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Superfood and Functional Food

An Overview of Their Processing and Utilization

Edited by Viduranga Waisundara and Naofumi Shiomi

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Meet the editor

Editor, Dr. Viduranga Waisundara obtained her PhD from the Department of Chemistry, National University of Singapore in Food Science and Technology in 2010. She was a lecturer at Temasek Polytechnic, Singapore, from July 2009 to March 2013. Following this, she re-located to her motherland Sri Lanka and spearheaded the Functional Food Product Development Project at the National Institute of Fundamental Studies from April 2013 to October 2016. She is currently pursuing independent writing projects in Kandy, Sri Lanka. Dr. Waisundara is a prolific writer with many research publications and articles in newspapers and magazines. She has also been an invited speaker in international conferences and participated in local school events in Sri Lanka to spread awareness on functional food and dietary habits.

Co-Editor, Dr. Naofumi Shiomi studied recombinant yeast as a researcher at the Laboratory of Production Technology of Kanena Corporation for 15 years until 1998 and earned his PhD degree in Engineering from Kyoto University. He now works as a professor at the School of Human Sciences of Kobe College in Japan, where he teaches biotechnology and life science in his "Applied Life Science" laboratory. He has studied bioremediation and biomedical science for 20 years at Kobe College and has published more than 40 papers and several book chapters. His recent research has focused on the prevention of obesity and aging.

Contents

Preface

This book primarily focuses on the usage and application of plant- and animal-based food products with significant functional properties and health benefits as well as their utilization in the development of processed food products. Many chapters focused in this book contain overviews on superfood and functional food from South America. It is common knowledge that there are many underutilized and undiscovered phytochemicals and, thereby, phytonutrients in this part of the world. Through these chapters, it is hoped that the readers would be able to obtain knowledge and information on some plant-based functional food products from South America, specifically Chile and Brazil.

It is of value and importance that details on the functional properties of apiculture products are also included in this book. Bee products are gaining rapid interest and enthusiasm given the substantiation of their usage for various disease conditions through scientific means.

Additionally, an area that is not widely discussed in academia—pet food with functional properties—is also included in this book. It is established that many domestic animals appear to be suffering from the same disease conditions as their human counterparts and, thus, remedying such conditions is rapidly gaining much interest as well.

While superfood and functional food appear to be a major topic of discussion on a global scale, the safety aspects of certain food products need to be evaluated as well. Additionally, given the global pandemic of obesity and the importance of weight management, the capabilities of superfood and functional food in mitigating obesity appear to be an area of interest. These two aspects are also covered through two chapters in the book.

It is hoped that this book will be of value to both scientific and nonscientific communities to make informed choices about food products with proven benefits and, thereby, partake in a global movement of remedying the disease conditions that appear to be of severe effect on the health and wellness. It is also recommended for readers to take a look at a related book, *Superfood and Functional Food – The Development of Superfoods and Their Roles as Medicine*.

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Section 1

Apiculture Products as Functional Food

Chapter 1

A Systematic Review of the Antioxidant Activity of Apiculture Products in Brazil

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Dark Luzia dos Santos Neto and
Sandra Maria Botelho Mariano

Additional information is available at the end of the chapter

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Abstract

The use of substances with antioxidant ability can be very important in the therapeutic prevention of diseases related to increased oxidative stress, such as cancer, heart disease and aging. Brazil has a great diversity of vegetation from which bees can collect resins. This study is a systematic bibliographical review on different electronic scientific databases through descriptors of antioxidant activity of bee products from Brazil. The identification of the articles and their inclusion occurred between the months January to MayW 2016. The bibliographic research was conducted in the following electronic databases: (1) Scientific electronic library online—SciELO; (2) Public library of science—PLOS Medicine; and (3) ScienceDirect. The articles selected were the ones that contained antioxidant activity evaluation of bee products from Brazil during 2011 and 2016. It was possible to observe a large number of articles published in this topic, but a compilation of data from all of these studies was necessary. Given there is a great diversity of vegetation in Brazil, a standardization process of the bee products in Brazil was conducted and by means of this process it was possible to draw a profile of the main antioxidants found in apiculture products in Brazil.

Keywords: phenolic compounds, DPPH, HPLC

1. Introduction

The use of substances with antioxidant ability can be very important in the therapeutic prevention of diseases related to increased oxidative stress, such as cancer, heart disease and aging. Brazil has a great diversity of vegetation from which bees can collect resins. This gives place to large chemical diversity among apiculture products collected in different regions and seasons.

This chemical diversity becomes important due to the derived biological properties. Given that apiculture products have great chemical diversity they must be chemically standardized before use to ensure quality, efficacy and safety and thus it is possible to correlate the type of product with its therapeutic application.

The word propolis is derived from the Greek word pro that means in defense and polis that means city or community; thus propolis means in community defense. It is a material in resin form with complex and varied chemical composition, which is gathered from various species and parts of plants (sprouts and exudates) and results in a substance of different colors and consistencies [1–3]. Propolis production in Brazil is estimated at around 100 tons per year. A large part of this percentage is for export, either in a raw form as in manufactured goods, reaching high prices in foreign trade and representing an important source of income [4, 5]. Among the apiculture products, propolis has been subject of pharmacological studies due to its antimicrobial [6], antiinflammatory [7] and antioxidant properties [8], among others. This biological potential is due to a synergism that occurs amongst its various constituents. Propolis is an important therapeutic alternative from an economic point of view because of its pharmacological efficacy since it is easy to obtain and presents pharmaceutical properties. The use of substances with antioxidant ability can be very important in the prevention and treatment of diseases related to increased oxidative stress, such as cancer, heart disease and aging [9].

Brazil has a great diversity of vegetation from which bees can collect resins. In addition, diversity increases when products are collected in different regions and seasons. Due to this chemical diversity, there has been intense research over the past decades to classify the different types of propolis and bee products found in Brazil [1, 10, 11]. Propolis needs to be chemically standardized before use to ensure quality, efficacy and safety. This way, it is possible to correlate the type of propolis and its therapeutic application, an essential task for a growing market and more demanding throughout the v one of the world's biggest suppliers of bee products [3, 11–13].

In this context, it is important to evaluate the published scientific studies about the antioxidant activity of bee products from Brazil.

2. Research methods

2.1. Study design

Study of systematic literature reviews on different scientific electronic databases through descriptors related to the antioxidant activity of bee products from Brazil. The identification of articles and their inclusion occurred between the months January to May 2016.

2.2. Eletronic databases

The bibliographic research was conducted in the following electronic databases:

1. Scientific Electronic Library Online—SciELO;

2. Public Library Of Science—PLOS Medicine;

3. ScienceDirect.

Additional information was obtained from manual search based on the references listed in the articles included in the review.

2.3. Search strategy

The searches were conducted through cataloged descriptors in Descriptor Health Sciences—DHS and Medical Subject Headings—MeSH, in Portuguese and English, contained in the title or summary of the studies. The combination of terms used together or separately, in the respective databases (SciELO, PLOS Medicine, ScienceDirect) was as follows:

- antioxidants;
- antioxidant response elements;
- phenolic compounds.

2.4. Selection and analysis of publications

For the selection of articles, a personal study was created with the following information: author, year, title, development period of the study, Province, city and research area, study design, descriptor used to locate the publication, objective and main results. An inclusion criterion was used in which the selected articles had to be original, published in international and national journals in English or Portuguese, published between 2011 and 2016 and indexed on one of the databases previously cited. Articles that contained evaluation data of the antioxidant activity of apiculture products from Brazil were selected for review.

2.5. Sampling

About 445.265 scientific articles about antioxidant activity in Brazil and in the world searched in the databases with the descriptors previously mentioned were identified. Of the total number of published articles between the years 2011 and 2016, 77 studies were related to the theme of antioxidant activity of bee products. About 60 articles were excluded for being repeated, in other languages or for not containing evaluation of products from Brazil. For this literature review, 17 studies were selected.

3. Results

For review, only articles containing analyses of evaluation of the antioxidant activity of bee products from Brazil, between 2011 and 2016, were selected. As shown in **Figure 1**, the number of published works on bee products is relevant. However, it is clear that the rate of work on this issue in Brazil is low.

The results obtained with the application of the described search strategy are presented in the logical framework of the study (**Figure 1**).

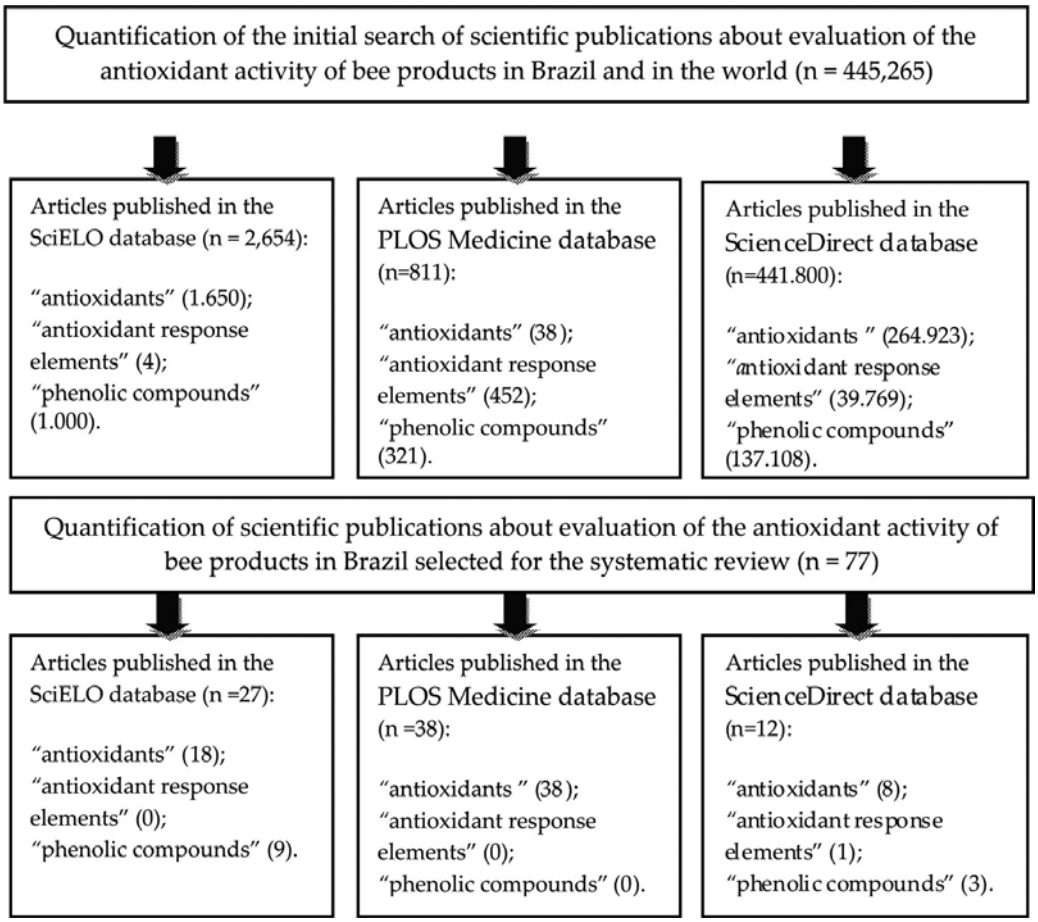

Figure 1. Logical framework of the systematic review, studies about evaluation of the antioxidant activity of bee products in Brazil between the years 2011 and 2016.

Table 1 presents a summary of the studies assessed in this study.

Given the large number of articles published in this topic was observed, a compilation of data from all of these studies was necessary. We can highlight the following identified substances from the main antioxidant substances of apiculture products from Brazil: triterpene compounds of the cycloartane, ursane and oleanane types as the main compounds, in addition to phenolic acids, protocatechuic and gallic acids. It is also important to highlight the high antioxidant potential of geopropolis from Brazil. The works presented here identified the following substances with antioxidant capacity: gallic acid, ellagic acid, catechin, gallocatechin, hesperidin, kaempferol, luteolin, morin, naringin, naringenin and rutin. Analysis of honey also shows the presence of antioxidant substances, especially gallic acid.

No.	Reference	Objectives	Main results/conclusions
01	[14]	Investigate the chemical composition of geopropolis produced by *M. fasciculata* collected from beehives of two phytogeographical regions of Maranhão and evaluate its antioxidant activity.	Geopropolis collected in Palmeirândia contained triterpene compounds of the cycloartane, ursane and oleanane type as the main compounds, in addition to phenolic acids, protocatechuic and gallic acid. In contrast, geopropolis collected in Fernando Falcão contained high concentrations of phenolic acids (gallic acid and ellagic acid) and exhibited high antioxidant activity, suggesting that the high levels of phenolic acids are responsible for the antioxidant property of this geopropolis. The chemical composition and antioxidant activity contribute to the identity and quality of the types of geopropolis produced by *M. fasciculata* collected in two phytogeographical regions of the Maranhão State, northeastern Brazil.
02	[15]	The objective of this study was to determine, among other analyses, the antioxidant capacity of propolis of stingless native bees (Meliponinae) of Tocantins, North of Brazil (State of Tocantins).	Propolis samples collected in the two regions of Tocantins presented physical and chemical characteristics that fit within Brazilian legislation for propolis quality. The flavonoid luteolin was restricted to the samples collected in Santa Maria of Tocantins, while, naringerin and rutin were restricted to the samples collected in Novo Acordo, demonstrating substantial differences between the pasture of the studied regions. And also evidence a high concentration of phenol compounds and good antioxidant capacity.
03	[16]	The objective of this study was to evaluate the effect of cassava starch coatings incorporated with propolis on the content of phytochemicals of nutritional interest and on the antioxidant activity of strawberries stored under refrigeration for 16 days.	The coating with 66% of propolis promoted higher Vitamin C content than fruits submitted to the other treatments at 8 and 12 days of storage.
04	[17]	Identify the antioxidant capacity of propolis samples collected by Apis mellifera L. bees, in four Brazilian regions and compare them using the coupled oxidation method of b-carotene system/linoleic acid.	From the propolis samples collected and analyzed by the coupled oxidation of b-carotene system/ linoleic acid, only two showed antioxidant capacity below 60%, both in the northeast. Samples of the southeast had the highest antioxidant capacity, followed by from the midwest, south and northeast. Pollen analysis revealed the predominant presence of Eucalyptus pollen, mainly from the southeast and south and a greater variety of pollen types in northeast samples.
05	[18]	Isolate phenolic substances of the extract in methanol of geopropolis, from species of *M. interrupta*, Mi5, grown in the National Institute of Amazonian Research—INPA by the Bees Research Group—GPA and MI6, grown in Ramal do Brasileirinho community in Manaus.	Analyses of geopropolis extracts of these species showed promising results, presenting antioxidant activities that are important for body maintenance and disease prevention, as well as being used as food (nutraceutical) by local people. From fractionation of the methanol extracts of geopropolis from *M. interrupta* with higher antioxidant activity was possible to isolate four flavonoid.

