumers to select high value nutritive food and estimate food quality is needed, especially regarding confectionery products.

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Chapter 17

Bitter Melon: A Multifunctional Medicinal Plant with Powerful Bioactive Compounds

Fadime Eryilmaz Pehlivan

Abstract

Nature is full of poisons as well as life-saving entities. Extracts of natural products in medicinal plants have been used for thousands of years in traditional medicine throughout the World. Bitter melon (*Momordica charantia)* is a member of Cucurbitaceae family, widely distributed in tropical regions of the World, that has been used in folk medicine for the treatment of diabetes mellitus, and its fruit has been used as a vegetable for thousands of years. It contains phytochemicals, flavonoids, triterpenes, saponins, ascorbic acid, steroids, proteins, and polysaccharides. This plant is a traditional herbal medicine, possesses various biological, medicinal activities and pharmacological functions, namely antidiabetic, anthelmintic, contraceptive, antimalarial, laxative, antihyperglycemic, antimutagenic, antiulcer, antilipolytic, antifertility, hepatoprotective, anticancer, antibacterial, antiviral, antitumor, immunomodulation, antioxidant, antidiabetic, and anti-inflammatory activities of *M. charantia* have been reported. Its fruit has a special bitter taste, parts of *M. charantia,* such as fruits, vines, leaves and even roots have been used as folk medicine for the remedy of diseases like toothache, diarrhea, and diabetes. It is also used for the treatment of eczema, gout, jaundice, pneumonia, psoriasis, and rheumatism. These beneficial effects are attributed to the various bioactive components of *M. charantia*, which are important sources of phytoconstituents used to treat various diseases since ancient times. This chapter reviews various aspects of the results of investigations involving *M. charantia* in the recent years, providing a comprehensive overview of the phytochemical application of *M. charantia* to attract more attention to their biological activities for better utilization of *M. charantia*; focusing on the review of benefits that bitter melon offers in terms of its potential as a source of bioactive compounds and its role in the control of different diseases.

Keywords: Bitter melon, medicinal, biologicl, bioactive compounds, phytochemicals

1. Introduction

Bitter melon (*Momordica charantia* L.) commonly called bitter melon, is also known as bitter gourd, bitter apple, bitter squash, balsam-pear, belongs to Cucurbitaceae family. It is a plant found in tropical and subtropical regions of Asia and Africa, where it is valued for its various health benefits. This traditional tropical plant has been claimed to have therapeutic effects for ages for its pharmacological activities, and nutritional properties due to its content of bioactive compounds.

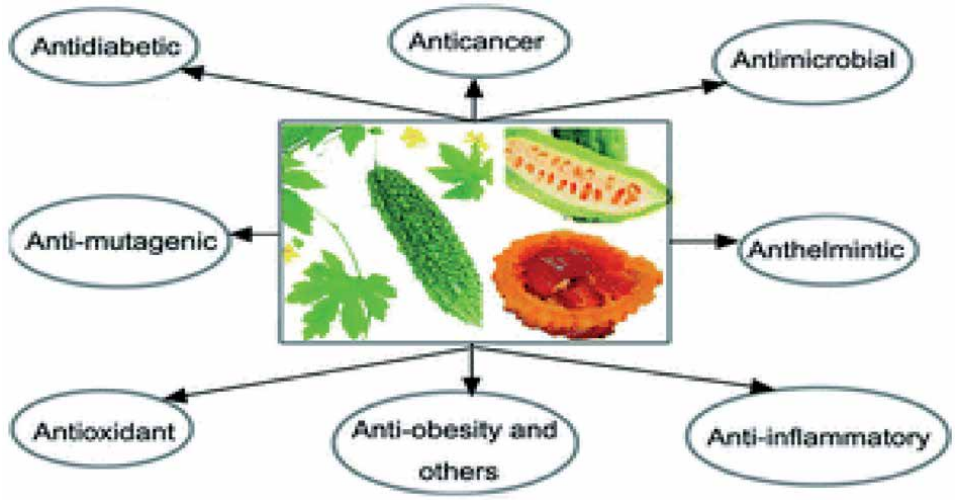

Figure 1.
Momordica charantia: *A popular health-promoting vegetable with multifunctionality [1].*

The presence of many bioactive compounds, some of which possess potent biological actions, this plant is used in folk medicine all over the world for the treatment of different pathologies. It has been used in a wide range of medical applications, such as diabetes, cancer, hypertension, obesity, bacterial and viral infections, and even AIDS (**Figure 1**) [1, 2]. It is also used for pain relief, against chronic fever, in cases of jaundice and illnesses of the liver or the digestive system [2]. In Turkish traditional medicine the oil obtained from the ripe fruits of bitter melon, macerated in olive oil warmed by the sun, was combined with honey, and used for the prevention and healing of gastric ulcers [3]. In African folk medicine it is mainly used for worm infections, inflammation, fever, syphilis, rheumatism, and skin diseases [4].

Momordica charantia L. is a widely cultivated medicinal plant around the world. All parts of *M. charantia* possess important medicinal properties, including anti-inflammatory, antidiabetic, anticancer, hypotensive, anti-obesity, antimicrobial, anti-hyperlipidemic, antioxidant, immuno-modulatory, anthelmintic, neuro-protective, as well as hepato-protective properties both in vitro and in vivo (**Figure 1**).

2. Nutritional value and chemical composition of *Momordica charantia*

Bitter melon (*Momordica charantia*) is an unique bitter tasting herbaceous medicinal plant, cultivated in tropical and subtropical regions of many countries; which is one of the nature's most valueable gifts although it is one of the discarded vegetables by people, just because of its bitter taste. All parts of the plant, including the fruit, taste very bitter, mainly because of the presence of three pentacyclic triterpenes, momordicinin, momordicin and momordicilin. It contains lipids, fiber, protein, carbohydrates, calcium, sodium, potassium, iron, manganese, copper, phosphorus and vitamins. It also contains phytochemicals, vitamins, antioxidants, and bioactive chemicals. It is a plant high in health-beneficial compounds such as antioxidants, flavonoids, phytosterols, and saponins. Since antiquity, it is used in different countries as a folk medicine traditionally. It possess rich nutritive values among cucurbits and being a good source of medicinal products, it contains carbohydrates, proteins, fibers, vitamins (C, A, E, B1, B2, B3, and B9 as folate), and minerals (potassium, calcium, zinc, magnesium, phosphorous and iron) [5, 6]. Fruits are reported to contain vitamin C, A and P, thiamine,

riboflavin, niacin, and minerals with 93.2% of water content, while protein and lipids account for 18.02 and 0.76% of its dried weight, respectively [7, 8]. Its seeds also represent a good source of lipids, polyunsaturated fatty acids and conjugated linolenic acid [9].

Bitter melon has been associated with anti-cancer, anti-microbial, anti-inflammatory and anti-diabetic properties. The medicinal values of the bitter gourd fruit are linked to its high content of phenolics, which act as antioxidants. Phenolic compounds containing phenolic acids, coumarins, lignins, tannins, lignanes and flavonoids are among the secondary metabolites that are abundant in the plant. *M. charantia* is also a good source of phenolic compounds, which can protect from oxidative damage by acting directly on reactive oxygen species and to activate endogenous defense systems [10]. The biological activity of *M. charantia* depends on its major phytochemical constituents, containing phenylpropanoids, and other bioactive compounds, such as polyphenols, phenolic acids, flavonoids, essential oils, fatty acids, amino acids, lectins, sterols and saponins, tocopherols, monoterpenes, sesquiterpenes, [11, 12], including cucurbitane-type triterpenoids, cucurbitane-type triterpene glycosides, and some proteins present in fruits, seeds, roots, leaves and vines [13]. The most prevalent chemical constituents are cucurbitane-type triterpenoids, the bitterness of *M. charantia* is the consequence of cucurbitane-type triterpenoids: cucurbitacins, momordicines I and II and triterpene glycosides: momordicosides, exhibiting a broad range of biological activities, mainly anti-inflammatory and anti-diabetic [14]. Different major constituents found in different varieties and different parts of the plants are summarized below (**Table 1**) [14, 15].