No.	Reference	Objectives	Main results/conclusions
06	[1]	This study aimed at verifying whether there is positive correlation between the identity and quality criteria for propolis and the biological activities exhibited by the extracts of propolis.	It was established that G12 propolis has a high content of total flavonoid and phenolic compounds, which gives it an excellent quality according to the Brazilian legislation.
07	[19]	Identify the chromatographic profile of phenolic acids and flavonoids using solid phase extraction (SPE) and liquid chromatography reversed-phase (RP-HPLC) and conduct study of antioxidant activity in honeys from three different species of bees *A. mellifera* (Africanized) exotic species, *Melipona flavolineata* (yellow uruçu) and *M. fasciculata* (gray uruçu) native species. The honey samples were obtained from four Pará State municipalities.	From 36 analyzed samples of honey, the presence of 13 phenolic compounds and three unidentified compounds (Ph1, Ph2 and Ph3) was observed. For most of the honeys, major compounds identified as gallic acid and quercetin were analyzed. The AC and ACP methods were able to distinguish the phenolic composition of the analyzed honeys for the species studied. This factor that indicates possible selectivity of the species in relation to the botanical origin of honeys. In general, analyzed honeys showed significant antioxidant activity, especially the darker honeys which also had higher levels of polyphenol.
08	[20]	The objectives of the present study were to produce and evaluate a propolis extract for use in skin care products.	Reasonable stability was noted and the preferred formula was the one that used combination of propolis extract with tocopheryl acetate.
09	[21]	Comparison between the antioxidant activities of ethanolic extracts of propolis prepared using hydrous ethanol with different ethanol/water contents and different propolis concentrations.	It was noted that the DPPH• and FRAP values are dependent on the propolis concentration and the water/ethanol proportion used in the extraction. This correlation was also observed in phenolic contents and flavonoid contents.
10	[22]	The present work aimed at performing a bioassay-guided fractionation of red propolis samples from Igarassu (Pernambuco, Brazil) in order to determine the main constituents associated with its antimicrobial activity, especially against *Candida sp.*	The botanical origin of propolis samples is difficult to ascertain on the basis of only one palynological analysis and a more definite confirmation depends of analysis comparing the chemical profile of the samples with the chemical profile of resins and extracts from plants found near the hives. It should be stressed that red propolis has been suggested as being the only propolis type derived from a plant from the leguminosae family (*D. ecastaphyllum*), rich in inisoflavones such as genistein and formononetin. Although flavonoids exhibit pleiotropic activity affecting several different targets and the synergistic effects cannot be discarded, our results suggest that the isoflavone formononetin is responsible at least partially for the antimicrobial activity of red propolis.
11	[23]	This work aims at optimization the extraction process of bioactive compounds, evaluate antioxidant activity and also conduct the chemical characterization of propolis, using the high performance liquid chromatography technique.	It was concluded that the analyzed propolis has a promising phenolic content and antioxidant activity. Three phenolic acids derived from hydroxycinnamic acid were identified, common in Brazilian propolis.

No.	Reference	Objectives	Main results/conclusions
12	[24]	This paper aims to identify the pollen types and quantify the total phenolic compounds in propolis samples produced in the arid territory of Bahia—Brazil	The levels of the total phenolic compounds found in the propolis samples from the territory of the arid region of Alagoinhas fulfill the standards of the Brazilian legislation. We recommend that more analyses should be conducted in order to obtain more data that corroborate the information contained here.
13	[25]	We evaluated the effects of phenolic compounds from three propolis-based products with different concentrations of propolis and levels of alcohol on feed intake, digestibility (ruminal and intestinal) and blood parameters in lactating dairy cows.	The propolis-based products have positive effects on protein metabolism in the rumen, without interfering with any other parameter evaluated. The propolis concentration and alcoholic level used in this study influences the amounts of flavonoids and phenolic acids in the propolis-based products, which may interfere with the observed effects on ruminal metabolism and digestive parameters.
14	[26]	This study assessed the polyphenolic profile and the antioxidant and antibacterial activities of monofloral honeys produced by Meliponini in the Brazilian semiarid region.	Honeys from Ziziphus joazeiro Mart. (juazeiro) and Croton heliotropiifolius Kunth (white velame) showed the highest total phenolic contents (TPCs) and the greatest antioxidant activity in assays with DPPH and ABTS + radicals. Malícia's honeys showed the greatest quantities of myricetin, quercetin and kaempferol among the studied honeys.
15	[27]	This study aimed to assess the fatty acid composition of milk, the antioxidant quality of milk and blood lipoperoxidation of dairy cows whose diet was supplemented with flaxseed oil containing a propolis-based product (PBP) with or without vitamin E.	Under the studied conditions, the improvements of milk fat quality, of the oxidative properties of milk and the blood's resistance to oxidation, were reached with PBP supplementation and E vitamin.
16	[28]	The aim of this study was to evaluate antioxidant properties of lyophilized bee pollen extract (LBP), to determine the phenolic profile by liquid chromatography and to evaluate the effect of LBP on the oxidative stability of pork meat sausage.	The LBP (lyophilized bee pollen extract) extract exhibited strong antioxidative effects in pork sausage, probably due to high antioxidant activity and the presence of the phenolic compounds in bee pollen; which has potential to be used in pork sausage.
17	[29]	Evaluation of antioxidant activity as well as the determination of phenolic compounds and antimicrobial activity, among other analyses of honey samples from south Brazil.	In the analyzed samples, the bioactive compounds found in a larger amount were the phenolic compounds. With respect to antimicrobial activity, we can highlight the relevance against Gram-positive microorganisms.

Table 1. Reference, objective, main results and conclusions, studies about evaluation of the antioxidant activity of bee products in Brazil between the years 2011 and 2016.

In addition, with respect to methods of extraction and preparation of propolis extracts, the composition of propolis extracts varies with the concentration of propolis, especially with the water/ethanol content of hydrous ethanol used in the extraction. Oldoni et al. observed the influence of standardization of extraction. The results of their study confirmed that the optimization of the extraction conditions is important to obtain extracts that are rich in phenolic compounds and antioxidant activity of propolis [23].

Since propolis has great chemical diversity, it needs to be chemically standardized before use to ensure quality, efficacy and safety and this way, it is possible to correlate the type of propolis and its therapeutic application, an essential task for a growing market and more demanding throughout the world. Brazil is a pioneer in these practices, since it is considered one of the world's biggest suppliers of bee products [3, 11–13].

Brazilian propolis were classified in the most prevalent types, resulting in 12 groups or types based on their geographical origin, chemical composition and plant origin: five in the south, one in the southeast and six in the northeast. A new type of propolis from a mangrove region from the State of Alagoas had its botanical origin identified as Dalbergiaecastophyllum, a species of legume and was ranked as the 13th type of northeast Brazilian propolis. These findings confirm the great Brazilian biodiversity that has become the subject of several scientific research studies throughout the world [3, 12, 13, 30, 31].

In this context, it is important to note that the evaluation of propolis samples from different geographical and climatic regions means that there are variations in the chemical composition and, therefore, biological activities may differ. Thus, when performing pharmacological studies, we should not indiscriminately compare propolis samples from different regions, neither assign a proven activity in a given sample to other samples from different regions. For this reason, publications on evidence of biological activities should include the physical and chemical characterization of the used propolis [32].

Moreover, there is smaller variation in the chemical composition of propolis found in temperate regions of the planet, where its main bioactive compounds are flavonoids: apigenin, quercetin, hesperetin, rutin, luteolin, genistein, daidzein, anthocyanidin, kaempferol, among others. Although flavonoids are the most extensively studied components of propolis, they are not the only ones responsible for their pharmacological properties. Several other compounds have been linked to its medicinal properties [12, 13, 24].

4. Conclusion: key results

In order to assist in the standardization process of apiculture products which are greatly diverse due to the variety of vegetation that exists in Brazil, it was possible to draw a profile of the main antioxidant substances of apiculture products from Brazil. It was possible to identify several substances of antioxidant capacity in bee products from Brazil, mainly in propolis and geopropolis, propolis extracts and honey.

Standardization has not yet been observed in scientific research and legislation in Brazil. Therefore, the types and quantification of substances with antioxidant capacity become too variable, thus, interfering in a more general analysis of the country's propolis. In this review, we observed that the chemical substances in propolis are determined by the flora of the region.

Additionally, since Brazil has great flora diversity, it is complex to follow a standardization process for the substances found in propolis.

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Chapter 2

Bee Products as Functional Food

Banu Yucel, Erkan Topal and Mustafa Kosoglu

Additional information is available at the end of the chapter

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Abstract

The studies that reveal the impact of the bee products on overall health are accompanied by new researches every year, and the importance of these researches are gradually on the rise. Bee products that are used as food and food supplements and drug concentrations in the historic process are drawing the attention with their marvellous characteristic features. The search for nourishment of the body on behalf of healthy living is currently being searched by many people. Therefore, the consumption of products that protect the health appears as the primary preference of people. In the light of this recent tendency, food sector is now offering well-supported products that are suitable for this preference. At this point, bee products such as honey, pollen, bee bread, royal jelly and propolis gain importance as functional food with their nutritious features that help in protecting the health. In this article, within the consideration of the researches that evaluate bee products as functional food, we aim to introduce the prominence of bee products in our nourishment and overall health.

Keywords: Bee products, functional food, health, prebiotic, probiotic

1. Introduction

At the present time, alterations in food production and consumption occur constantly. Standardized food production gradually gives its place to mass tailor-made production. Throughout this process, consumer needs also change and progress. In every aspect of life, the search for comfort increases its importance. Functional foods come to the light as a result of the search for comfort to fulfil certain needs. Functional food products become prominent within the several product groups particularly due to their direct effect on human health [1].

Functional foods are a highly important sector of the food market that grow rapidly. Within this concept, the interest towards the pro- and prebiotic foods increases by degrees. Foods that

beyond their basic nutritional features make positive contribution to our health are named as 'functional foods'. The term functional food underlines the positive relation of nutrition with health [2–5]. The International Food Information Council (IFIC) defines functional foods as 'foods and food components that may provide benefits beyond basic nutrition'. According to the International Life Science Institute (ILSI), the concept of functional foods refers to the 'biologically active components in foods that have the potential to optimize physical and mental well being' [6–13].

The most important target groups of the functional foods are women and elders. The new conception assimilates Hippocrates, the founder of the medicine, nearly 2500 years old philosophy 'Let food be thy medicine and medicine be thy food', and therefore pays more attention to the healthy nutrition. Nowadays, due to reasons such as the increase in the treatment expenses, labour loss, length of life and the portion of the old people inside the population and people's desire of living a quality life, people start to expect more from the food items [14].

According to the data of the research that is made to reveal the consumers' awareness, acceptance and approach towards the functional foods, mineral water, whole-grain diet biscuits and whole-grain breakfast cereals are the most frequently consumed functional foods in the order. The functional food increases the level of the beneficial bacteria in the gut, helps lose weight and supports child development. These three features of the functional food are emphasized by customers as 'the top 3 factors that convince them to consume functional foods' in the research [15].

Education level has advanced hand in hand with the economic development; therefore, people's demand to healthy foods has increased alongside with these progressions. The concept of functional food occurred for the first time in Japan in the 1980s. Functional foods are put on display for customers as an active food trend at the beginning of the 1990s in America and in the middle of 1990s in the countries of Europe.

According to the Foods for Specified Health (FOSHU) affirmed in Japan in 1991, it is stipulated that in order for a product to get functional food licence it is required to meet with certain criteria. Some of these criteria are stated in relation to bee products. Functional foods should help the improvement of the quality of nourishment, and protection and preservation of health, the compound of the product should not differ from the similar type of foods' nutritional substance components within normal conditions, the product should be a well-used food in daily diets, as all bee products have [16–19].

2. Bee products as functional food

Bee products are accepted as 'functional food' by adding them in other food products to increase their nutritional value or used alone with its natural and rich nutritional content and high bioactive components. Honey, pollen, bee bread, royal jelly and propolis have high nutritional value and beneficial effects that affect the human health in a positive manner. Bee

products are rich in proteins, simple sugars, essential amino acids and monounsaturated fatty acids. These features strengthen the immunity, help the body fight actively with bacteria and stimulate the quality tissue regeneration, and consequently protect overall body health and treat it [20] (see **Figure 1**).

Figure 1. Honey bee on combs and bee products on sale *(photographed by M. Kosoglu and E. Topal).*

Phenolic compounds	Honey (μg/100 g)	Pollen (μg/100 g)	Bee bread (μg/100 g)
Gallic acid	3.05	3.68	6.17
Protocatechuic acid	7.08	N.D.	91.25
Chlorogenic acid	N.D.	N.D.	N.D.
p-OH benzoic acid	8.98	N.D.	N.D.
Caffeic acid	79.90	N.D.	N.D.
Syringic acid	38.17	4.80	N.D.
Catechin	N.D.	N.D.	N.D.
Vanillic acid	41.40	5.30	N.D.
Epicatechin	N.D.	N.D.	N.D.
Ferulic acid	N.D.	6.62	28.38
Ellagic acid	N.D.	N.D.	N.D.
Rutin	56.66	50.80	217.20
P-coumaric acid	N.D.	N.D.	N.D.
O-coumaric acid	N.D.	3.25	N.D.
Quercetin	247.40	N.D.	N.D.
T-cinnamic acid	22.26	27.08	586.40
Apigenin	N.D.	N.D.	N.D.
Kaempferol	67.10	179.53	623.50

Table 1. Phenolic compounds of some bee products.

Bee products include many biochemical components that can be found on almost all functional foods. Proteins, saccharides, fatty acids, prebiotics, probiotics, fibre, phytochemicals, bioactive peptides, mineral matters, vitamins and organic acids can be shown as examples to these components. Additionally, phenolic acids, flavonoids and carotenoids that are found in the structure of bee products affect diseases such as cancer, arteriosclerosis, cardiovascular diseases, weakening of the immune system, Parkinson's disease, Alzheimer, arthritis and photoageing dramatically as preventive and curative matters [21]. Especially flavonoids inhibit the efficacy of the enzyme systems that include lipid peroxidation, platelet aggregation, capillary permeability and lipoxygenase [22].

Bee products' functional food features do not remain limited with these contents. Phenolic components in its structure make positive impact on body's immune system in a crucial level [23]. Even in bee products which are procured by monofloral production, it is seen in **Table 1** that the phenolic component amount shows an alteration [22, 24] (**Table 1**).

The functional food feature and biological effect of bee products are fairly high [25, 26] (**Table 2**).

Product	Biological effect	Functional effects
Honey, pollen, bee bread, royal jelly, propolis	Antibacterial, fungicide, antiviral, antioxidative, immunomodulating and immunoactivating, anti-inflammatory, analgesic	Growth inhibition of pathogen bacteria, fungi, and viruses, anticancer, stimulate immune difference against inflammation
Pollen, royal jelly, propolis	Radioprotective, anti-arteriosclerotic, enhances Ca absorption	Protect against radiation, artherosclerosis and osteoporosis
Honey	Prebiotic (oligosaccharides), probiotic (contains probiotic bacteria)	Stimulates healthy digestion by promoting the growth of good intestine bacteria (*Bifidus*, etc.)
Royal jelly	Antihypertensive, vasodilative, increases reproduction and oxygen uptake of cells and has an effect on central and peripheral nervous system	Cardioprotective, stimulating and energizing against stress and fatigue, protection of the central nerve system

Table 2. Biological and functional effects of bee products [25, 26].

2.1. Honey

Honey is a bee prodomponents in its structure. Honey mainly consists of water and sugar that is formed from fructose and glucose. It includes ascorbic acid, pantothenic acid, niacin vitamins and additionally mineral substances such as manganese, phosphor, potassium and zinc [27] (see **Figure 2**).

Honey is a functional food that contains inulin and fructo-oligosaccharide (FOS) and it has prebiotic features that have beneficial effects to our gastrointestinal system. As widely known, prebiotics are undigested carbohydrates which increase the activity and number of both

column bacteria and probiotics (living organisms that regulate the intestinal microbial balance). According to the identified data, prebiotics stimulate the immune system and help the inhibition of carcinogenesis inside the column [28, 29]. As a result of prebiotics' fermentation in large intestine, substances such as lactate, short-chain fatty acids (acetic, butyric and propionic acids), hydrogen gas, carbon dioxide and methane are produced. The intestine's pH drops significantly. The drop in the intestinal has many benefits such as inhibition of microorganisms that can potentially have detrimental effects, degradation of secondary bile acids, increment of the solubility and absorption of minerals such as Ca, Mg, Fe and Zn [30]. Undigested carbohydrates that are known as prebiotics include lactulose, lactitol, oligosaccharides and inulin. Undigested oligosaccharides are found naturally in fruits, vegetables, milk and honey [31].

Figure 2. Honey (*photographed by E. Topal*).

Inulin is a term that is used for heterogeneous mixture of fructose polymers that are found commonly in the form of stored carbohydrates in nature and has a polymerization degree that alters between 2 and 60°. The units that have lower polymerization degree (2–20) are called fructo-oligosaccharide or oligofructose [32]. The consumption of 4–10 g of fructo-oligosaccharides in daily basis creates bifidogenic effect, thus increasing the number of beneficial bacteria [33].

Honey is not only a functional food but also has many positive impacts on human health. Due to these impacts, it is used countless times in folkloric medicine and its usage as a drug put on the records centuries ago for us to read the crucial role of the honey in our life [34]. In addition to that, honey comes forward as an important antioxidant source due to its vitamin C, flavonoids and phenolic content [35, 36]. Honey's strong biological activity originates from its rich phenolic content. Flavonoids stimulate antibacterial, antiviral, anti-inflammatory and

vasodilator effect [21, 22]. Flavonoids and phenolic acids form the most common group of plant phenolics [37]. Phenolic acids and flavonoids act as free radical scavengers due to their reducing agents besides their antioxidant and anticarcinogenic effects [38–40]. Flavonoids increase the mucosal content of prostaglandins and have an important inhibitory effect on the gastric mucosa, thus preventing ulceration. Mucosal content of prostaglandins has an important inhibitory effect on acid secretions, preventing the formation of peptic ulcers [22].

Antibacterial feature of honey that comes from its osmotic effect is an outcome of the sugar molecules in the setting which drain the water around and leave so little to the microorganisms that cause the bacteria in the honey to die from dehydration. Alongside with this osmotic effect, hydrogen peroxide that is constituted from glucose oxidase in honey creates the same level of antibacterial activity. By the time that hydrogen peroxide decomposes, it produces highly effective free radicals and consequently these situation cause bacteria to die. Due to this feature, it can be understood that honey defends the body successfully against the destructive effect caused by oxidative stress [34, 41].

In a research aimed at studying the long-time storage of the honey as a functional food, honey and pollen are fermented by probiotic lactobacillus and conserved at 40°C for 7 days to examine the prebiotic cells' liveliness. In consequence of the examination, it is found out that prebiotic stains preserve their liveliness at a significant level [42].

Honey's role as a protector of the overall health and creator of an antioxidant effect makes it useful in the food sector for long ages. The sugar known as 'Maillard Reaction' forms a composition of aldehyde-ketone and amine interaction that has an antioxidant feature. In other words, honey diminishes the microbial activity that starts after cutting the meat which gets harmful for the health [43]. Furthermore, the usage of the honey in chicken meat makes its water-holding capacity to increase, prevents boiling of loss, balances pH, gives a light colour to the meat by Maillard reaction and provides richness in flavour [44–46]. Correlative to its fructose content, honey enhances the flavour of the meat and increases its nutritional value due to its 'functional food' feature [47].

The antioxidant effect of honey can be evaluated with the lipid peroxidation system. Honey stops the bacterial growth in dry-aged and wet-aged meats and even acts like a bactericide when it gets together with propolis. The strong antioxidative and antibacterial activities of honey clean the active oxygen that cause meat to go bad [48]. Additionally, it is found out that in chicken meats that have been marinated with honey, the formation of heterocyclic amine that occurs as a result of Maillard reaction after cooking drops at a rate of 92–99%. As a reason behind this drop, it is submitted with the acidic structure of honey that prevents the occurrence of amine [49]. Phenolic and flavonoid compounds inside the honey's composition increase the capacity of the antioxidants and prevent the activation of hydrogen peroxide [48]. According to a conducted research, chicken meats that have been marinated with different portions of honey significantly prevent bacteria growth by comparison with the group that has not been treated with the honey whatsoever [46].

2.2. Pollen

Pollen is a bee product, which serves as an important food supplement thanks to its content that not only includes rich food substances as a male reproductive unit of the flower but also consists of salivary juices of honeybee. It is accepted as a 'natural drug concentration' due to its content that consists of enzymes, coenzymes, steroids, vitamins, antibiotics, microelements, carotenoids and flavonoids. Pollen's strong antioxidant feature originates from high level of polyphenols in its content. Due to this feature, it is considered as a functional food/food supplement [50]. Generally obtained from bees from various plants, pollen is accepted as 'the most distinguished food in the World' [51]. The composition and chemistry of pollen is not standardized. The type and characteristic of pollen alters in accordance with the kind of the plant that it is obtained from, the season, climate, environmental aspects and the age of the plant. The alterations, which depend on the structure of the plant that the pollen is acquired from, change the compounds of pollen and the level of impact that it has on health. For instance, meanwhile a willow's pollen is rich in vitamin C, a clover's and willow's pollens are rich in flavone matter. This content protects the body from sclerosis, spasmolysis and radioactive effect. Due to willow's and cherry's pollens' chlorogenic acid content, they protect vascular health, act as an anti-inflammatory and regulate thyroid and pituitary glands' secreted hormones. In addition to the effects of the pollens acquired from one plant type only, pollens collected from thousands of various types of flowers in the nature have exceptionally beneficial effects. For that reason, it is stated that the consumption of mixed pollens has positive impact on health [41] (see **Figure 3**).

Figure 3. Pollen (*photographed by M. Kosoglu*).

Pollen's important antioxidant level is mostly connected with the phenol compounds [52]. In the conducted research made in Brazil, in 25 examples which are collected on certain days for 9 months, isoquercetin, myricetin, quercetin, luteolin, selagin, kaempferol and isorhamnetin substances are identified. Even though the flora that the bee worked on has changed, it is noted that there were not excessive differences in phenolic composition level [53].

Pollen is accepted as a dense nutraceutical substance based on its phenolic compounds and high antioxidant activity. It regulates the blood flow in lecithin vessel. Pollens stimulate the production of polyphenols and biocides in liver and clean the liver by decreasing the oil level. At the same time, pollen is a food supplement that is extremely rich in selenium which plays a crucial role in protecting the cardiovascular health. The consumption of the pollen, which is considered as both 'functional and super food', is recommended for 15–20 g a day, two times a year in weekly cures for 3 weeks [54].

According to the results of the research made to examine the effects of physico-chemical and microbiological features of yogurts produced by honey, pollen and probiotic yeast, it is identified that there are significant increases in the amounts of oil and protein in yogurt due to the pollen addition [5]. Another study revealed that the probiotic yogurt produced by adding pollen has a positive impact on both physico-chemical and sensorial features [55]. Unfortunately, the positive impact of the addition of pollen in the yogurt on senses of taste and smell is revealed to be not the same in the case of adding the pollen in milk. It is reported that even though the addition of pollen in milk does increase the probiotic feature, sensorial features are effected negatively [56].

A conducted research presented that in order to extend the shelf life of meats, pollen can be added to the mincemeat as an antioxidant and antimicrobial agent. It is revealed that the lipid oxidation and microbial growth are prevented by pollen supplementation [57].

Adding pollens inside the whole-grain biscuits is a common way of consuming pollens. It is stated that adding an amount of 5% of pollen as a food supplement inside the biscuits does not affect the oil amount but does affect the amounts of sugar and protein at a significant level [58].

2.3. Bee bread

Bees mix pollen with honey and their digestive enzymes and then the whole composition will be fermented by the lactic acid. The final state of this mixture is called 'bee bread' which is an extremely valuable product. In apiculture, this valuable product can be put onto the market as a nutraceutical human food/food supplement. Bee bread includes a significant amount of proteins, vitamins and one of the natural antioxidants, the phenolic compounds. The flora that is found at the region where colonies are present has a significant impact on the compounds of the bee bread. Honey bees ferment hidden pollens inside the comb with various enzymes and the addition of honey then stores it as bee bread. This implementation provides a more advantageous storage against the risk of nutrition loss of the dried and frozen pollen [20] (see **Figure 4**).

The positive effect it has on health and its content that is full of rich polyphenols make bee bread medically the focus of interest [59]. In a research, which has examined the bioactive features of cherry pollens and bee bread, it is found out that these bee products have high antioxidant and anti-inflammatory capacities with their content, including 1371 mg/100 and 1428 mg/100 g high phenolic compounds [24]. A converted research made in Colombia to analyse the bee bread, flavonoid and phenolic matters' content are found 3.2 ± 1.0 mg

(quercetin/g) and 8.9 ± 3.1 mg (galia acid/g.), respectively. Additionally, ferric-reducing antioxidant power (FRAP) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) antioxidant activities are reported at a level of 46.1 ± 13.0 versus 61.5 ± 10.2 mmol (trolox/g), respectively, and according to the data bee bread can be digested with more ease and biologically used at a higher level than pollen. This case shows the fact that bee bread has a higher nutritional level and more amount of usable bioactive compounds than pollen. According to the obtained results, as a functional food supplement bee bread is a product that has a high level of utilization potential [60].

Figure 4. Bee bread (*photographed by M. Kosoglu*).

2.4. Royal jelly

It has different colours changing from cream to dark yellow, an acidic structure and an astringent taste. Royal jelly includes all the food substances necessary for the larva's growth. It is quiet rich in proteins, oils, sugars, hormones, vitamins and mineral matters. Chemically, royal jelly comprises water (50–60%), proteins (18%), carbohydrates (15%), lipids (3–6%), mineral salts (1.5%) and vitamins together with a large number of bioactive substances such as 10-hydroxyl-2-decenoic acid with immunomodulating properties, antibacterial protein, fatty acids and peptides. The royal jelly also demonstrated significantly improved recovery from 5-fluorouracil-induced damage [61, 22]. The quality of the royal jelly is evaluated by 10-hydroxy-2-decenoic acid (10-HDA) level in its content and the level of the matter is expected to be found among 1.4 and 1.8% levels. This value can differ due to the vegetation of the region where royal jelly is obtained from and the implementation techniques in harvesting royal jelly. Unlike honey, the mineral matter composition in royal jelly is not affected by the topography nor the flora [62] (see **Figure 5**).

Figure 5. Royal jelly in cells and harvesting royal jelly (*photographed by M. Kosoglu*).

Royal jelly is a very complex bee product in terms of the content of compounds. Components in its compound target various biological functions both known and unknown, and therefore they play a crucial role on the biomedical effect of the royal jelly [63].

Royal jelly stimulates cell renewal, production and metabolism and due to these effects it creates liveliness, health, energy and high immunity and vigour in all the tissues of the organism. Its content is rich in natural hormones, vitamins, essential fatty acids, amino acids, sterols, phosphor compounds and acetylcholines. Acetylcholine is effected in the transmission of the neurons' messages and regulation of the work of endocrine glands. Being rich in building blocks of life, royal jelly's rich content of the gelatinous amino acid, which is the fundamental component of collagen and therefore the royal jelly, has an anti-ageing effect. The gammaglobulin in the content of the royal jelly effects the body's capacity to fight with the infections and acts as a strengthening factor to the immune system. With its decanoic acid content, royal jelly has a strong antibiotic effect to many bacteria and fungus. Due to its fight with tumours and metastasis, it is reported that royal jelly is used in oncology, psychiatry and neurology, geriatrics, bone and cartilage tissue regeneration, vessel stiffness, growth and development. It is actively used to stop physical exhaustion. Royal jelly not only lowers the cholesterol, total lipid, phospholipid, triglyceride and b-lipoprotein levels in blood but also lowers the overall cholesterol and acts as a vasodilator. Because of the fact that it consists of inulin-like peptides, royal jelly shows hypoglycaemic and immunological effects. In the case of feeding premature and undernourished babies with 8–100-mg royal jelly, it is reported that there were significant improvement on their overall health condition, weight gain, increase on the levels of red blood cells and haemoglobin [41–65]. In addition to all these features, the significant impact of the consumption of royal jelly has on sperms and its mobility and the positive results obtained from the infertility treatment when consumed regularly are reported [66].