M. charantia is one such sample that holds rich phytochemicals and is an effective agent in dietary regimens to prevent against different maladies. Brief about the *M. charantia*, it is used as a vegetable in many countries but since time immemorial, it is also used for administration of numerous ailments comprising wide range of pharmacological activities for instance, antioxidant, anti-inflammatory,

Major Bioactive Components	Functions	Distribution	References
Polysaccharides	Antioxidant, antidiabetic, immune enhancement, neuroprotective, antitumor	Various parts of plants	[11–14, 16, 17]
Peptides and proteins	RNA N-glycosidase, polynucleotide adenosine glycosidase (PAG), DNase-like, phospholipase, superoxide dismutase, anti-tumor, immune suppression, antimicrobial	Seed	[18–21]
Lipids	Antitumor, antioxidant	Seed, flesh	[11–14, 16, 17]
Terpenoids	Anticancer, antioxidant, antidiabetic, hypoglycemic, cancer chemoprevention	Stem, leave, fruit	[11–14, 16, 17, 19–22]
Saponins	antihyperglycemic, hypolipidmic, antiviral	Fruit, root, seed	[18–21, 23–29]
Phenolics	Antioxidant, anti-inflammation, immune enhancement	Fruit, pericarp, seed	[11–14, 16, 17, 19–22]
Sterols	Antimicrobial	Pericarp, fruit	[25–31]

Table 1.
Major bioactive components of bitter melon and their related functions [15].

antimicrobial, antidiabetic, antiobesity, antiulcer, anticancer, hypotensive, and blood cholesterol lowering effects that are demonstrated in **Figure 1** and **Table 1**. The following is an overview of its common pharmacological activities.

3. Antioxidant and anticancer activity of *Momordica charantia*

Secondary metabolites are attracting attention for their effects in preventing diseases due to oxidative stress, which leads to degeneration of cell membranes and leads to many pathological diseases. They play a major role in preventing disease due to oxidative stress, which leads to many pathological diseases [11–14]. Recent investigations have shown that antioxidants with free radical scavenging properties have great importance as therapeutic agents in preventing aging process and in scavenging free radical mediated diseases [11–14]; such as diabetes, atherosclerosis and other complications [15].

Many studies have shown that *M. charantia* is a good natural source of antioxidants such as bioactive phytochemicals mainly include polysaccharides, saponins and phenolics; that possess an activity against oxidant damage in vitro and in vivo [32]. *M. charantia* and its ethanolic extracts are analyzed to contain high antioxidant activities that are well correlated with phenolic compounds [32]. Bitter melon and its extracts are demonstrated to have stronger antioxidant activity than other solvent extracts, by increasing the activities of catalase and levels of reduced glutathione, bitter melon is proved to exhibit inhibited stress-induced lipid peroxidation [32, 33].

Several phytochemicals, including bitter melon extracts, are described to possess potentials in anticancer therapies [34]. The extracts of bitter melon have been investigated for their potential use as anticancer agents; suggesting that dietary consumption of bitter melon could help to lower risk of several cancers [34]. *M. charantia* extracts and its monomer components have shown strong anticancer activity against various tumors such as lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin cancer and prostate cancer [35]. Furthermore, bitter melon extracts are shown to decrease human prostate cancer cell growth due to the selective induction of apoptosis [35, 36].

Anticancer properties of bitter melon extracts are reported to have ability to modulate several deregulated signaling pathways in different type of cancer, like MAPK pathway, through the modulation of cell cycle proteins, thereby inducing cell cycle arrest, inducing apoptosis or other cell death pathways. Cucurbitane-type triterpene glycosides have been showed to have significant antitumor activity in hepatic carcinoma derived cell lines [36].

It is well known that tumor progression toward malignity is strongly related to chronic inflammation that is responsible for tumor invasion of surrounding normal tissues and angiogenesis. Bitter melon components are suggested to exert their antitumor effects by modulation of the inflammation status.

4. Antiinflammatory activity of *Momordica charantia*

Inflammation is known to be a complicated immune process that can be defined by the sequential release of mediators such as pro-inflammatory cytokines, including interleukin (IL)-1, interferon (IFN)-c, IL-6, IL-12, IL-18, tumor necrosis factor (TNF), and the granulocyte-macrophage stimulating factor. It is settled by anti-inflammatory cytokines such as IL-4, IL-10, IL-13, IFN-a, and the transforming growth factor (TGF)-b [37]. Inflammation is known as an overall a

protective response against xenobiotics, but chronic inflammation is also known to be detrimental to tissues; causing in chronic inflammation-derived diseases, such as autoimmune diseases, cardiovascular diseases, systemic lupus erythematosus (SLE), aging-associated diseases, such as Alzheimer's or Parkinson's disease; and cancers. It is known that oxidative stress and inflammation activate each other and oxidative stress plays a role in chronic infectious diseases [37]. Chronic inflammation is shown to promote tumor initiation and malignant progression of many cancers, considering the importance of inflammatory changes in different cancer types, preventing or reversing inflammation has become an important approach to control cancer progression [16, 37]. Thus, inhibition of the overproduction of inflammatory mediators, especially pro-inflammatory cytokines IL-1b, IL-6, and TNF-a, may prevent or suppress a variety of inflammatory diseases [16, 37].

Dietary habits contribute to a chronic state of inflammation, which can alter gut microbiota and immune status. Various dietary components have demonstrated to modulate chronic inflammatory conditions and to be helpful in their therapy [16]. Bitter melon dietary supplementation has been widely studied to treat several diseases, such as obesity and cancer, promising to possess hypoglycemic and lipid-lowering properties [17]. Chronic inflammation is involved in the pathogenesis of different diseases, such as metabolic syndrome, obesity, cancer, cardiovascular disease, and a neurodegenerative diseases [16, 17]. In diabetic patients, inflammation contributes to increase blood glucose concentration in developing cardiovascular diseases and obesity. The beneficial properties of *M. charantia* appear to be due to anti-inflammatory and antioxidant activities by acting on several important signal pathways involved in inflammation [38].

Several investigations suggest that oxidative stress plays a role in chronic inflammatory diseases; which are closely related in pathophysiological processes that can activate each other [39–41]. Bitter melon has shown to have beneficial properties dependent on its anti-inflammatory and anti-oxidant activities [41–43]; regulating inflammation mainly through NF-κB signaling pathway inhibition, reducing TNF-α production [44]. It is also reported that bitter melon extracts reduced TNF-α-induced expression of inflammatory markers, including inducible NO synthase, p65 subunit of NF-κB, TNF-α, and IL-1β [45]. The bitter melon containing diet also reported to normalize serum levels of the cytokines suggesting its role in reducing inflammation, obesity and insulin resistance in obese mice [46]; suggesting that bitter melon supplementation may be useful as a preventive agent in individuals at risk for inflammatory-related diseases [47]. Therefore, bitter melon has anti-inflammatory effects by acting on several important signaling pathways involved in inflammation.

Xanthine oxidase, which is a key enzyme for the induction of hyperuricemia and gout, it is involved in many inflammation related diseases [48]. Cucurbitane-type triterpene glycosides isolated from bitter melons fruits is proved to inhibit xanthine oxidase activity [49]. Antioxidant compounds in bitter melon showed potential natural antioxidant activity to inhibit the lipid peroxidation [50]. demonstrating anti-inflammatory effects of phenolic compounds present in the bitter melons extract [51].

The wound-healing activity of the olive oil macerate of *M. charantia* was investigated in wound models. Experimental data have shown that *M. charantia* has wound-healing and anti-inflammatory effects [52]. *M. charantia* has been investigated and reported to play a role in suppressing gastric inflammation against gastric ulcers from ethanol [53]. It is also shown that *M. charantia* can suggest an alternative in reducing the need for analgesic drug consumption by reducing pain and improving symptoms in diseased individuals [54].

5. Antidiabetic (Hypoglycemic) activity

Diabetes mellitus is known as a metabolic disease that is the fastest growing diseases in the world, and is characterized by hyperglycemia resulting from defects in insulin secretion and insulin action [55]. Drugs obtained from the plants used all over the world to treat diabetes. Bitter melon is used to treat many diseases with good medicinal values, but more emphasis is given to its anti-diabetic properties. As an antidiabetic drug, bitter melon has been widely used in different countries for thousands of years is suggested as a remedy for the treatment of diabetes [56].

It contains active compounds, including charantin that reduce blood sugar levels. In addition, bitter melon contains lectin which also reduce blood glucose level that is a major factor of hypoglycemic effect [57]. Some other compounds present in bitter melon such as steroids, inorganic, triterpene, proteid, lipid and phenolic compounds also offer anti-diabetic properties. The aqueous extract of bitter melon fruits is reported to stimulate insulin secretion of β cells in pancreatic islets isolated from obese-hyperglycemic mice [23, 24, 57]. Antidiabetic mechanism of bitter melon extracts is suggested to enhance insulin secretion by the islets of Langerhans, reducing glycogenesis in liver tissue, enhancing peripheral glucose utilization and increasing serum protein levels [23, 24, 57].

Oral administration of the aqueous extract *M. charantia* fruits are observed to lower blood glucose level in diabetic rats [24]; they are shown to stimulate insulin secretion of β cells in pancreatic islets isolated from obese-hyperglycemic mice [24]. They are also indicated to play a role in the renewal of β cells in diabetic rats or recovery of destroyed β cells [24]. *M. charantia* fruit juice is examined to reduce blood glucose levels in diabetic rats due to enhancing insulin secretion by the islets of Langerhans, reducing glycogenesis in liver tissue, enhancing peripheral glucose utilization and increasing serum protein levels [23, 24, 57].

6. Antihyperlipidemic activity and weight loss

Hyperlipidemia is a major health problem and associated with diabetes due to increase in morbidity and mortality. High blood lipid concentration is associated with cerebrovascular disease, ischemic heart diseases, and atherosclerosis. *M. charantia* is proved to have an antihyperlipidemic effect. A component of *M. charantia*, metformin, and other components such as alkaloids, flavonoids, saponins, tannins, and triterpenes have suggested to lower total cholesterol level in diabetic rats. Moreover, bitter melon has been described to repair damaged β-cells, resulting in increasing the levels of insulin and its sensitivity [23, 56, 57]; by enhancing the release and synthesis of thyroid hormones and adiponectin, and also by inhibiting the activity of glucosidase that inhibits the absorption of glucose. The action of AMPK (adenosine-5-monophosphate kinase) is shown to be increased by bitter melon consumption that is associated with fat release from fatty tissues and glucose uptake and thus resulting in weight loss [23, 24]. Diabetic rats that are treated with *M. charantia* extract are also exhibited significant reduction of blood lipid levels [23, 24]. Bitter melon has also shown to reduce the cholesterol and trigliceride levels by increasing Apo-A-1 (Apo lipoprotein A-1) which is basic protein component compulsory for HDL synthesis [23, 24]. Recently, it is observed that bitter melon has ability to reduce body weight and the accumulation of high fat due to its anti-hyperlipidemic activity [24].

7. Antimicrobial activity

M. charantia extract has been suggested to have broad-spectrum antimicrobial activity, clinical signs of broad-spectrum antimicrobial activity has been delivered by the extract of bitter melon leaf [25]. Polysaccharides isolated from *M. charantia* have been reported to have significant inhibitory effects on bacteria. It has been shown that the main components of *M. charantia* responsible for antimicrobial functions are polysaccharides [26]. It is also suggested that antibacterial properties of *M. charantia* oil is related to its high trans-nerolidol and conjugated linolenic acids content [25, 26]. *M. charantia* is a basis of natural products which derived from plant with antimicrobial activity. A component of bitter melon, α-momorcharin, due to its ribosome-inactivating protein (RIP) ability is effectual in inhibiting the fungal and bacterial growth [25, 26, 30]. It has been reported that *M. charantia* polysaccharides have a good bacteriosis activity in *B. subtilis*, *S. aureus*, *S. typhimurium* and *E. coli* and the most obvious effect is the effect on *S. aureus* [26]. Essential oils of *M. charantia* seeds have significant inhibitory effect on *S. aureus*, while having less impact on *E. coli* and *C. albicans* [26, 30]. It has been demonstrated that the whole plant extract has antiprotozoal activity and methanol, water, and ethanol extract of the bitter melon leaves are considered to have an antibacterial action against *Salmonella, Pseudomonas aeruginosa, E. coli, Bacillus*, and *Streptococcus* chain [26, 30]. *M. charantia* fruit also has an anti-*Helicobacter pylori* activity that is a causal agent of ulcer [26, 30].

8. Anti-parasitic (anti-anthelmintic) activity

Helminthic infection is a problem, which is caused by nematodes, cestodes, and trematodes. The main target of helminthic infection is gastrointestinal system that affects human and livestock's in the world. Nowadays, in spite of using medicines, functional foods such as bitter melon is considered as an important therapeutic medicinal food with anthelmintic action. The presence of functional ingredients including saponins, momordin, momordicoside, momordicin, the worms are paralyzed by inhibiting the acetyl cholinesterase [30]. Anthelmintic effect of *M. charantia* also include the inhibition of arachidonic acid metabolism, mico nicotinic agonists, oxidative phosphorylation inhibition, increased calcium permeability, acetyl cholinesterase inhibitors, and β-tubulin binding [24, 30]. Saponins are observed to affect the permeability of the cell membrane of worms and lead disintegration and vacuolization of tegument; irritating the mucous membrane channel gastrointestinal of worms that interfere with the absorption of food [30]. Flavonoid compounds especially apigenin is shown to inhibit larval growth and inhibit the arachidonic acid metabolism which may lead to the degeneration of neurons in the worm's body and lead to death [24, 30].

9. Anti-HIV activity

Bioactive components which are present in *M. charantia* are analyzed to be useful in the management of HIV infection [31]. Ethanolic extracts from leaves and stems of *M. charantia* have shown highly antiviral activity [31]. The root of *M. charantia* is suggested to have moderate anti-HIV-1 activity [31]. Bitter melon proteins is examined to inhibit HIV activity, depress the expression of the virus core protein [27]. Compounds such as momorcharin, and lectin are isolated from

M. charantia, these compounds have a protective effect against viral infections. That are shown to have a strong influence on HIV, but these compounds are not well absorbed in patients. In infected people, oral intake of *M. charantia* is demonstrated to slow the progression of HIV [27, 31]. Leaf extracts are provided immunostimulant effects against viral infections particularly HIV and has an ability to treat various viral diseases [27, 31].

10. Wound healing activity

Growth factor deficiencies, neovascularization, abnormalities such as impaired immune response and decreased synthesis of collagen are known to be associated with diabetes and to the delayed wound healing [18, 19, 28, 29]. Treatment with *M. charantia* fruit ointment is suggested to enhance wound closure in diabetic rats, and upregulate TGF-β expression in wound tissue, which plays an important role in regulating cell growth and differentiation [18, 19]. The juice of *M. charantia* is demonstrated to have a healing potential against psoriasis, scabies, and ringworm. In rats modeling wound healing potential of *M. charantia* fruit powder has been assessed with a significant response by powder ointment in terms of period of epithelization, wound-contracting ability and wound closure time [19].

11. Immunomodulatory activity

In vitro experiments have shown that *M. charantia* polysaccharides can increase immunity by stimulating the activation of lymphocytes and macrophages [20]. It has been reported that the water-soluble polysaccharide isolated from *M. charantia* may increase endothelium-derived relaxing factor production in the cell proliferation, the development of the inflammatory and immune response, and stimulate splenocytes and thymocytes [21].

12. Other biological activities

There are also some reports on other bioactivities. Components in *M. charantia* have an inhibitory effect on gastrointestinal nematodes [22]. Momordin is reported to have hypotensive effects [58]. A fruit extract has been demonstrated to possess activity against *Helicobacter pylori*, which could induce stomach ulcers [59]. It has been reported that *M. charantia* polysaccharides have a neuroprotective effect that can reduce neuronal death caused by thrombin in primary hippocampal neurons [60]. The hepatoprotective effect of *M. charantia* water soluble polysaccharides has been investigated on the CCl4 liver damaged mouse model [61].

13. Toxicity and side effects

Although the plant is basically harmless to human body under normal conditions, it may induce adverse reactions according to different uptakes, processing methods, physical differences and other conditions. There have been reports of toxicity since 1960s, mainly including acute toxicity, chronic toxicity and reproductive toxicity.

Intake of *M. charantia* leaves is known to be used to prevent childbirth in India [62]. Moreover, the aqueous extract was reported to significantly decrease

hemoglobin concentration of albino rats [63]. *M. charantia* lectin had a cytotoxic effect, which significantly inhibited DNA and protein synthesis in human peripheral blood lymphocytes of normal or leukemic cells [64]. Clinical findings indicating that long-term use of *M. charantia* at high doses may cause kidney conditions should be tested by better organized clinical trials. People who report allergies to other herbs from the Cucurbitaceae family should avoid the use of *M. charantia* [65].

14. Conclusion

It is known that the majority of the world population prefer traditional folk medicine products to industrial products. One of the main reason for the increased interest in herbal medicinal products is that natural products will be considered less toxic, but this is often a false perception. In health problems, many components of vegetable origin obtained from natural products have the potential to act as supplements, alone or in mixtures. Due to the synergistic effect, many active compounds may have therapeutic potential much higher than the effects they can give alone when given as a herbal preparation. It is helpful for different diseases such as inflammatory, leukemic, diabetic, mutagenic, mycobacterial, microbial, tumor, ulcer, aphrodisiac, viral, astringent, carminative, cytotoxic, hormonal, depurative, hypotensive, immuno-stimulant, etc. Bitter melon is the most important medicinal plant having good therapeutic values. Therapeutic importance bitter melon is traditionally famous due to its medicinal importance. It has as anticancer, antiviral, antidiabetic, anti-inflammatory, immune-stimulant and cholesterol reduction properties. It contains many phenolic compounds, due to this reason, it has antioxidant and antimutagen properties. Its fruit, leaves, stem and roots all are used for the treatment of many diseases such as hyperlipidaemia, digestive disorders, microbial infection and menstrual problems. It has antiviral and anticarcinogenic properties which can boost the immune system. Other medicinal properties such as wound-healing activity have revealed that the bitter melon extract has high amount of therapeutic compounds for the regeneration of tissues that encouraged the proliferation of dermal fibroblasts of human.