2.5. Propolis

Propolis is a resinous substance that bees collect from the exudates of plants and which they use to seal holes in the bee hive [67]. Propolis, too, forms part of traditional medicine, and chemical analysis has pointed to the presence of at least 300 compounds in its composition. It

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is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%) and other organic compounds (5%). Among these organic compounds, we may find phenolic compounds and esters, flavonoids in all their forms (flavonoles, flavones, flavonones, dihydroflavonoles and chalcones), terpenes, beta-steroids, aromatic aldehydes and alcohols, sesquiterpenes and stilbene terpenes. Caffeic acid phenethyl ester (CAPE) is a biologically active ingredient of propolis with several interesting biological properties, including apoptosis, metastasis and radiation sensitivity of cancer cells [68–71] (see **Figure 6**).

Figure 6. Raw propolis and honey bee (*photographed by M. Kosoglu*).

The impact that propolis has on cancer bases on especially its regulative and strengthening role on the immune system. Based on this strong effect of propolis, whether propolis shows cytotoxic and apoptotic features in acute lymphoblastic leukaemia cell lines or not is searched, and found out that due to its caffeic acid and phenethyl ester content, propolis shows a significant level of cytotoxic effect and stops tumour growth [72]. According to the results of the research which searches especially for the inhibitory effects of propolis on osteogenic sarcoma cell growth, due to the anticarcinogenic effect of the apoptosis mechanism induced with the propolis extract, it is reported that propolis can be beneficial in cancer treatment [73]. Along with many polyphenols and antimetastatic effects propolis has, it is been observed that the compounds CAPE obtained from populus' propolis and Artepillin C obtained from *Baccharis propolis* show antitumoural effects [74].

The usage of propolis is recommended by patients who take radiotherapy during their cancer treatment. Propolis acts almost like a protective barrier and prevents the radioactive ray from affecting the healthy cells and makes it effective only to the distorted tissues. By this way, the level of radiation entering inside the patient's body decreases. Additionally, due to the

strengthening feature of propolis on the body's immune system, it supports patient's body functions and therefore increases body's resistance. Propolis should be implemented as a complementary supportive care that is accompanied by conventional surgery, chemotherapy and radiotherapy. It is crucial to use it with an ethical and responsible approach and in right doses and for enough time [75].

Propolis and its derivates have the capacity to inhibit virus propagation. Several *in vitro* studies have shown the effect of propolis on the DNA and RNA of different viruses, among them Herpes simplex type 1, Herpes simplex type 2, adenovirus type 2, vesicular stomatitis virus and poliovirus type 2. The effects observed involve a reduction in viral multiplication and even a virucidal action [22].

The antiseptic, antibiotic, antibacterial, antifungal and antiviral features of propolis come from its galangin, caffeic and ferulic acid content. In converted researches, propolis is used in branches and diseases such as dentistry, oto-rhinolaryngology, ophthalmology, gynaecology, dermatology, digestive system diseases and pulmonary diseases besides its cancer and radiation treatment [76].

The antioxidant, antimicrobial and antifungus effects of propolis are benefited by food technology. In a converted study, 0.02 and 0.4% of ethanolic propolis extract (EEP) and 0.28% of potassium sorbate are added in meat products that are mixed with stored fats and it is stated that meat products treated with propolis have a longer shelf life than the ones mixed with potassium sorbate [77]. Similar studies determine the fact that propolis has a significant level of antibacterial effect and propolis extracted in 0.3% ethyl alcohol extract and in water can be used successfully in meat products as a natural coating compound [78, 79]. In these studies, propolis forms a surface such as a film badge during the marination and its prevention of the microbial activity is remarked. Additionally, the utilization of propolis decreases the level of the total volatile nitrogen content (TVB-N) and thiobarbituric acid (TBA) that affects the processed meats badly. Due to these features of propolis, it is recommended for use as a decent preservative in processed and unprocessed meats [77].

In a research that has specified the physico-chemical and microbiologic alterations in meat's structure, as a result of treating the beef hamburger meats with propolis ethanol extract (PEE) and freezing them for 8 months at −18°C, it is observed that propolis extends the shelf life without affecting the quality of meat negatively [80].

The impact of different propolis concentrations (250, 500 and 1000 ppm, respectively) on the microbial load on the surface of the Roumy cheese is searched and it is found that propolis inside the 1000 pm concentration completely prevents the production of mould and stigmatosis in the cheese; therefore, the utility of propolis in foods as an economic and natural preservative is indicated as a result of the research [81].

In a research examining the bactericidal and bacteriostatic activity against the *Escherichia coli bacteria in vitro*, 20% EEP is recommended to be evaluated as a natural food preservative due to its neutralization of *E. coli bacteria* [82]. In a similar way, the antibacterial effect of the propolis on some of the fermentative bacteria in yogurt is examined and the inhibitory impact of the propolis even at low concentration on the normal bacteria growth is stated [83].

In a research that has identified the antifungal effect of the propolis extract on the six types of yeasts which cause unpasteurized fruit juices to go bad, it is revealed that propolis can be used as an alternative to the chemical preservatives in fruit juices due to its antibacterial effect [84].

3. Conclusion

The improving technology and changing living conditions alter human life and nutritional habits, and as a conclusion the thought of preventing diseases by being nourished with functional and super foods enriched with natural preservatives and substances that protect the health become extremely popular. More studies are needed on the contribution of the magnificent bee products have on our health and nourishment in order to create more awareness and influence the production of qualified bee products and increase the conscious selection and consumption of the bee products made by consumers. Making multidisciplinary scientific researches about the protective effects of the bee products as functional foods that meet with the common trend of 'not getting sick' will significantly contribute to spreading the results of the researches all around the world and improving the sector.

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Section 2

Functional Food from South America

Chapter 3

The Revival of Quinoa: A Crop for Health

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Additional information is available at the end of the chapter

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a basic food in pre-hispanic Andean communities, used not only as a food but also for medicinal purposes. The interest in quinoa has increased because of its plasticity to adapt to environmental conditions: it tolerates frost, salinity and drought; it grows on marginal and arid soils and high altitudes. The nutritional quality of quinoa is well recognized: protein content ranges 13–17 g/100 g, with an amino acid score above 1.0 and it is gluten free. The grain contains starch and free sugars, with a glycemic index ranging 35–53, depending on the cooking time. It also contains bioactive phytochemicals such as dietary fiber, carotenoids, phytosterols, squalene, fagopyritols, ecdysteroids and polyphenols. The composition of quinoa varies among ecotypes and is affected by environmental factors: some amino acids and phytochemicals augment under stress episodes. The rationale for the revival of quinoa and its reintroduction into the diet is related with the epidemiological situation, which includes diseases that exhibit risk factors that may be reduced with a balanced nutritious diet, in which quinoa plays a major role, being considered as a "superfood." Moreover, it is one of the crops selected by Food and Agriculture Organization (FAO) to offer food security.

Keywords: quinoa, *Chenopodium quinoa* Willd., ancient crop, nutritional quality, chemical composition, bioactives, health, crop plasticity

1. Introduction

Since 1998, the WHO has considered obesity as an epidemic affecting the globe, a condition related to more deaths than undernutrition in the whole planet. Obesity is associated with various noncommunicable diseases (NCD) such as cardiovascular diseases, cancer and diabetes, among others. Globally, two out of three deaths each year are attributable to NCD. In this context, it is very important to take into account some alimentary traditions and the social value of food practices that have been lost with time. Most of the traditional culinary practices, beliefs, attitudes and meanings of certain foods have been neglected and traditional crops have been left aside, missing the food cultural practices of different regions.

An outstanding food crop that has been almost lost is quinoa (*Chenopodium quinoa* Willdenow), a South American dicotyledonous primary crop (an indehiscent achene: a seed-like fruit with a hard coat) that has become an extremely popular food product in the last decades. The seeds (approximately 2.5 mm in length and 1.0 mm in diameter) are flat white, yellow, red, brown and black, whereas the seed coats have a brown color and possess excellent nutritional properties (**Figures 1** and **2**).

1.1. Quinoa plant: origin and botanical properties

Chenopodium quinoa Willd. is an annual gynomonoecious plant with an erect stem, alternate leaves and flowers clustered together to form the inflorescence in a panicle that measures from 15 to 70 cm long [1]. The basic chromosome number of quinoa is $x = 9$ and their somatic chromosome number is $2n = 4x = 36$, suggesting that it is an allotetraploid plant [2]. Measurements of chromosome arm length ratios in quinoa indicate an allopolyploid, which is consistent with it high degree of self-fertility and low levels of inbreeding depression seen in this species [3].

Figure 1. Chilean quinoa plants.

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Figure 2. Collected seeds of quinoa.

Quinoa was one of the basic foods in pre-Hispanic communities of the Andean Region, grown for over 7000 years mainly in the current locations of Peru, Bolivia, Ecuador, Chile, Argentina and Colombia, from 2° North latitude (Colombia) to 47° South latitude (Chile) [4–6]. The name refers to "the mother grain" by the Andean people and it was used not only as a food but also for medicinal purposes. The colonists suppressed its cultivation and the remaining crops that survived were cultivated practically hidden in small areas [7]. The locals have preserved quinoa in its natural state, including its many varieties, as food for present and future generations.

Quinoa represents a cultural heritage in many Latin-American countries. It has survived from extinction in different agroecological zones, ranging from the extremely dry Altiplano highlands at 4000 m above sea level with average rainfall of 150 mm per year to coastal zones of central and southern Chile, where soils are clayish and rainfall is above 1000 mm/year [8]. It spread throughout the central and north-central Andean valleys and southwards into the Araucanian coastal region and adjacent Patagonia, diversifying into its five principal ecotypes. The crop is produced mainly in Bolivia, Peru and Ecuador, with efforts to cultivate it worldwide and the diversity has been described by five major ecotypes linked to the geographical region: Altiplano (Peru and Bolivia), Inter-Andean valleys (Bolivia, Colombia, Ecuador and Peru), Salt lands (Bolivia, Chile and Argentina), Yunga (Peru, Bolivia and Argentina) and Coastal (Chile) [9, 10].

Miranda et al. [11] observed genetic differentiation among the geographic distribution of quinoa genotypes, which were expressed in morphological, yield responses, chemical composition and functional properties in a common garden assay of six selected genotypes. Using this model, the high capacity of adaptation of the seeds to different environments has been demonstrated [12]. Moreover, these properties of quinoa seeds allow this crop to be used under environmental extreme conditions in countries facing challenges such as drought and salinity under very diverse agroclimatic conditions globally [1].

There are currently more than 6000 varieties of quinoa cultivated by farmers [13]. Due to the wide range of genotypes (including 250 varieties), the possibilities of adaptation to many abiotic stresses abroad have increased significantly the interest of quinoa cultivation [14]. The plant exhibits an enormous adaptability to different environments, including the harsh conditions that characterize much of the Andean zone. Therefore, the production has spread through many different countries, including Japan, Australia, Spain, Germany, England, Sweden, Denmark, the Netherlands, Italy, France, Finland, Kenya, Ethiopia, India, the USA, Canada, among others. Many reports indicate that quinoa is an interesting alternative crop for the use of deteriorated and poor soils [5] and it has been successfully tested in various countries in Asia, the Near East and North Africa [6]. In fact, the enormous plasticity of quinoa includes tolerance to frost, salinity and drought, it has the ability to grow on marginal and arid soils and is also adapted to high altitudes [15–18]. The strong tolerance to drought and salinity allows it to resist the current and future challenges of the global climate change, including water shortage [15]. The plant adapts well to climates ranging from desert dry weather to relative humidity from 40 to 88%, with temperatures from −4°C to 38°C.

Several genotypes of quinoa are able to maintain a high photosynthetic efficiency under water-deficit conditions [19, 20] and to quickly reestablish photosynthesis after a period of rehydration [21–24]. Quinoa shows an extraordinary physiology of adaptation to stress, particularly its highly efficient use of water [8], that is, the quantity of grain obtained per liter of water used is another useful criterion for comparing quinoa with cereals. Martinez [25] reported 500 L water per kilogram quinoa, a significantly lower water-use footprint compared with rice (2497 L/kg) or maize (1222 L/kg), figures that are even greater if one considers also quantity of protein per kilogram. Crop production is acceptable with rain amounts of 100–200 mm [26]. The drought tolerance of quinoa has been attributed to a reduction in leaf area [23, 24, 27], the presence of calcium oxalate vesicles in leaves, which could reduce the transpiration rate [22, 28] and their branched and dense root system, which is able to penetrate into 1.5 m sandy soil [22, 27].

Regarding the metabolism of quinoa during periods of drought stress, it has been suggested that the induction of antioxidant molecules related with nitrogen metabolism is very important [29]. In fact, drought increases the amount of glutamine in quinoa leaves, which is the main form in which nitrogen is translocated to the grains [30]. Therefore, drought stress episodes increase the content of various amino acids, including Phe, Val, Trp and Met. These changes in quality could compensate the decline of the seed yield under stressful conditions. It has been suggested that the ornithine cycle and induction of amino acids could play a key role in the response to water scarcity and subsequent restoration under conditions of rehydration [29, 30]. Moreover, the aromatic amino acids Phe, Tyr and Trp are the main precursors of bioactive

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secondary metabolites, including the biosynthesis of flavonoids and alkaloids [31], most of which exhibit healthy properties [32]. The physiological relationship between the induction of amino acid synthesis and the production of healthy secondary metabolites is under investigation.

2. Quinoa: a traditional crop and a "superfood"

2.1. Nutrients in quinoa

The proximate analysis of quinoa seeds is shown in **Table 1**.

Quinoa proteins are recognized for their high amount [18, 33–40] and good quality, which was reported for the first time by White et al. in the 1950s [41], who described that the quality of quinoa protein was equal to that of whole dried milk protein when fed to rats. Later, it was reported that pigs fed cooked quinoa grew as well as those fed dried skimmed milk [42]. Proteins exhibit a high content of Lys (4.8 g/100 g) and Thr (3.7 g/100 g), which are in general the limiting amino acids in conventional cereals [43], along with a good albumin/globulin balance and an amino acid score above 1.0 [38, 44–46]. The excellent quality of protein is maintained even taking into account that the amino acid profile is affected by environmental factors [47].

	References							
Component	**[18]**	**[34]**	**[36]**	**[37][a]**	**[37][b]**	**[38]**	**[39]**	**[40]**
Protein	16.8	12.9	13.1	14.7	12.8	14.1	16.5	12.6
Carbohydrates	51.4	63.7	59.9	59.1	68.4	57.2	69.0	67.3
Lipids	5.9	6.5	5.7	6.4	6.2	6.1	6.4	5.7
Fiber	12.1	13.9	11.7	1.9*	1.5*	7.0	1.9*	3.0*

*Expressed as Crude Fiber.
[37] [a] var. Regalona.
[37] [b] var. Ancovinto.

Table 1. Proximate analysis of quinoa seeds (mean values, g/100 g DW).

Several methods to obtain protein isolates have been described [35, 48], consisting mainly of 11S globulins and 2S albumins, the main contributor of sulfur amino acids Cys and Met, which are limiting in legumes and they also contain interesting amounts of Arg [49]. Also, various high protein-rich fractions of interest can be obtained for the food industry [50]. An additional nutritional advantage of quinoa is that it may be consumed by celiac patients, since it is considered a gluten-free grain because it contains low concentrations of prolamins [51] and has a distant phylogenetic link with gluten containing cereals such as gramineas (wheat, barley and rye). In spite of this, the ability of quinoa cultivars to stimulate gliadin-specific T cell lines and other immune responses is still under investigation [52].

Quinoa seeds have moderate lipid content (5–9 g/100 g), with an interesting fatty acids profile. Compared with rice oil, quinoa oil contains over 20 times more unsaturated fatty acids. The main saturated fatty acid is palmitic (16:0, around 10%), whereas the main unsaturated fatty

acids are oleic (18:1n-9; 20-30%), linoleic (18:2n-6; 49–57%) and α-linolenic (18:3n-3; 8.5–12%), corresponding to 87–88% of the total [34, 37]. The oil also contains various tocopherols [39, 46] and other minor lipid constituents.

Among carbohydrates (51–70 g/100 g), the grain contains starch and free sugars (glucose, fructose, sucrose and maltose). Another healthy property of quinoa grains is their glycemic index (GI), which represents a ranking of carbohydrates on a scale from 0 to 100 according to their impact on blood sugar levels during the 2 h following consumption. For quinoa, the GI ranges 35–53, depending on the cooking time, which are considered low values on the glucose reference scale [53], whereas rice GI values range from 75 to 89 [14]. This property is related with the dietary fiber content of quinoa (7–14 g/100 g), since the fiber contained in the grain affects the digestibility of nutrients, including carbohydrates and the absorption of glucose occurs at a lower rate through a longer area in the gut, lowering the postprandial peak of blood insulin. Most fiber is insoluble, containing galacturonic acid, arabinose, galactose, xylose and glucose, whereas the soluble fiber is composed mainly of glucose, galacturonic acid and arabinose and arabinose-rich pectic polysaccharides [54]. Additionally, the intake of quinoa has been associated with satiety and appetite control in animal models [55] and humans [56], although further studies are required on this subject.

The grain is also a good source of minerals, exhibiting high amounts of potassium, calcium, magnesium, copper, iron, manganese and zinc [57–59], which are higher than those of conventional cereals [60] and the calcium-phosphorus ratio (1:0.7–3.9) is better than that of cereals (1:7.8–54.0) [34]. Among vitamins, the B complex is outstanding [61], with a high level of folate [38].

2.2. Bioactives in quinoa

Quinoa is often considered a natural functional food, a property that represents a benefit for health that is generally associated with the presence of bioactive phytochemicals. The crop is recognized as a good source of multiple bioactives, including dietary fiber, carotenoids, phytosterols, squalene, fagopyritols, phytoecdysteroids and phenolic compounds [40, 61–66].

Among phenolics, the seeds contain flavonoids such as quercetin and kaempferol glycosides, ferulic acid, phytic acid (the main storage form of P in the plant) and tannins [67–69]. Most of the phenolics in quinoa exhibit antioxidant activity [70–72] and the total antioxidant capacity is further increased by non-phenolic compounds [73]. The interest on phenolics is not only due to their antioxidant properties but also since they present antiallergic, anti-inflammatory, anticarcinogenic, cardiovascular protective properties, among other beneficial effects for health [74, 75]. In a comparative study, Gorinstein et al. [76] showed that pseudocereals have higher antioxidant activity than some cereals (e.g. rice and buckwheat), whereas Laus et al. [77] reported that antioxidants from quinoa seeds may be more readily accessible than those in wheat species. Hirose et al. [78] observed that the amounts of phenolics such as quercetin and kaempferol in quinoa grown in Japan are higher than those of conventionally used edible plants. On the other hand, when cooking or dehydrating quinoa an increase in temperature leads to a reduction in the total phenolics content [79].

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Quinoa leaves also contain a high level of phenolics [80], which exhibit anticarcinogenic effects in vitro, linked with inhibitory effects on the proliferation, motility and cellular competence of cancer cells [81]. However, the effects depend on the technological processes and the food matrix in which quinoa grains or leaves are included. For instance, Swieka et al. [82] formulated supplemented bread with phenol-rich quinoa leaves and observed an improvement of the antioxidant activity of the product obtained, although not as high as expected, probably due to the blocking of reactive groups of phenolic compounds by bread components. Some phenolic compounds in quinoa also inhibit α-amylase and α-glucosidase activities, enzymes involved in the breakdown of starch and derivatives, which allows for better control of intestinal glucose absorption and therefore of postprandial glycemia [68, 83]. Moreover, quinoa seeds are also a source of a different kind of phenolics: isoflavones, among which the main are genistein and daidzein [66]. These molecules are usually named as "phytoestrogens," due to their structural similarity with β-estradiol (an estrogen) and exhibit a wide range of beneficial effects [84].

Among the secondary metabolites, betalains are quantitatively important in several genotypes of quinoa. In fact, quinoa belongs to one of the 13 families of betalain producers [32]. These chromoalkaloids are water-soluble pigments containing nitrogen and include the red-violet betacyanins and the yellow betaxanthins. Studies performed with several genotypes of quinoa indicate that contrastingly with the *Amaranthus* genus, where the principal betalains are amaranthine and isoamaranthine, in quinoa, the main compounds are betanin and isobetanin [85]. Recently, it has been proposed that betanin is a good scavenger of reactive oxygen species and prevents low-density lipoprotein (LDL) oxidation and DNA damage [86].

Another type of secondary metabolites in quinoa is phytoecdysteroids (polyhydroxylated steroids), structurally related to insect molting hormones, that have been implicated in plant defense since they protect them against nonadapted insects and nematodes [87]. The seeds contain ecdysteroids in amounts ranging from 450 to 1300 μg/g [88]. The main form is 20-hydroxyecdysone (30 μg/g) and several minors have been reported in a range of 3–9 μg/g, including makisterone A, 24-epi-makisterone A, 24,28-dehydro-makisterone A and 20,26-dihydroxyecdysone [89]. Dini et al. [43] showed that quinoa flour contains both 20-hydroxyecdysone and kancollosterone and Nsimba et al. [73] described the presence of a new set of ecdysteroids. The ecdysteroid content of quinoa seeds from different sources shows significant variations. These molecules are rather stable during food processing, representing an intake of 20-hydroxyecdysone that may have positive effects on human health (**Figure 3**) [65].

A characteristic feature of quinoa grains is the presence of saponins (triterpenoid glycosides) in the outer layer. These secondary metabolites are utilized by the plant as a predator repellent and exhibit a series of pharmacological properties [90, 91] and impart a bitter taste. Consequently, saponins are reduced for debittering by various methods that remove the hulls (abrasive processes, washing). The amount in the grains depends on the cultivar and can be classified into "sweet" (<0.11%) or "bitter" (>0.11%) [92].

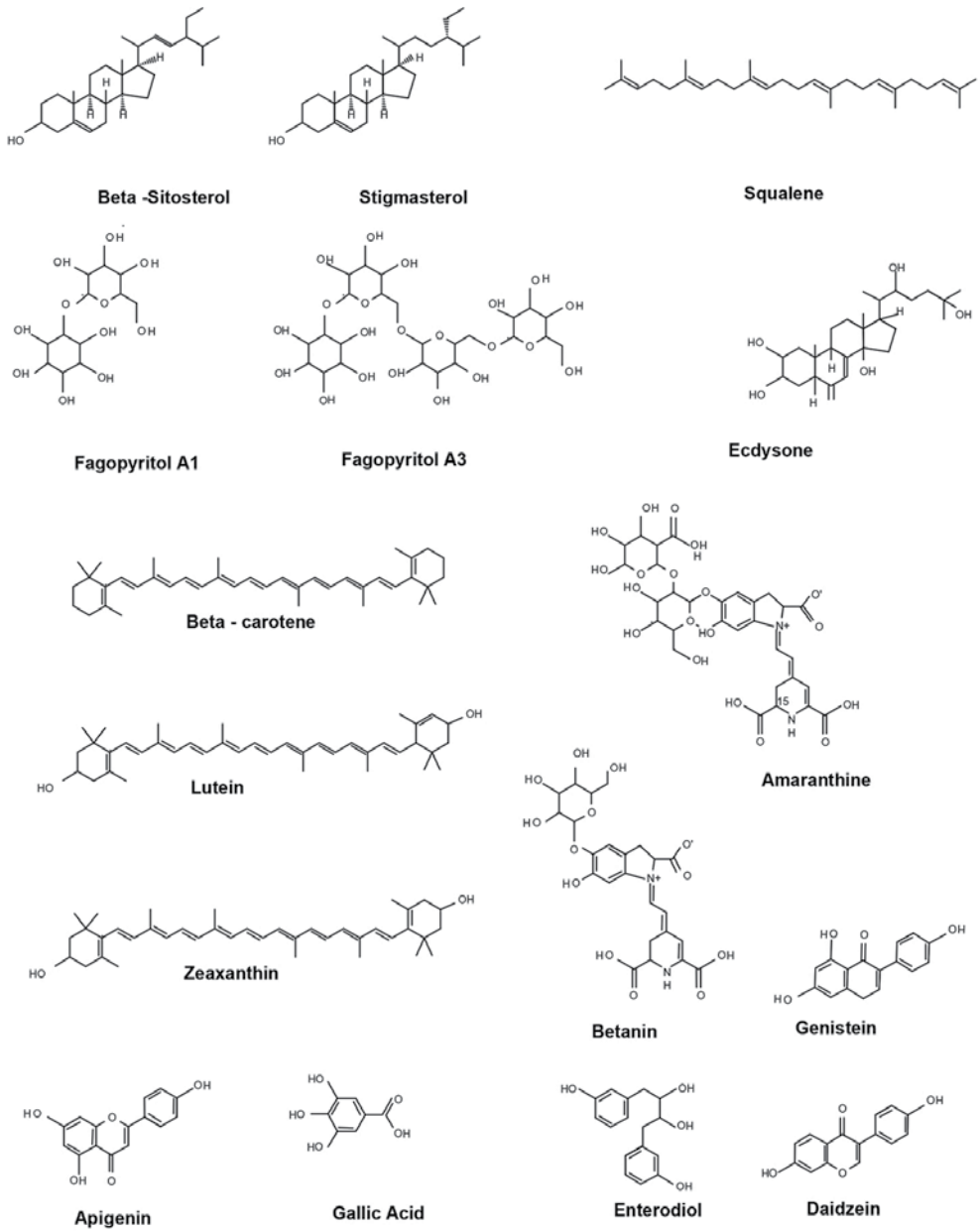

Figure 3. Bioactive molecules in quinoa.

Although all the grains exhibit excellent nutritional properties, it is necessary to take into consideration that the chemical composition of quinoa varies among ecotypes, that is, according to groups of cultivars and/or landraces defined according to distributional, ecological, agronomic and morphological criteria due to strong genetic variability in addition to environmental differences in the Andean region [93]. Moreover, the nutritional composition

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varies in relation with the environmental stress factors and several research groups have described changes in nutritional aspects of seeds as a result of environmental stress episodes. For instance, Panuccio et al. [94] reported that under high salt conditions, phenolic content and antioxidant capacity of quinoa seeds increased. Miranda et al. [11] compared two Chilean genotypes grown under arid and cold-humid environments, showing that in cold rainy zones the size and weight of the seeds increased, whereas under hot arid conditions, phenolic compounds and components of proximate analysis (except proteins) increased.

The quality and amount of protein in the seed has also led to the search of bioactive peptides, among which antihypertensive angiotensin I converting enzyme (ACE) inhibitory peptides has been demonstrated [95–97]. On the other hand, protein ingredients not only provide nutrition but also good technological properties to facilitate food processing. The technological functional properties of quinoa proteins are well recognized, since they provide emulsifying capacity and emulsion stability, which affect foods by acting on the membrane matrix that surrounds the oil drop in an emulsion, preventing its coalescence [98]. Moreover, quinoa proteins show a high foaming capacity and stability [99].

The nutritional properties of quinoa and specifically the high quantity and quality of protein, allow the use of protein isolates in the formulation of various foods. A series of patents have been described in relation with their production, processing and uses. Just to mention a couple of examples, patent US 7563473 B2 relates to "quinoa protein concentrate" (QPC), which contains at least about 50 wt% protein which is food grade and/or pharmaceutical grade and methods of preparing such protein concentrates as well as starch, oil and fiber from quinoa grain, whereas patent US 20100196569 A1 involves grain products having a reduced bitter flavor with a sweet taste or crunchy texture, among many others. Another line of work is related with the multiple industrial uses of the saponins obtained from quinoa grains, including their processing, for example, in the pharmaceutical industry as immunological adjuvants, to stimulate nonspecific immunity, as well as to enhance an immunological response to a selected antigen and to enhance mucosal absorption of some drugs. As such given examples, many other uses of quinoa seeds and coproducts have been described.