Bitter melon is also shown to express anti-HIV activity with alpha- and beta-momorcharin proteins that are present in seeds, leaves, and fruit. Investigation on the bioactivities of *M. charantia* has developed rapidly. Application of bitter melon in food and medicinal fields are still in the initial processing stages; the health benefits are still far from being fully utilized. Due to its numerous health functions, the plant can be utilized in tumor therapy, lowering blood glucose and other aspects of clinical applications with broad prospects under the premise of ensuring safety.

As conclusion, bitter melon has different medicinal properties due to the presence of high bioactive compounds that is beneficial for the human health This chapter provides information about nutritional profile and food value of bitter melon to highlight the bioactive composition that is linked to its therapeutic effects, aiming to fully utilize bitter melon and add further value to this medicinal plant.

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Chapter 18

Evaluation of the Effect of Fruit Juice Containing Bacillus Coagulans Probiotic Supplement on the Level of Immunoglobulins A, M and Lymphocytes in Two-Speed Athletes

Elahe Ebrahimi, Maryam Golshahi, Samane Yazdi and Mohammad Mehdi Pirnia

Abstract

Probiotics exert beneficial effects on their host health by creating microbial balance in the digestive system. The role of some probiotic strains in strengthening the immune system and reducing the risk of diseases, especially respiratory infections, has been proven in previous studies. Aim: The aim of this study was to evaluate the effect of probiotic supplementation containing *Bacillus coagulans* on the Runner athletes immune system. In this study, the effect of *Bacillus coagulans* probiotic on immunoglobulins A, M and monocytes count 60 male athlete sprints Evaluates that which were randomly divided into two groups of 30.For 3 months, the experimental group received a daily glass of probiotic juice containing 10^9 cfu / ml containing probiotic supplement and the control group received plain and no supplemental juice. During the study period, once every 2 weeks, One day after exercise (running 200 meters), blood samples were taken from all participants Then In the collected samples, IgA, IgM and lymphocytes were evaluated. Consumption of probiotic juice containing 2×10^9 f cfu/ml *Bacillus coagulans* probiotic supplement showed a significant difference in the amount of IgA, IgM and Lymphocyte between the experimental group and the control group. The results of this study showed that the consumption of juice containing probiotic supplement *Bacillus coagulans* can increase the level of immune factors IgM, IgA, lymphocytes and prevent the occurrence of diseases, especially respiratory infections, by improving the function of the immune system.

Keywords: IgA, IgM, probiotic juice, immune system, lymphocytes, athletes

1. Introduction

Hard and under pressure training combined with stress caused by attending various competitions by reducing the physical and mental strength of individuals

causes dysfunction in the immune system of professional athletes. Intense training, insufficient rest and improper nutrition are the factors that induce stress in athletes and make them prone to several health complications like immune depression, inflammatory dysregulation, increased respiratory tract infections, and mental stress [1–3]. Many epidemiological studies have reported that the symptoms of respiratory tract infections increase 1 to 2 weeks after strenuous endurance competition. The biological balance of the body's organisms improves host health, improves immune system function, and increases the body's defenses. Microbiota plays an important role in the physical performance of the host [4–6]. Dietary nutrients improve energy gain during exercise, which can bring many metabolic benefits to an athlete during exercise and recovery. Studies have shown that metabolic activity and related pathways increase in the microbiome of athletes compared to sedentary individuals [7–9]. Many studies have shown the benefits of probiotics such as reducing toxins, increasing immunity and resistance to infections, production of vitamins and nutrients, production of organic acids, reduction of allergic reactions and respiratory infections, arthritis and modulation of immune responses. Investigating the link between probiotic use and physical function, which shows that probiotics protect the body against undesirable physiological changes that may be caused by strenuous exercise. Probiotics can improve intestinal barrier properties [10] and antioxidant status [11]. Decreased physical performance and immune system strength following chronic fatigue in athletes who exercise intensively has been proven in many studies. Nieman et al. (2019) showed that regular and continuous exercise increases the strength of athletes' immune systems, while with heavy and intermittent exercise, the opposite results have been reported. The results of some studies show that heavy exercise, although It does not harm the organs, but it disrupts the immune system [12]. Humoral immunity in athletes is often assessed by measuring mucosal immunoglobulins A (IgA) and M(IgM) especially the amount of secretion changes from tissues in sports activities. Decrease in immunoglobulin After the intense sports activities, Increasd the possibility of upper respiratory tract infections. Lymphocytes play a very important role in the immune system due to the secretion of antibodies. Increasing the level of immunity and reducing the risk of disease in athletes, in addition to Increase their performance, will reduce treatment costs and improving immune function [13–19], so in this study, the effect of consumption of probiotic juice of *Bacillus coagulans* on the level of some immune system factors such as IgA IgM and lymphocyte Has been studied.

2. Materials and methods

In this study, probiotic juices containing 2 × 109 cfu *Bacillus coagulans* were prepared. 60 male runner athletes with an age range of 18 to 20 years and an average weight of 68 to 72 kg were randomly divided into two groups of 30 people. The criterion for participating in the study of complete physical health and was non-smoking. During the 12 weeks of the study, the experimental group received a glass of probiotic juice containing *Bacillus coagulans* daily, and the control group received fruit juice Without supplemental.

2.1 Collect blood samples

Every once two weeks, One day after exercise (Running a distance of 200 meters) 8 ml of blood was taken from all subjects Blood samples were collected in heparin tubes and immediately transferred to the laboratory. To separate the blood serum, samples were centrifuged at 5000 rpm for 15 minutes.

2.2 Measurement of immunoglobulin A& M levels

In the present study, the levels of immunoglobulins A and M were measured using ELISA kits (ab196263 andab214568). The test steps were performed according to the protocol recommended by the kit manufacturer (abcam Inc., USA) And light absorption of the samples was read by ELISA reader (Biotech microplate reader ELX800).

2.3 Lymphocyte cell count

Lymphocyte counts were performed by flow cytometry. Blood samples were collected in heparinized tubes and then centrifuged. The cell layer was washed with phosphate buffered saline (PBS) twice, then 10 µl of monoclonal antibody was added and the samples were incubated in the dark for 20 min. To remove excess antibodies, wash twice with buffer. to measure adsorption Optical samples of fluorescent isothiocyanate fluorescein fluorochrome (ftic) were used. Then the tubes containing the sample were placed in a flow cytometer and the results were reported based on percentage.

2.4 Statistical analysis

Analysis of variance (ANOVA) was used to determine the effects of probiotics. Sample t-test was used to identify differences within the group (Beginning and end of the experiment). Values less than 0.05 were considered significant. All statistically The analysis was performed using Graphpad Prism software (version 6).

3. Results

All participants completed the test. Taking a probiotic supplement did not cause any side effects. During the first month of the experiment, no significant change in the level of immune factors was observed. With increasing probiotic supplementation time, the experimental group had a significant increase in the levels of immunoglobulin A, immunoglobulin M and lymphocytes compared to the control group, That was arrangement it IgA (258 vs. 233 ug/dL) (**Figure 1**), IgM (159 vs. 133 ug/dl)

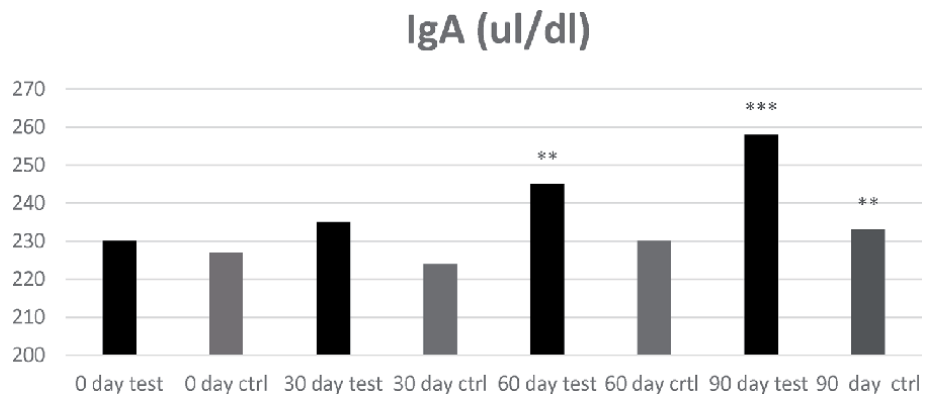

Figure 1.
*Immunoglobulin A level in experimental and control groups during the study time (0–90 days). * standard deviation, ** means that the difference in the results in each row of values is not significant, *** means that the difference in results in each row is noticeable, (P < 0.05) different.*

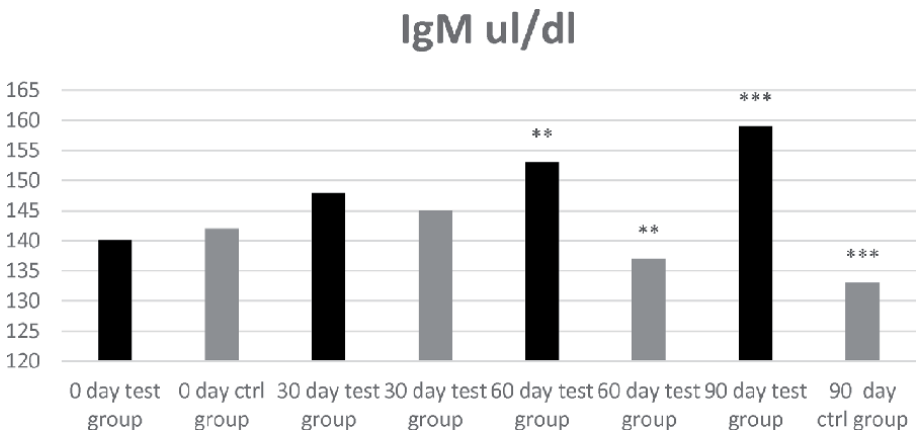

Figure 2.
*Immunoglobulin M level in experimental and control groups during the study time (0–90 days). * standard deviation, ** means that the difference in the results in each row of values is not significant, *** means that the difference in results in each row is noticeable, (P < 0.05) different.*

Group	Time (Day)	0	30	60	90
TEST		32 ± 4.1 (Cell/µl)	32.7 ± 2.6 (Cell/µl)	36.8 ± 5.3** (Cell/µl)	37.6 ± 4.6*** (Cell/µl)
Control		32.1 ± 3.9 (Cell/µl)	32.3 ± 3.5 (Cell/µl)	31.0 ± 4.3 (Cell/µl)	33.6 ± 3.1* (Cell/µl)

**Standard deviation.*
***means that the difference in the results in each row of values is not significant.*
****means that the difference in results in each row is noticeable.*
(P < 0.05) different.

Table 1.
Lymphocyte cell count in both experimental and control groups one day after exercise (two 200 m speed) in terms of%.

(**Figure 2**) and lymphocytes (37.6 vs. 33.6 cell/ul) (**Table 1**). In addition, there was a significant reduction in the incidence of respiratory infections among the supplement group compared to the control group.

4. Discussion

Athletes due to their readiness for various competitions endure a lot of physical and psychological stress.These tensions can lead to decreased immune system function and the development of multiple infections. Probiotics are natural compounds with a variety of benefits to increase the quality of life of people by improving their health by improving the function of the immune system [20–22]. The results of this study showed that consumption of fruit juice containing the probiotic *Bacillus coagulans* has a positive effect on improving the health and quality of life of athletes by improving the function of the immune system and reducing the risk of respiratory infections. A 2007 study by Kakonen et al. found that runners who took probiotic supplements were less likely to develop respiratory infections than other athletes [23]. The results of a 2016 study by Ahanchian et al. Also showed the effect of taking probiotic supplements on the prevention of respiratory infections [24]. In this study, it was found that taking probiotic supplements over time shows a positive effect on health. The results of the Zhang et al. study also showed that

taking probiotic supplements Lactobacillus casei after 16 weeks has a significant effect on increasing the level of immunoglobulins A [25]. Based on this, it can be concluded that to evaluate the beneficial effects of probiotics, it is necessary to take these natural supplements regularly and intermittently.

Michalikova and colleagues examined the effect of taking helveticus Lafti L10 on humoral safety in athletes. The results of this study showed that taking this probiotic supplement for fourteen weeks significantly improves the immune status of athletes [26]. The effect of *L. fermentum* supplement on respiratory and immune system of runners was evaluated by Batatinha and colleagues. The results of this study showed that taking this supplement has a positive effect on athletes' health [27]. Consumption of probiotics restores or maintains the balance of intestinal microbiota, reduces oxidative stress, and improves cardiovascular function in athletes. Strengthening the control system and inhibition of free radicals was observed in athletes using supplements containing several probiotic strains (L. rhamnosus® and *L. paracasei*) [28, 29]. Decreased gastrointestinal discomfort and endotoxin content in athletes during multi-component probiotic supplements (L. acidophilus CUL-60, L. acidophilus CUL-21, B. bifidum CUL-20, B. animalis subsp. Lactis CUL-34) Has been [30]. The results of the study by Strasser et al. Showed that the use of multicomponent probiotic supplements including (B. bifidum, B. lactis, *E. faecium*, L. acidophilus W22, *L. brevis* and L. lactis) impaired tryptophan levels during intens exercise By increasing serotonin levels, exercise induced stress is reduced and athletes' mental state is improved [31].

Probiotics directly affect the function of endocrine immune cells, modulate the immune system and strengthen the immune system, and most importantly, have a positive effect on the immune system without causing a harmful inflammatory response. Therefore, it seems that probiotics are a natural and healthy method that can increase host resistance in the face of injury and stress [32]. The effect of taking the probiotic supplement L-fermentum was evaluated in another study in 2014. The results showed that the level of influenza-specific immunoglobulin increased significantly with the use of this supplement compared to the control group [33].

The effect of consuming a mixture of probiotics on increasing the number of immune cells, including lethal t cells, cd4 cells, lymphocytes and monocytes, has been proven in a study by Wu et al. [34]. Considering the importance of maintaining health and improving the performance of athletes during competitions, based on the results of this study and other studies conducted in this field, it was found that taking probiotic supplements will have many positive effects on the health of people, especially professional athletes.

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Chapter 19

Oilseeds as Functional Foods: Content and Composition of Many Phytochemicals and Therapeutic Alternatives

Aicha O. Cherif

Abstract

Oilseeds composition has been studied extensively, but recently it has been thoroughly investigated considering especially the phytochemicals representing the minor components. This interest is connected with the activity of such compounds against cardiovascular diseases, lipid oxidation, protein cross-linking and DNA mutations and hemostasis function, which prevent the attack of biomolecules by free radicals. This chapter book could aim to give an overview of the different uses of several oilseeds as bioactive foods, focusing on their active constituents (phytosterols, polyphenols, tocopherols, tocotrienols, and carotenoids) and their content in oilseeds. We will also focus on the beneficial aspects of theses nutraceuticals in human health.

Keywords: oilseeds, phytosterols, phenolic acids, tocopherols, tocotrienols and carotenoids

1. Introduction

Phytochemicals in plant material have raised interest among scientist, producers, and consumers for their roles in the maintenance of human health and in assessing the protective status of people from chronic degenerative disorders. These biologically active compounds have been reported to elicit several biological effects, including cardioprotective, anti-inflammatory, anticancer, and others. Plant derived phytochemicals have been the focus of recent research due to their health promoting effects [1].

Phytochemicals are bioactive non-nutrient chemical compounds found in plant foods, such as fruits, vegetables and grains which may be potent effectors of biologic processes and have the capacity to influence disease risk via several complementary and overlapping mechanisms. Nowadays, thanks to technological and scientific advances, it is possible to extract, characterize, and evaluate bioactive compounds from foods and medicinal plants [2].

From phytosterols to polyphenols, fat to polyphenols, it is the combination of these components that lead to good health and well-being. Furthermore, many of the characteristic components of oilseeds are known to have positive effects on health, capacity and well-being, and can be used to design functional foods.

Vegetables, fruits and nuts are all rich in phenols, flavonoids, isoflavonoids, phytosterols and phytic acid–essential bioactive compounds providing health benefits. In fact, with the increasing demand for edible oil, plant sources have become the target for research to explore their quality and functional properties. Thus, there are many in vitro, in vivo, randomized, and clinical studies evaluating the ability of bioactive compounds to provide health benefits. Also, their beneficial health effects starting from major diseases and health conditions that are in the first places of death worldwide, including cardiovascular disease, cancer, diabetes, neurodegenerative diseases, and aging [3].

Therefore, this chapter is intended to describe the main bioactive compounds such as sterols, polyphenols, tocopherols, and carotenoids. It will also encourage a large consumption of this species in local and international markets and its possible industrial use, especially in the food.