The grain shows a high versatility for culinary uses, but other parts may also be used in cooking: the parts of the plant that have been used as food ingredients include the seed, leaves, stems and roots. The mostly used form of quinoa is the cooked grain (soups, stews), followed by various other forms such as toasted seeds, tender leaves (soups, crepes, pancakes, tortillas), flour (bakery products such as breads, biscuits, cookies, muffins), as well as nutrition bars, granolas, confections and various beverages, fermented or not. Quinoa grains and by-products (e.g. hay) are also used for animal feed.

The nutritional quality of quinoa grains is well recognized, even by agencies such as the National Research Council and the National Aeronautics and Space Administration (NASA) [100], which included quinoa as part of the controlled ecological life support system (CELSS). As described, this ancient crop is nutritious and healthy, with high adaptability that can withstand food processing and can also be used as a replacement for allergenic nuts and seeds. It can support sustainable production and FAO selected it as one of the crops destined to offer food security, by promoting quinoa as part of a FAO strategy to encourage the cultivation of traditional crops [101].

3. Conclusion

The rationale for the reintroduction of quinoa into the diet is strongly related with the epidemiological situation prevailing, which is similar in many nations around the world: growing rates of child obesity, high prevalence of obesity during/after pregnancy in women, high rates of NCD such as cardiovascular, diabetes, cancer, which are associated with the major causes of death. From the nutritional point of view, quinoa represents an excellent source of nutrients and bioactive phytochemicals that contribute to a healthy diet and, on the other hand, supplies good quality protein to support children's healthy growth. The chemical composition of different cultivars is outstanding, although it may be affected by the environmental and climatic factors. Taking into account all its properties, quinoa is currently promoted as an extremely healthy food ("superfood"), the so-called food of the twenty-first century.

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Chapter 4

Functional Properties of Brazilian Propolis: From Chemical Composition Until the Market

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Additional information is available at the end of the chapter

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Abstract

Propolis is a product obtained from resins and exudates of different plants from different regions in order to protect the comb, with peculiar organoleptic, chemicals and biological properties. Considering this, this chapter presents the types of Brazilian propolis as the types available nowadays, their chemical compositions, as well as, some of their important biological properties enabling employing them as important health food, such as antimicrobial, antioxidant, and immunomodulation action. Various "*in vivo*" and clinical trial studies, conducted in different regions, on the safety and dosage of propolis, technologies used to obtain propolis extract, and several innovative presentations of this promising bee product are also presented in this chapter. Finally, this chapter aims to present the regulatory affairs, potential market for propolis around the world, and perspectives for a near future.

Keywords: Brazilian propolis, green, red, brown, chemical composition, antimicrobial, immunomodulatory, antioxidant, extraction process, innovation, regulatory affairs, potential market

1. Introduction and a brief history

The antique civilizations always used bee products as valuable therapeutic resources in their medicinal practices. The history of medicine of the Assyrian civilizations, Chinese, Tibetan, Inca, Egyptian, and also the Greco-Roman is very rich and possess records of centenary formulations, including propolis to treat or prevent diseases. Old Egyptians, Greeks, and Romans used propolis to treat wound, cutaneous lesions, ulcers, and chirurgical interventions [1].

In Egypt, propolis was used as one of the main ingredients used in the formulations to embalm cadavers. It was also used by Aristotle, Dioscorides, Pliny, and Galneo as an antiseptic and wound-healing. The Greeks, including Aristotle and Hippocrates, adopted it as an internal and external healing. Pliny Roman historian refers to propolis as a medicine to reduce swelling and relieve pain [2].

The term "propolis", "pro" in favor of and "polis", "city of bees", which means in defense of the honey comb, was described in the sixteenth century in France [3], and in the seventeenth century, propolis was considered an official drug by London Pharmacopoeia [4]. In the subsequence centuries, propolis has attracted growing interest due to its medical properties, especially in Eastern Europe. In 1908, the first scientific article about propolis chemical properties and composition [5] was published, indexed on Chemical Abstracts. In 1968, the abstract of the first patent was published on Chemical Abstracts [6].

In South Africa, during the war in the end of the nineteenth century, propolis was largely used because of its healing properties [3] and in the Second World War it was used by several Soviet clinicians [2].

In the last decades, propolis has gained wide acceptance as traditional medicine in several parts of the world. This disseminated interest in propolis in several countries encouraged a large number of studies considering chemical and biological properties of propolis [7].

Nowadays, in the Brazilian market and in several other countries, it is possible to find propolis in different presentations, such as liquid or powder extract: in bottles, capsules, tablets, vaporizers, syrups, creams, and among others, aiming to act as an antimicrobial [8–10], antioxidant [11], immunoregulatory [12–14], anti-inflammatory [12, 15, 16], antiviral [17] agent, besides several other functionss. A very large number of publications endorsing these biological benefits in "*in vitro*", "*in vivo*," and in some clinical trials, is be discussed further in this chapter.

Thus, this chapter presents recent studies about Brazilian types of propolis such as green, red, and brown, considering their chemical composition and some biological properties such as antimicrobial, antioxidant, and immunoregulatory, safety aspects, extraction process, and technology associated, regulatory aspects, potential market and challenges, as well as tendencies for a near future.

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2. Chemical composition of Brazilian propolis

Propolis is formed by a complex set of components collected by *Apis mellifera* from different parts of plant resins (twigs, flowers, pollen, buttons, and exudates of trees) which are deposited in the hive with saliva and enzymes of the insect to seal the cracks and maintain the temperature (**Figure 1A**) [18].

Figure 1. Presentation of different types of Brazilian propolis. (A) *Apis mellifera* collecting Green propolis from *Baccharis dracunculifolia* species, (B) green propolis on the intelligent collector, (C) brown propolis and (D) red propolis. Figures (A) and (B) were gently donated by César Ramos, Natucentro Company. Figures (C) and (D) were gently donated by Felipe Galeti Miguel, Apis Flora Company. Both companies are associated with ABEMEL.

It consists of resin (50% of the mixture is composed by flavonoids and phenolic acids), wax (30%), essential oils (10%), pollen (5%), and other organic substances (5%). Among the present compounds, it can be consisted of hydrocarbons, alcohols, aliphatic and aromatic acids, esters and its derivatives, aldehydes, ketones, flavonoids, fatty acids, terpenoids, amino acids, sugars, lignans, vitamins, minerals, etc. [19].

The chemical composition of propolis differs significantly according to the geographic region where resins were collected due to the flora of each region, allowing the selection of different plants as source of resin [20].

When this product is derived from Europe or China, for example, the main plant metabolites found are flavonoids and phenolic acids, unlike the stemmed ones from southeastern Brazil, which, besides phenolic compounds, contain high amounts of terpenoids and prenylated derivatives of p-coumaric acid [21]. This difference in composition reveals the collection of resinous material, in temperate zones, from poplar, especially species of *Populus* and in

southeastern Brazil, especially from *Baccharis dracunculifolia* DC (Compositae), popularly known as "vassourinha do campo" [22].

Regarding Brazilian propolis, it was further divided into 12 classes according to Park et al. [23]: the first five ones originate from the south and have the colors yellow, light brown, dark brown, light brown, and greenish brown, respectively. Regarding propolis found in the Northeast, it was divided into six groups such as reddish brown, greenish brown, dark brown, yellow, dark yellow, and yellow. Finally, the last of these classes regards the kind of propolis that comes from the Southeast and is known to have a greenish brown or green color and so-called green propolis (**Figure 1B**) [23]. After 2007, the 13 types of propolis was added: this new kind comes from the mangroves of the Brazilian states of Sergipe, Alagoas, Paraiba, Pernambuco, and Bahia. Among the Brazilian propolis, the green, the brown (**Figure 1C**), and the red (**Figure 1D**) ones are the most studied and relevant to the Brazilian economy due to their biological activities and exports to other countries, such as Japan [24].

2.1. Green propolis

Green propolis is composed of large amounts of phenolic compounds such as artepillin C, baccharin, kaempferide, isosakuranetin, dihydrokaempferide, drupanin, *p*-couma ric acid, caffeic acid, aromadendrin, caffeoylquinic acid derivatives, and other compounds, such as the triterpene lupeol-3-(3'R-hydroxy)-hexadecanoate. The key source of these compounds is *B. dracunculifolia* [12, 25–30] (**Figure 2**).

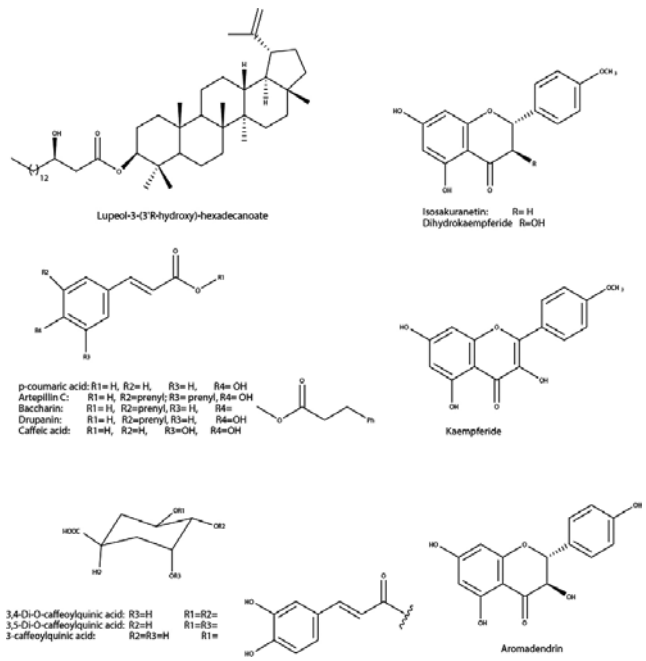

Figure 2. Chemical structures of compounds found in Brazilian green propolis.

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Regarding the volatile compounds found in Brazilian green propolis, the major ones are sesquiterpenes, such as (E)-nerolidol, β-caryophyllene, spathulenol, and δ-cadinene. Further more, other compounds such as selina-3,7(11)diene, benzenepropanoic acid and longipinene were also identified. These compounds are responsible for the pleasant aroma in this bee product and are also responsible for many biological activities reported in the scientific literature. [31, 32]. (**Figure 3**).

Considering the large amount of artepillin C presents in green propolis (**Figure 4**), in addition to other biologically active compounds, the commercial value of Brazilian green propolis in the international market is high [16].

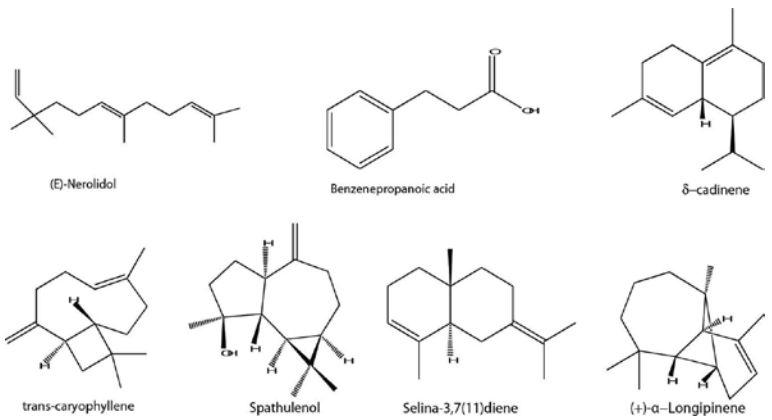

Figure 3. Volatile compounds from Brazilian green propolis.

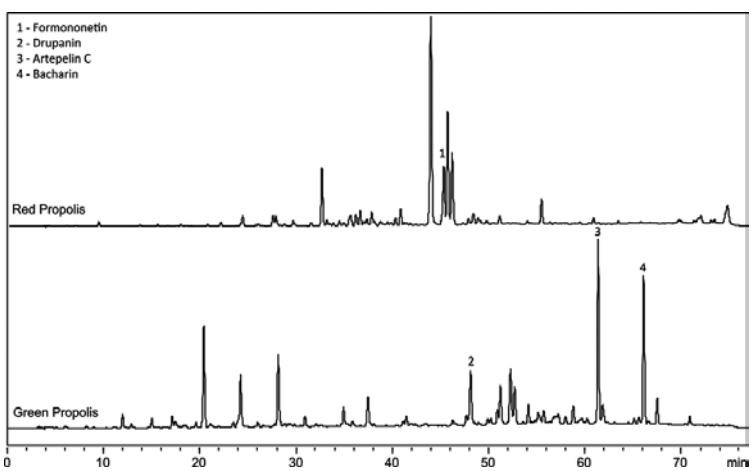

Figure 4. Chemical fingerprint for red and green propolis. Chromatographic profile was obtained using HPLC-DAD, Shimadzu Shim-pack CLC-ODS column (4.6 mm · 250 mm, particle diameter of 5 μm, pore diameter of 100). Green propolis conditions followed according Berretta et al. [8] and red propolis, Cavendish et al. 2015.

2.2. Red propolis

Many of chemical compounds of red propolis, as well as green propolis, have been determined. Some of them are elemicin, isoelemicin, methyl isoeugenol, formononetin, biochanin A, isoliquiritigenin, liquiritigenin, medicarpin, homopterocarpan, quercetin, and vestitol. In the lipophilic extract, the majority of the compounds found are polyprenylated benzophenones—guttiferone E, xanthochymol, and oblongifolin A (**Figure 5**). Because the isoflavones— 7 formononetin, biochanin A, pinocembrin, and medicarpin—are abundant in red propolis, they are used as chemical markers for identifying red propolis (**Figure 4**) [33, 34].

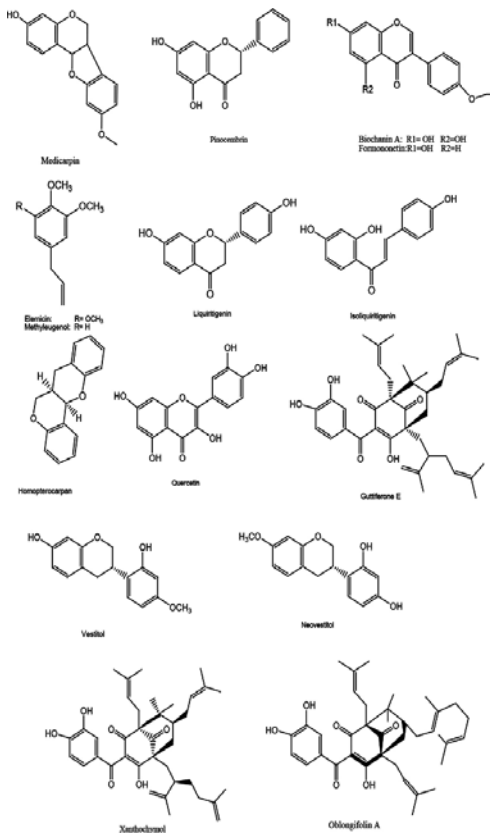

Figure 5. Chemical structures of some compounds found in Brazilian red propolis.