2. Phytosterols (DESM, MMS, DIM)

Phytosterols also called plant sterols, are minor components of vegetable oils and from a major proportion of the unsaponifiable fraction of lipids which can occur in vegetable oils either in free form or esterified with fatty acids [4]. The individual sterols and their relative proportions can be used to determine the identity of the oil and to detect adulterations. Phytosterol contents in vegetables are known to vary due to different factors such as variety, season, extraction, and other technological procedures [5].

Furthermore, phytosterols are known to lower serum mow-density lipoprotein (LDL). Cholesterol levels by reducing intestinal cholesterol absorption. Clinical studies confirmed that phytosterols have hypocholesterolemy, anti-inflammatory and anticarcinogenic effects [5]. Therefore, phytosterols have been added to several functional food products such as yoghurt, milk and some vegetable oils. These types of products are now available on the market and have been scientifically proven to lower blood LDL cholesterol by around 10–15% as part of a healthy diet [5].

2.1 Structure

Phytosterols (PS) are by-products of the isoprenoid biosynthetic pathway via squalene from acetyl-coenzyme A [6]. They are 28or 29-carbon alcohols and resemble cholesterol in vertebrates in term of both functions, i.e. by stabilization of phospholipids bilayers in plant cell membranes as in structure, given the four-ring steroid nucleus, the 3β-hydroxyl group and often a 5, 6-double bond (**Figure 1**) [7].

They have been categorized into three subclasses: on the basis of the number of methyl groups at the C_4 position: (4-DEMS) none (4-desmethylsterols or "sterols"), (4-MMS) one (4α-monomethyl-), and (4-DIMS) two (4,4′-dimethylsterols or triterpenes alcohols) [8, 9]. The main compounds of 4-desmethylsterols class are β-sitosterol, campesterol, stigmasterol and Δ^5- avenasterol (**Figure 2**). 4,4′-dimethylsterols (triterpenes alcohols) and 4α-monomethylsterols are metabolic intermediates in the biosynthetic pathway leading to the end-product, 4-desmethylsterols, but they are usually present at lower levels than 4-desmethylsterols in most plant tissues [8–11]. The major components of 4,4′-dimethylsterols are β-amyrin, α-amyrin, cycloartenol and 24-methylencycloartanol (**Figure 2**). In the case of 4α-monomethylsterols, we evoke mainly Obtusifoliol, gramisterol and citrostdienol (**Figure 2**). Generally, plant sterols are 4-desmethylsterols because they do not contain any methyl groups at the fourth position of the sterol ring structure [12].

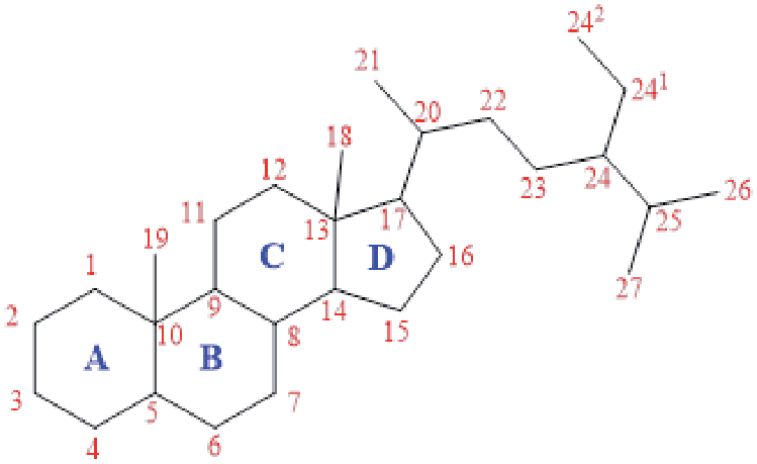

Figure 1.
Steroid structure.

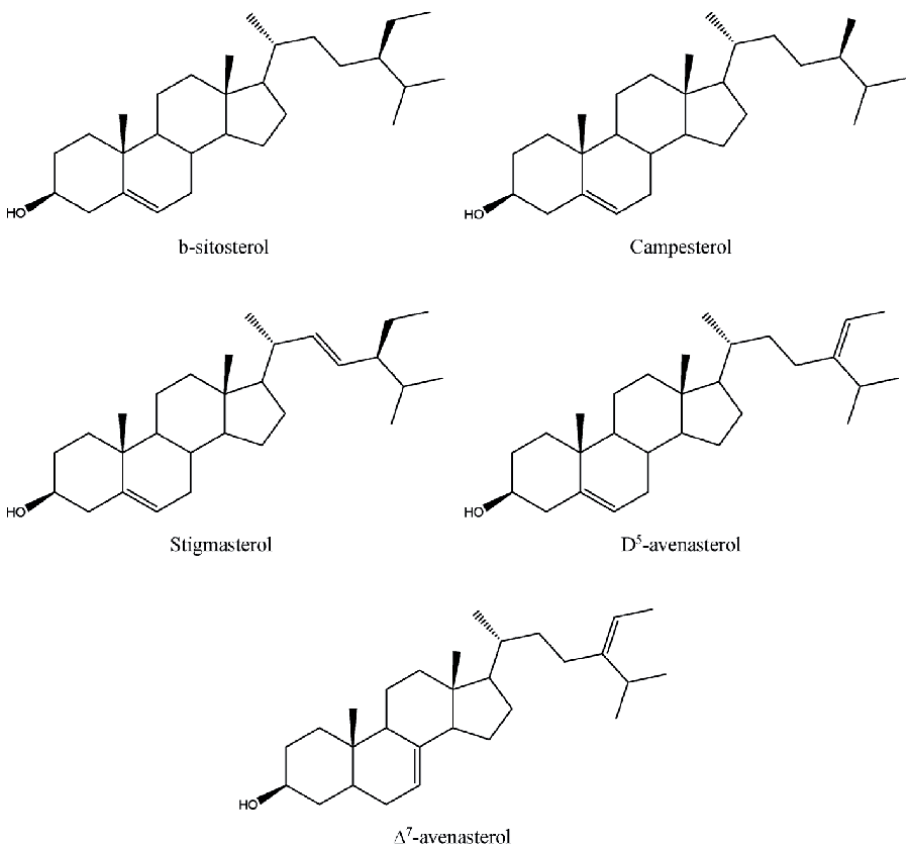

Figure 2.
Detailed chemical structures of b-sitosterol, Campesterol, Stigmasterol, D⁵-avenasteroland and D⁷-avenasterol.

2.2 Plants as source of phytosterols

Vegetable oils, oilseeds, and nuts are the richest sources of phytosterols. β-sitosterol (29C), campesterol (28C) and stigmasterol (29C) are the most common sterols [4, 12]. The total PS content and profile can vary according to

variety, agronomic and climatic conditions, maturity, extraction and refining methods [13, 14].

The impact of cultivar on seed oils phytosterols content was demonstrated and many major phytosterols components were identified on some seed oils of commercial importance such as rapeseed, soybean, and sunflower have been regarded as rich sources of phytosterols [15].

2.2.1 Health benefits

Several studies have demonstrated the PS protect against many chronic ailments such as cardiovascular diseases [16, 17], cancer [18], ulcers [19], diabetes [20], and inflammation [21].

4-desmethylsterols contribute to lowering serum cholesterol levels [22]. In fact, it have been reported that they have the capacity to reduce dietary cholesterol absorption in the intestine [22]. They are also considered to have anti-inflammatory, anti-bacterial, anti-atherosclerotic, anti-oxidative, anti-ulcerative, and anti-tumor properties in humans [8–23], as well as contributing to the oxidative and thermal stability and shelf-life of vegetables oils [24]. In vivo studies have shown that diets enriched with PS (2%, w/w) contributed to improve lipid profiles and decreased atherosclerotic lesions in apolipoprotein E-knockout (apoE-KO) mice [20]. In addition, they are useful emulsifiers for cosmetic manufacturers and supply most steroidal intermediates and precursors to produce therapeutic steroids [25].

PS have been reported to have a protective effect against various forms of cancer such as breast [21], prostate [26], lung [4], liver and stomach [27], and ovary and colon cancers [28].

Triterpenes compounds are also important bioactive secondary metabolites, due to the wide range of their biological activities. They show mainly antimicrobial, cytotoxic, antitumoral, antiviral, anti-inflammatory- hepatoprotective, antifeedant and insecticidal activities [29].

3. Phenolic acid

Phenolic phytochemicals are the most abundant secondary metabolites and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols (tannins, stilbenes and lignans) [30].

Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants [31]. They are plant secondary metabolites, and they have an important role as defense compounds. Phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes.

3.1 Structure

They are defined as chemical substances possessing one or more aromatic rings with one or more hydroxyl groups in their structures [32].

The term "phenolic acids" (PA), in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxycinnamic and hydroxybenzoic

structures (**Figure 3**). Hydroxycinnamic acid compounds are produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure and are characterized by hydroxylated aromatic rings [33]. These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation, and cancer. Indeed, tumor cells, including leukemia cells, typically have higher levels of reactive oxygen species (ROS) than normal cells so that they are particularly sensitive to oxidative stress [34]. Many papers and reviews describe studies on bioavailability of phenolic acids, emphasizing both the direct intake through food consumption and the indirect bioavailability deriving by gastric, intestinal, and hepatic metabolism [35]. In addition, Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical, and medical studies. In recent years, the importance of antioxidant activities of phenolic compounds and their potential usage in processed foods as a natural antioxidant compounds have reached a new level and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [36].

3.2 Plants as source of phenolic acid

The hydroxycinnamic acids are more common than are the hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, and ferulic. Indeed, caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represents between 75 and 100% of the total hydoxycinnamic acid content of most fruit. Concentrations generally decrease during ripening but increase as the fruit increases in size [37]. The hydroxybenzoic acid content of edible plants is generally very low, except for certain red fruits, black radis and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight [37]. Tea is important source of gallic acid; tea leaves may contain up to 4.5 g/kg fresh wt [37]. It has been stated that the most important PA derivatives are in the rapeseed oil including 2.6 dimethoxy-4-vinylphenol (257 μg/100 g) and ferulic acid (5.6 μg/100 g), while vanillic acid (11.4 mg/100 g) is in pumpkin seed oil and ferulic

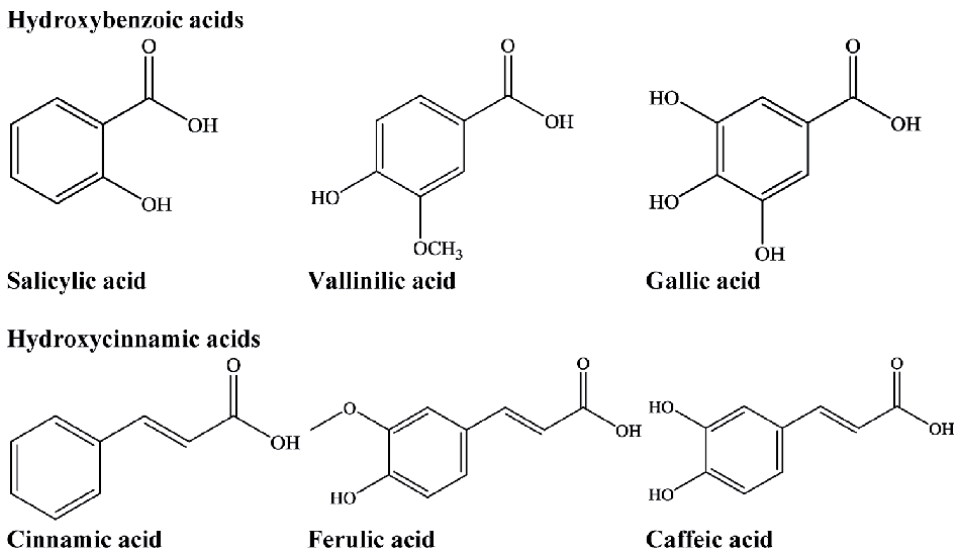

Figure 3.
Structure of the important naturally occurring phenolic acids.

acid (5.8 mg/100 g) is in corn oil. The total amount of PA was determined as 79 mg gallic acid/kg oil in soy oil, 124 mg gallic acid/kg oil in canola oil, 8397 mg gallic acid/100 g oil in palm fruit, and 20–43 mg synapic acid/100 g oil in rapeseed oil [38]. PA content and profile in plant oil generally depends on the variety, environmental conditions, extraction methods, and storage conditions [39].

3.3 Health benefits

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical, and medical studies. Phenolic compounds in many plants are polymerized into larger molecules such as the proanthocyidins (PA; condensed tannins) and lignins. Moreover, phenolic acids may arise in food plants as glycosides or esters with other natural compounds such as sterols, alcohols, glucosides, and hydroxy fatty acids. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as *Staphylococcus aureus* are some of biological activities of phenolic acids [40]. Varied biological activities of phenolic acids were reported. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, and antidepressant activities [41]. Moreover, a phenolic compound can interrupt the radical chain reaction by donating a hydrogen stom to the free radicals and therefore converting itself to a radical. PA can also act as metal chelators and oxygen scavenger and helps then against diseases associated with oxidative stress [42].

4. Tocols

4.1 Structure

Tocopherols and tocotrienols, together abbreviated as tocols, are natural lipophilic antioxidants that protect oxidation in vegetable oils [43]. Tocopherols

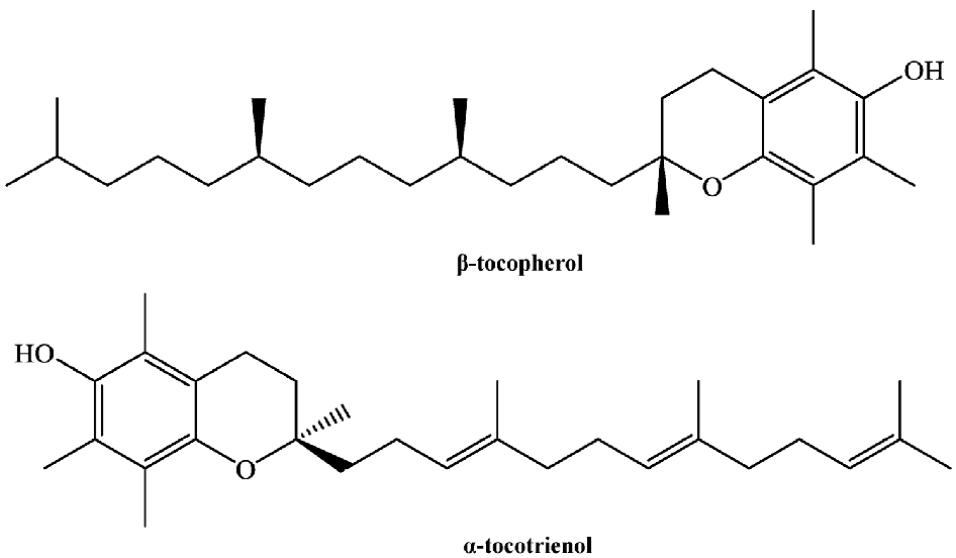

Figure 4.
Structure of the important naturally occurring tocopherols.

(vitamin E) are the most important and effective lipid-soluble compounds constituting a family of antioxidants with several health benefits [44]. Vit E comprise a chromanol ring with a C16 phytol side chain and are reclassified in two types according to which the side chain is either saturated (tocopherols) or contains three double bonds at carbons 3, 7 and 11 (tocotrienols). The presence of methyl (-CH_3) group in the aromatic ring of tocopherol (**Figure 4**) makes this compound stable to heat, alkali, or acid. However, this vitamin undergoes degradation and isomerization under certain stress conditions, such as oxidizing agents or UV light, yielding four major vitamers: isomers (i.e., α-, β-, γ-, and δ-tocopherols) (**Figure 4**) and differ in the matylation patter of the benzopyran ring with three methyl groups (at C-5, C-7, and C-8) [45].

Among these homologs, α-tocopherol exhibits 100%biological activities, 30, 15, and 5% for β-, γ-, and δ-tocopherols, respectively. Seeds often dominate other plant parts in terms of the abundance of total-tocopherols (T-tocopherol), although α-tocopherol, a form of vitamin E that is most biologically active, is often only a minor component [44].

4.2 Plants as source of tocopherols

For tocopherols, the richest dietary sources are vegetable oils and the products made from these oils. Genetic factors and cultivars differences predominantly drive the expression of tocopherol compounds in seed oil exist. Vegetable oils contain not only α-tocopherol but also other tocopherols, especially γ and Δ tocopherol [46]. The soybean and corn oils are usually dominated by γ-tocopherol while in olive oil the more abundant form is α-tocopherol [47]. The tocopherol contents in seed oils range from 2 to 8 mg/100 g of coconut oil to 113 to 183 mg/100 g of corn oil [48]. The amounts of tocopherols in vegetable oils vary according to variety, extraction method, and refining [49].