In addition to these compounds, De Mendonça et al. [33] identified caffeic acid, ferulic acid, umbellic acid, p-coumaric acid, genistein, kaempferol, cathechin, dalbergioidin, epicatechin, daidzein, 2′-hydroxyformononetin, evernic acid, naringenin, calycosin, (7S)-dalbergiphenol, thevetiaflavone, cycloartenol, guttiferone C, and other compounds, using LC-Orbitrap-FTMS, a powerful tool to detect compounds because it does not require chromophores such as ultraviolet detector and it can detect very low amounts.

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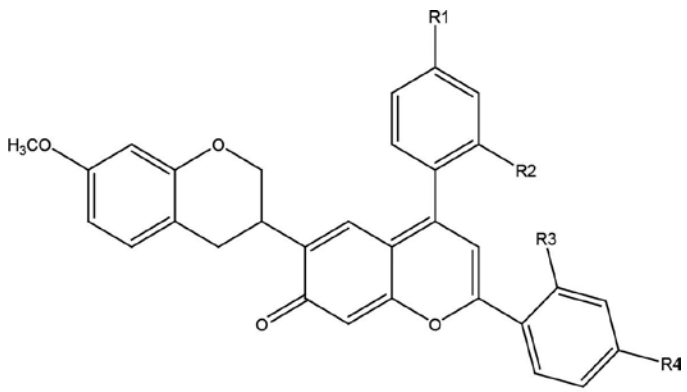
Red propolis has these chemical constituents, mainly due to the collection of resin from *Dalbergia ecastophyllum* by the bees. Its red color is due to the presence of cationic C_{30} isoflavans, retusapurpurins A and B (**Figure 6**). This is characteristic of the propolis found in Brazilian Northeast, found especially in hives nearby mangroves in the states of Sergipe, Bahia, Alagoas, Paraiba, and Pernambuco. This bee product has attracted wide interest because of its numerous biological activities, such as cytotoxic against several cancer cell lines, antibacterial, antifungal, anti-cariogenic, antioxidant, antiproliferative, anti-inflammatory, and others [24, 33, 34]. In addition to its extracts, numerous biological activities of the isolated compounds have been described [24].



Retusapurpurin A: R1= OH R2=H R3=OCH_3 R4=OH
Returapurpurin B: R1=OCH_3 R2=OH R3=H R4=OH

Figure 6. Chemical structures of isoflavans, retusapurpurins A and B.

Righi et al. [35], besides the compounds already described, found alkanes such as *n*-tricosane, *n*-pentacosane, *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane, and *n*-tritiacontane in the apolar red propolis extract (hexane extract). They also identified other compounds as β-amirin, *α*-amirin, lupeol, methylguaiacol, trans-anethole, resorcinol, anisylacetone, *cis*-asarone, farnesol, and among others.

Nunes et al. [36] determined 34 volatile (**Table 1**) compounds in Brazilian red propolis and found that the major ones are trans-anethole, *α*-copaene, and methyl-*cis*-isoeugenol (**Figure 7**). They found that the chemical composition remains relatively constant during the year, considering that 17 out of the 34 compounds were detected every season of the year. But, the compounds δ-cardinol, ß-gurjunene, isocaryophyllene, and δ-cadinene were found only in the sample collected in October and the alkanes, the 1,8 cineol, and *α*-selinene in the sample collected in July. This can be explained by the visitation of bees, in rainy seasons of shrubs and in dry seasons of woody plants: when the apiculture pasture changes, the chemical composition of propolis also changes. Similarly, as trans-anethole, the other red propolis compounds show biological activities such as analgesic, anesthesic, antigenotoxic, and antioxidant, making the volatile fraction pharmacologically interesting.

Class of the compound	Compound	Class of the compound	Compound
Monoterpene	*p*-Cymene	Sesquiterpene	α-Cubebene
	Limonene		α-Copaene
	1,8-Cineole		β-Gurjunene
	Linalool		β-Caryophyllene
Aromatic compound	Naphthalene		α-Bergamotene
Alcohol, aldehyde or ketone	4-Hydroxy-4-methyl-heptan-2-one		Farnesene
	6-Methyl-5-hepten-2-ona		Є-β-Farnesene
Phenylpropanoid	Octanal		D-Germacrene
	Nonanal		α-Selinene
	n-Decanal		Isocaryophyllene
	Anisaldehyde		β-Bisabolene
	n-Dodecanal		δ-Cadinene
	trans-metil isoeugenol		Cadinene
	Estragole		δ-Cadinol
	Trans-anethole	Aliphatic hydrocarbon	Tetradecano
	Methyl-*cis*-isoeugenol		Pentadecano
	Elemicin		Hexadecano

Table 1. Compounds found in Brazilian red propolis.

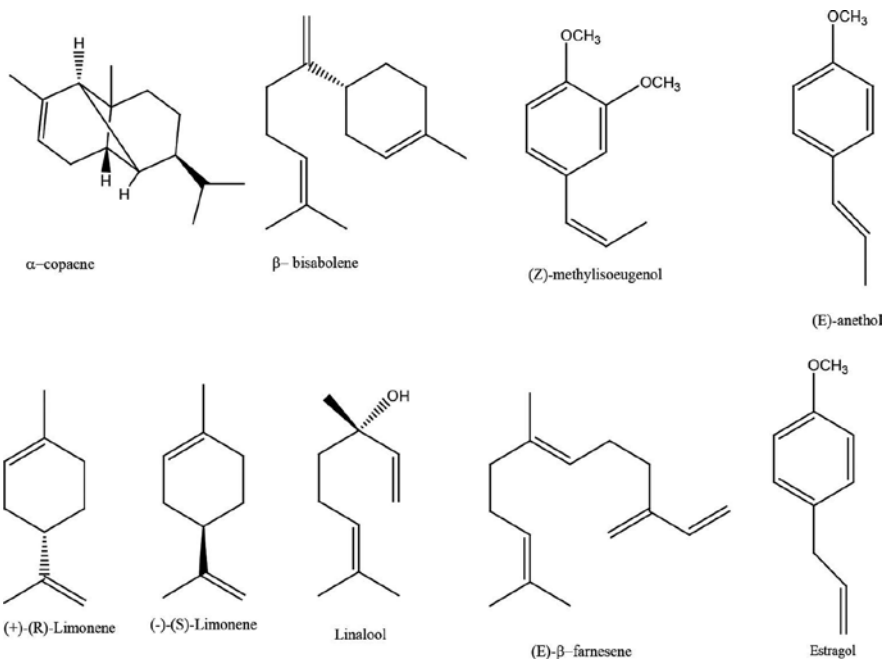


Figure 7. Some volatile compounds from Brazilian red propolis.

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2.3. Brown propolis

The brown color is characteristic of propolis from different areas, but regarding Brazilian brown propolis, it is usually referred as the one that comes from the south of the country. Although many chemical compounds present in this product and their biological effects have already been identified, this type of propolis has not been well studied as red and green Brazilian propolis and many scientific reports on brown propolis are relatively old. Its botanical source seems to be mostly *Araucaria*, although some compounds found on it are also present in *B. dracunculifolia* [37].

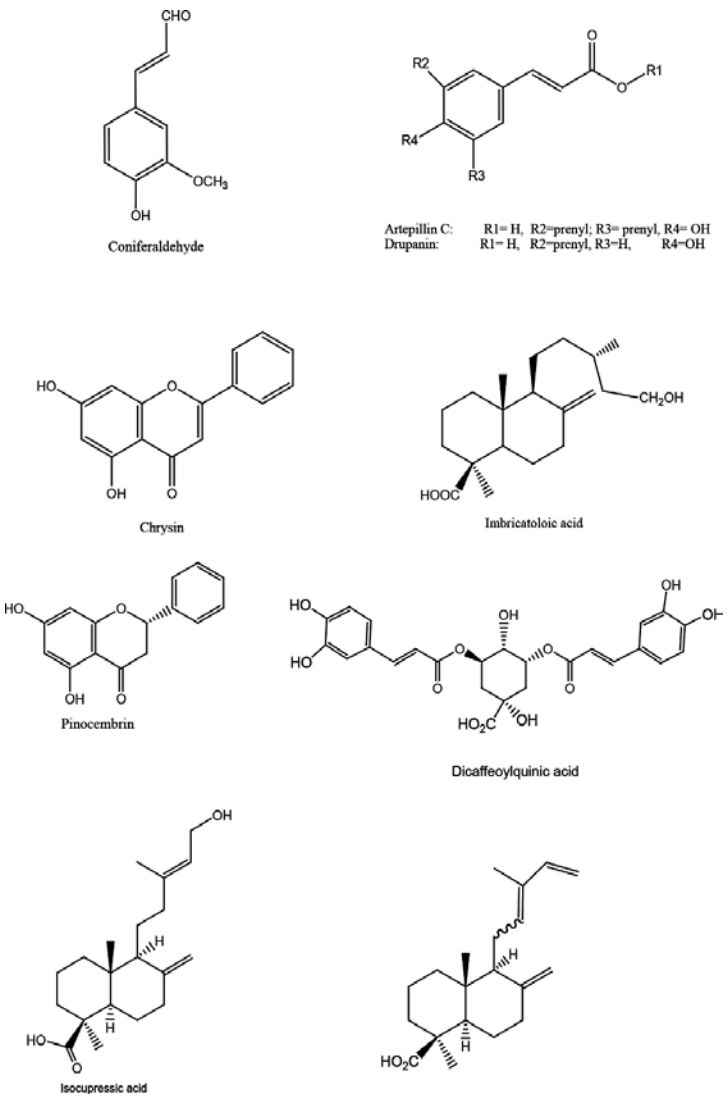

Figure 8. Chemical compounds of brown propolis.

The main compounds identified were: coniferaldehyde, 2,2-dimethyl-6-carboxyethenyl-2h-1-benzopyran, drupanin, pinocembrin, dicaffeoylquinic acid, and artepillin C, isocupressic acid, acetylisocupressic acid, imbricatoloic acid and a mixture of *cis* and *trans* isomers of communic acid [38–40] (**Figure 8**).

Among the numerous biological effects reported for brown propolis and its isolated compounds, it has been observed that both brown propolis and some of its isolated compounds have antimicrobial effect. In addition, it was possible to determine which compounds are responsible for such activity, highlighting the importance of chemically know product widely used by population [38, 41]. Moreover, brown propolis, as well as green propolis, has a significant preventive effect against oxidative stress in skin [42].

Brown propolis collected in Mato Grosso do Sul due to the significant amount of phenolic compounds in ethanol extract shows high antioxidant and antigenotoxic activities. Its volatile fraction is composed mainly of the sesquiterpenes spathulenol and (E)-nerolidol (**Figure 3**), which show an antimicrobial effect against *Cryptococcus neoformans, Enterococcus faecalis*, and *Staphylococcus aureus*. They were not mutagenic, considering that the antimicrobial activity is not because of DNA damage induction [43, 44]. The brown propolis collected from Mato Grosso also showed antimicrobial activity [45].

Therefore, considering that Brazil has a unique flora, among all types of Brazilian propolis three types of propolis are highly noticeable: green, red, and brown propolis due to their singular chemical composition, leading to their biological effects, culminating in the high value in the international market of Brazilian bee products.

3. Biological properties

3.1. Antimicrobial activity

Antimicrobial properties of Brazilian propolis are well-documented, including the antibacterial, antifungal, and antiviral activities. The biological activities of propolis are related to its chemical composition that varies with the collection period of the resin and the flora of the region visited by bees [46]. Therefore, in Brazil there are different types of propolis, since the different geographical regions of the country have a diversity of plant species. The most popular types of Brazilian propolis are green and red propolis.

Brazilian green propolis, whose most important plant source is *B. dracunculifolia*, has been extensively studied. Several studies have shown the activity of green propolis against several pathogenic bacteria, including Gram-positive bacteria (*S. aureus, Staphylococcus epidermidis, Streptococcus pneumoniae*, and *Kocuria rhizophila*) and Gram-negative bacteria (*Haemophilus influenzae, Porphyromonas gingivalis, Porphyromonas endodontalis*, and *Prevotella denticola*) [9, 10, 46–48]. The last three bacteria cause periodontal diseases, which affect the periodontal tissues (tooth supporting tissues). Furthermore, green propolis is active against cariogenic bacteria, such as *Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius, Streptococcus sanguinis*, and *Lactobacillus casei* [48, 49]. However, some Gram-negative bacteria are not

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susceptible to green propolis, such as *Escherichia coli* and *Pseudomonas aeruginosa* [9, 10, 46]. *E. coli* can cause urinary tract infections and gastroenteritis, among others, while *P. aeruginosa* is associated with nosocomial infections, since it is an opportunistic bacterium.

Antifungal activity of green propolis has been reported against all three morphotypes of *Candida albicans* (yeast, pseudohyphae, and hyphae) [50]. At the cellular level, green propolis is able to induce apoptosis and secondary necrosis in yeasts, as showed in a study using *Saccharomyces cerevisiae* as a model organism [51]. Green propolis is also active against filamentous fungi (molds), such as *Trichophyton rubrum, Trichophyton tonsurans*, and *Trichophyton mentagrophytes* [52], which cause dermatophytosis. Ngatu et al. [53] reported the antimycotic effect of green propolis in patients with tinea pedis interdigitalis and tinea corporis caused by *T. rubrum*.

Green propolis also has the capacity to inhibit virus propagation. Shimizu et al. [54] reported that the ethanol extract of green propolis exhibited moderate efficacy in limiting herpetic skin lesions in mice infected with herpes simplex virus type 1 (HSV-1). Urushisaki et al. [17] showed the anti-influenza effect (H1N1 influenza virus) of the water extract of green propolis and its caffeoylquinic acids, which may have a cytoprotective action by affecting the internal cellular process. Takemura et al. [55] also reported the anti-influenza effect of the water and ethanol extracts of green propolis and their 3,4-dicaffeoylquinic acid, which enhance viral clearance by increasing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in the lungs of mice infected with H1N1 influenza virus.