4.3 Health benefits

Plant lipids containing high level unsaturated fatty acids are prone to oxidation, therefore lipophilic antioxidants such as tocopherols are often found to co-exist with plant lipids, protecting the integrity and vitality of the plant [50]. Tocopherols and tocotrienols are vitamin E homologs, serving as strong antioxidants and having many essential physiological functions such as anticoagulant, essential regulator of metabolic processes including inflammation and cancer in humans [51]. Vit E is also indispensable for immune defense. It has been suggested that tocopherols, acting as hormones or as secondary donors of genetic information, control the expression of some genes [48]. Vit E deficiency causes the damage of cellular membranes resulting from oxidation of the unsaturated fatty acids in lipids, and vitamin E deficiency can also display itself as muscular pain and progressing muscular disorder [48].

Meanwhile, the importance of tocotrienols in human health both as vitamin E and bioactive components has received renewed recognition in recent years. Tocotrienols have hypocholesterolemic, anti-cancer and neuroprotective properties.

5. Carotenoids

5.1 Structure

Carotenoids are natural pigments with yellow, orange, and red colors. More than 600 carotenoids have been identified in nature. Their physiological functions

in promoting health are as pro-vitamin A and as antioxidants quenching singlet oxygen radicals (**Figure 5**). Carotenoids generally have a 40-carbon skeleton of isoprene unites cyclized at one or both ends [11]. The majority of carotenoids that occur in nature are in trans-form. Because of the long series of conjugated double bonds in the central part of its chemical structure, carotenoids exhibit light absorbing and unique singlet oxygen quenching capability [52]. Carotenoids can be classified as carotenes and xanthophylls, based on their chemical structure. α-carotene, β-carotene and lycopene are the predominant non-polar functional carotenoids and lutein is the primary polar functional carotenoids [53] (see **Figure 5**). Carotenes contain only a parent hydrocarbon chain without any functional group, while those bearing oxygen-containing functional group are called xanthophylls (e.g. astaxanthin, lutein, zaeaxanthin). Carotenoids can even be classified as pro-vitamin A (e.g. α-carotene, β-carotene, and β-crytoxanthin) and non-provitamin A compounds [53]. Actually, carotenoids present various substitutions: terminal ring systems joined by the chromophore-bearing chain of conjugated double bonds (e.g. β-carotene), hydroxyls at the terminal rings (e.g. zeaxanthin, lutein), ketone-groups with or without additional hydroxy groups (e.g. astaxanthin and canthaxanthin), aromatic rings (e.g. synechoxanthin), and the rare monocyclic carotenoids (e.g. torulene). There are over 700 carotenoids, from which 40 are ingested in human diet from fruits and vegetables.

5.2 Plants as source for carotenoids

Carotenoids distribution in plants is associated with the de novo synthesis that occurs in the differentiated plastids of roots, flowers, fruits, and seeds. Their accumulation can be subdivided as chloroplasts (green plastids), chromoplasts (yellow, orange, and red plastids), amyloplasts (plastids containing starch), elaioplasts (lipid containing plastids), leucoplasts (colorless plastids), and etioplasts (dark-matured precursors of the chloroplast) [53].

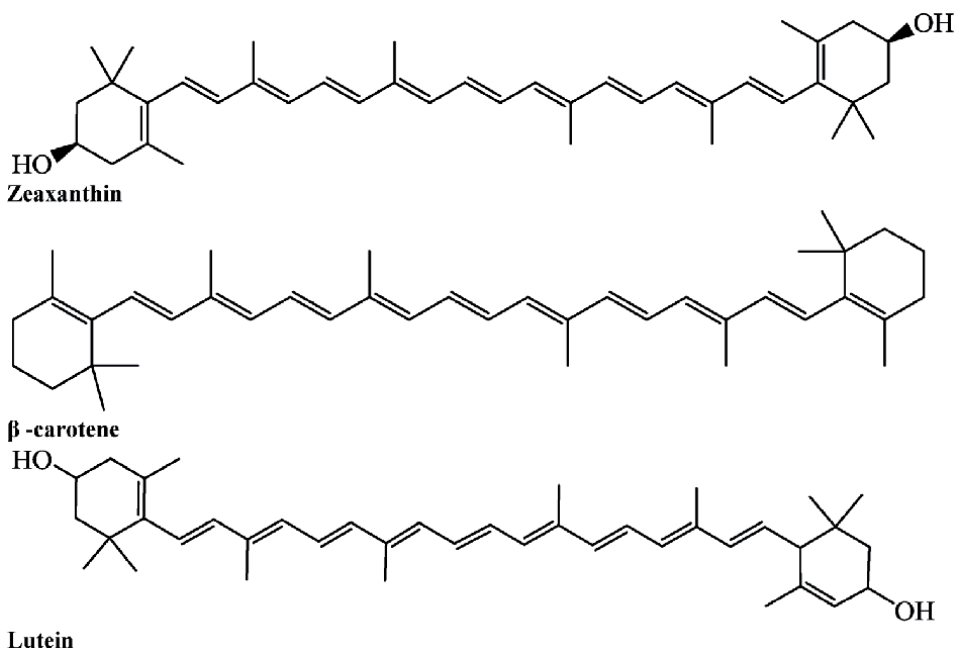

Figure 5.
Structure of the important naturally occurring carotenoids.

Because plants can synthesize carotenoids de novo, they are widely distributed in plant-derived foods and the composition is enriched by the presence of small amounts of biosynthetic precursors and derivatives of the major carotenoids. In general, the level of carotenoids is directly proportional to the intensity of color. Egg yolks, dairy products, fruits, vegetables, legumes, grains and seeds are their major food sources. In green leafy vegetables, b-carotene is predominant while in the orange-colored fruits and vegetables such as carrots, apricots, mangoes, yams, winter-squash, other carotenoids typically predominate. Yellow vegetables have higher concentrations of xanthophylls with a low provitamin A activity, but some of these compounds, such as lutein, may have significant health benefits. The red and purple vegetables and fruits such as tomatoes, red cabbage, berries, and plums contain a large portion of non-vitamin A active carotenoids. Tomato and watermelon are major sources of lycopene [53].

Higher plant usually contains similar carotenoids; however, their distribution differs quantitatively. It is known that the oil with the highest carotenoid content is crude palm oil (500-700 mg/100 g of oil). Carotenoid's content of the other crude vegetable oils is below 100 mg/100 g of oil [38].

5.3 Health benefits

Carotenoids, another group of lipid-soluble compounds synthesized by plants, are also strong antioxidants in addition to functions in plants' photosynthesis. Carotenoids are also essential to human health. More than 700 carotenoids have been identified in plant foods and human body, but the overwhelming majority (90%) in human diet is represented by β- carotene, α-carotene, lycopene, lutein, cryptoxanthin and zeaxanthin [51].

Carotenoids are characterized by a high reactivity due to their system of conjugated double bonds. They can readily suffer chemical transformation being oxidized by reactive species to a number of compounds. After ingestion, carotenoids suffer a series of modifications in the organism, namely through the reaction with reactive oxygen and nitrogen species (ROS and RNS, respectively). Interestingly, the way carotenoids react with ROS and RNS seems to depend on different factors, namely concentration of carotenoids, oxygen pressure, presence of other antioxidants, etc. Moreover, these factors may imply variations of the redox properties of carotenoids and of the oxidation products formed. These compounds, designed as oxidation products, are not yet fully studied and/or identified in biological tissues, but there are some studies relating them with the growth of several cancer cells and to oxidative effects. The increase of knowledge in this field seems to be truly important to establish the real impact of carotenoids and their oxidation products in human health [53]. Studies have shown that antioxidant carotenoids have protective effects against skin disorders, eye disorders, cancer, and cardiovascular diseases [54].

Carotenoids in oils play important role in the stability of the oil as a singlet oxygen quencher in addition to their coloring properties [55]. Carotenoids, together with PA and tocols, are involved in the oxidative stability of oils and have synergist antioxidant effects [56].

6. Conclusion

Oilseeds, like other plant sources, provide an important reservoir of myriad of phytochemicals. However, little has been researched about the relationship between ripening index of the seed bearing and oil fatty acids, bioactive compounds, and

antioxidant activity. This should be considered to have a clearer understanding of the preharvest effect on seed oil nutritional qualities and antioxidant properties. For instance, the drying time of seeds would have a considerable effect on extracted oil quality. In view of the thermolabile nature of bioactive compounds such as tocopherols and polyphenols, the effect of seed drying and pretreatments for seed drying on oil antioxidant compounds and capacity deserves more research. This would assist in quantifying losses of bioactive compounds losses and instituting preventive measures at this stage of seed oil processing.

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The phytochemicals present in functional foods play a vital role in boosting immunity and promoting health. This book provides a comprehensive overview of the importance of functional foods and antioxidants and their scavenging activity for preventing various health-related disorders. This book also covers the therapeutic and medicinal potential of various bioactive compounds for a healthy lifestyle, as well as examines different products containing functional ingredients that demonstrate health-promoting potential.

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