Batch-to-batch variability is a common problem in the manufacture of propolis extracts. Since medicinal use of these extracts must rely on appropriate quality requisites, batch-to-batch reproducibility is essential to ensure consistent quality. Therefore, Berretta et al. [8] developed the propolis standardized extract (EPP-AF®), an ethanolic extract which contains green propolis and has batch-to-batch chemical reproducibility. Furthermore, it has several biological activities, including antibacterial and wound-healing activities [8]. **Figure 9** shows some results obtained by our research group, showing the antibacterial activity of EPP-AF® and extracts of brown, red and green propolis.

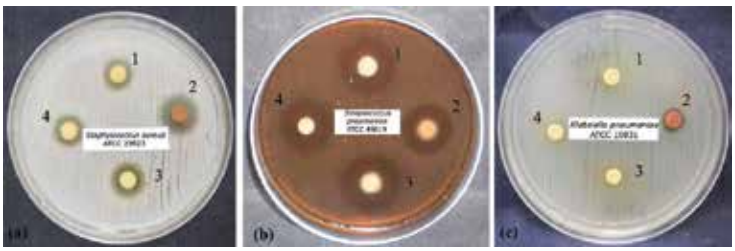

Figure 9. Zones of inhibition (disk diffusion method) provided by 1: Extract of brown propolis from the south of Brazil; 2: Extract of red propolis from the northeast of Brazil; 3: Extract of green propolis from the southeast of Brazil; 4: Propolis standardized extract (EPP-AF®); (a): Staphylococcus aureus ATCC 25923; (b): Streptococcus neumonia ATCC 49619; (c): Klebsiella neumonia ATCC 10031.

Our research group also has developed and evaluated different pharmaceutical forms of green propolis extracts, including propolis ethanolic extract (PEE), propolis water extract (PWE), propolis soluble dry extract (PSDE), and propolis matricial microparticles (PMM). With respect to antifungal activity (*S. cerevisiae* and *C. albicans*), PEE was the most potent followed by PWE, PMM, and PSDE [50]. The same results were obtained against *Lactobacillus* species (Gram-positive bacteria) (data not published yet).

Brazilian red propolis, in its turn, is produced from resinous exudates of *D. ecastophyllum*, found mainly in Northeastern Brazil (states of Alagoas, Bahia, Paraíba, Pernambuco, and Sergipe) [56]. Ethanolic extracts of red propolis showed activity against Gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) [56–58]. These results are very interesting, since green propolis is not active against *E. coli* and *P. aeruginosa* [9, 10, 46]. Red propolis also has antifungal activity. Siqueira et al. [52] reported its activity against some dermatophytes (*T. rubrum, T. tonsurans*, and *T. mentagrophytes*).

Isoflavone formononetin is one of main chemical compounds in red propolis. Das Neves et al. [59] evaluated the activity of this compound against some bacteria (*S. aureus, S. epidermidis*, and *P. aeruginosa*) and yeasts (*C. albicans, Candida tropicalis*, and *C. neoformans*). The MIC value was 200 μg/ml for all bacteria and 25 μg/ml for the yeasts [59]. (6aS,11aS)-Medicarpin is the other chemical compound in red propolis, which exhibits a strong antibacterial activity, since MIC values of 16, 32 and 32 μg/ml were obtained against *S. aureus, B. subtilis*, and *P. aeruginosa*, respectively [57].

Kamuyama et al. [60] evaluated the use of green propolis to control microorganisms in minimally processed carrot. The study involved the comparison between: (i) carrot sanitation with 200 mg/l of total available chlorine, (ii) chlorinated solution "A" together with edible film with 0.4% propolis solution, and (iii) carrot sanitation with 0.4% propolis solution, prepared from 25% propolis alcoholic extract. Mesophilic and psychrotrophic aerobic bacteria, mold, and yeast were counted during the storage of samples of processed carrots at 10°C. The results demonstrated that the results for all treatments were similar to mesophilic and psychrotrophic bacteria. For mold and yeast count, the application of treatments (ii) and (iii), in the end of study, was similar to T0, suggesting that the use of propolis as a food preservative is viable and promising.

Borges et al. [61] evaluated the antibacterial and antifungal properties of different concentrations of a propolis hydroalcoholic extract in fresh pork sausage. This product is target of microbiological contamination, with consequent commitment of "shelf-life" and ability to cause diseases, factors that stimulate food companies to use synthetic preservatives as sodium nitrate, which possess high toxicity.

Interestingly, the results demonstrated that propolis extract (0.03 g/100 g of food) used showed greater antibacterial and antifungal results when compared to sodium nitrate.

3.2. Antioxidant activity

The propolis antioxidant property is one of the most studied biological activities worldwide. This biological property presents outstanding importance in the general benefits that propolis

may bring to human health as the free-radical scavenging capacity of propolis compounds may be closely related to the anti-inflammatory, antimicrobial, anticancer activities, as well as, prevention of atherosclerosis, skin damages, ageing, and among others.

Several antioxidant methods are available to study propolis, i.e., the DPPH assay, scavenging of hydroxyl radical by the deoxyribose assay, inhibition of lipid peroxidation, inhibition of chemiluminescence produced in the H_2O_2/luminol/horseradish peroxide (HRP) system and inhibition of chemiluminescence produced in the xanthine/luminol/xanthine oxidase (XOD) system, and among others.

Propolis origin	Antioxidant activity/method	Reference
Brazil (marketed-standardized extract—PI 0405483-0)	0.016 μl/ml (IC_{50}) inhibition of lipid peroxidation 0.22 μl/ml (IC_{50}) inhibition of chemiluminescence produced in the H_2O_2/luminol/horseradish peroxide (HRP) system 0.005 μl/ml (IC_{50}) inhibition of chemiluminescence produced in the xanthine/luminol/xanthine oxidase (XOD) system 0.024 μl/ml (IC_{50}) scavenging of hydroxyl radical by the deoxyribose assay	[11]
Campo grande Brazil (raw-material*propolis from stingless bees)	3 μg/ml (IC_{50}) scavenging of DPPH No hemolyis—oxidative hemolysis inhibition assay 50–125 μg/ml—efficiency in inhibiting of AAPH-induced lipid peroxidation	[70]
Nan Province innorthern Thailand (raw-material)	Not indicated - scavenging of DPPH Observation: the authors inform the higher the ethanol amount in the ethanol aqueous solution, the higher the antioxidant activity	[71]
Alagoas, Brazil (brown propolis—raw-material)	8.01 μg/ml (IC_{50}) scavenging of DPPH—ethanolic extract	[33]
Mediterranean propolis	Most prepared extracts inhibited lipid oxidation—oxidation of sunflower oil method	[72]
Brazil aqueus extract	0.62 μg/ml (IC_{50})—inhibition of lipid peroxidation employing brain homogenate	[27]

Table 2. Antioxidant activity of propolis from several regions in the world.

Some of these methods mimic the physiological conditions found in human body, this is the case of lipid peroxidation assay, in which membrane fractions (from mitochondria or brain) are used. In this method, the addition of iron salts triggers the decomposition of lipid peroxides into peroxyl (LOO•) and alkoxyl (LO•) radicals that can abstract hydrogen from polyunsaturated acyl chains and propagate lipid peroxidation. Any antioxidant capable of scavenging LOO• and LO• will decrease peroxidation. However, other methods can be considered more accessible, such as DPPH, which can easily demonstrate, in large scale, the antioxidant capacity of propolis samples from different sources or different batches. According to Marquele-

Oliveira et al. [62], this method could even be employed as an alternative for worldwide characterization and standardization of natural products. A good correlation of the DPPH method was observed against lipid peroxidation assay. This assay is based on the ability of DPPH, a stable free radical, to be quenched and thereby decolorize in the presence of antioxidants resulting in a reduction in absorbance values. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine. The extension of the reaction will depend on the hydrogen-donating ability of the antioxidants [63].

Propolis antioxidant properties have been fully investigated and both propolis raw material and propolis commercial extracts have been studied. **Table 2** shows examples of the antioxidant profile and the method employed for each sample, focusing on their collecting origin. Phenolic compounds have been reported as the main propolis compounds responsible for the antioxidant property. The antioxidant role of polyphenols results from the donation of hydrogen atoms from an aromatic hydroxyl group to the free radical, leading to stabilization of the radical [64]. During the evaluation of propolis fractions (from Brazil), Wang et al. [65] observed a strong inhibition of lipid peroxidation using rat liver homogenate at a concentration of 2 mg/ml, and this activity was related to the presence of flavonoids. However, it is known that other than phenolic compounds, flavonoids are involved in the antioxidant activity of propolis. So a series of phenolic compounds, including flavonoids, were assessed against the peroxidation of linoleic acid in a micellar solution. The results demonstrated that polyphenols in general present higher activity than BHT (butylated hydroxytoluene), a well-known antioxidant [66]. In a study using cell culture, artepillin C has been proposed as a strong candidate to be responsible for the antilipoperoxidative activity of Brazilian propolis [67].

Santos et al. [68] assessed the antioxidant activity of flavonoids and reported that the presence of structural groups, i.e., the B ring dihydroxyl, double bond in C2 and C3 in conjunction with the 4-oxo function, and the additional presence of hydroxyl groups in C3 and C5 (except for quercetin and 3'-O-methyl-quercetin), were the most potent inhibitors of lipid peroxidation using mitochondria. This antioxidant activity was also due to Fe chelation, which may explain the activity of flavonoids and polyphenols which do not have the above described structural groups [69].

After screening the antioxidant properties of propolis around the world, not only in the presented references, but also in the vast literature about this topic, one can observe a wide variation of responses. On the one hand, the antioxidant ability of each extract is related to the type and amount of phenolic compounds present in each extract, closely dependent on the propolis origin. But, on the other hand, no standardization regarding the solid soluble amount in each sample is presented, making comparisons among them not adequate. However, the presence of antioxidant activity in every propolis source studied is clearly observed and this activity has special importance to propolis biological properties.

Tian et al. [73] have shown that ethanolic propolis extract (EPE) protects endothelial cells from oxidized low-density lipoprotein (ox-LDL)-induced apoptosis and inhibits atherosclerotic lesion development. This research group has also demonstrated the effect of propolis extract on endoplasmic reticulum stress-C/EBP homologous protein pathway-mediated apoptosis. Apoptosis, especially in macrophages present in atherosclerotic lesions, is considered as a

prominent feature of advanced atherosclerotic plaques, suggesting that macrophage apoptosis is closely related to the atherosclerotic development and subsequent plaque rupture, which is the prominent event that results in the majority of clinical manifestations of acute coronary syndrome such as acute myocardial infarction and sudden coronary death [74]. Thus, protecting macrophages from apoptosis is believed as an effective approach to attenuate plaque instability and combat acute vascular events.

Additional studies investigated the potential use of topically and orally administered propolis extracts to prevent UV irradiation-induced oxidative stress in skin. Brazilian propolis extracts both green and brown successfully prevent UV-induced GSH (endogenous antioxidant) depletion *in vivo* and are both promising antioxidant systems against oxidative stress in skin [75].

Propolis also due to its antioxidant properties was tested against acute lung inflammation (ALI) caused by cigarette smoke (CS) *in vivo*. The researchers observed that propolis (P) treatment (200 mg/kg) normalized all biochemical parameters in the CS+P group compared with the CS group, including nitrite, myeloperoxidase level, antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase), reduced glutathione/oxidized glutathione ratio, and malondialdehyde. Additionally, TNF-α expression reduced in the CS+P group when compared with the CS group. They suggested, therefore, the potential antioxidant and anti-inflammatory role for propolis with regard to ALI caused by CS in mice [76].

Regarding the influence of the propolis antioxidant activity in food preservation, when combined with heat treatment in apple juice, propolis (0.1 mg/ml) reduced the thermal treatment time and temperature needed to inactivate 5 log10 cycles of *E. coli*. No influence on organoleptic properties of the apple juice, which implies the possibility of obtaining a sensorially appealing, low-pasteurized apple juice with the functional properties provided by propolis was reached [77]. In another study, Costa et al. [78] studied the bifunctional biobased packing containing red propolis. In addition to the antimicrobial effect on coagulase-positive *Staphylococci* in cheese curds, the authors observed the reduced oxidation of butter during storage due to the antioxidant properties of propolis.

3.3. Immunoregulator

One of the biological effects of propolis is its immunomodulatory effect—by either enhancing or suppressing the immune system. This contradictory effect is probably due to its complex chemical variety, the presence in different geographic regions, and the different forms of extraction.

Little was known about the biological role of propolis until the 1990s, but recently numerous studies have been published, providing an important contribution to this research field.

Immunomodulatory as well as anti-inflammatory effects of propolis have been widely demonstrated both *in vitro* and *in vivo* [15, 79–82].

These effects are mainly related to its constituents, especially the phenolic compounds, including flavonoids as major components. Among the main types of flavonoids contained in

propolis are: pinocebrin, chrisin, and caffeic acid phenethyl ester (CAPE). In addition to flavonoids, propolis can also contain cinnamic acid derivatives such as caffeic acid and its esters, besides sesquiterpenes, quinones, and coumarins [83–85]. The typical constituents of Brazilian propolis, especially the Brazilian green propolis, are: caffeoylquinic acid and prenylated derivatives of cinnamic acid, such as artepillin C, p-coumaric acid, baccharin, and drupanin [23, 86, 87].

Despite the intensive search for the main constituent of propolis responsible for its immunomodulatory role, its effect seems to be associated with a combination of its different components [88].

Bachiega et al. [89] evaluated the propolis extract and its phenolic compounds, such as cinnamic and coumaric acids on cytokine production (IL-1b, IL-6, and IL-10) before or after macrophage challenge with LPS, to assess a possible immunomodulatory action. They observed a significant reduction in IL-6 and IL-10 in macrophages treated with the compounds only when the LPS was added before the stimulus, whereas the propolis extract was capable to inhibit the cytokine production both before and after the LPS addition. Thus, concluding that this efficiency could have occurred due to the synergistic effect of all compounds present in the extract [89]. On the other hand, the effect of polyphenolic compounds isolated from propolis and propolis extract was investigated on the growth and metastatic potential of a transplantable mammary carcinoma of CBA mouse. The results indicated that water-soluble extract of propolis (WSDP), caffeic acid (CA), quercetin (QU), and CAPE could be useful tools in the control of tumor growth in experimental tumor models [13].

The immunomodulatory activity of propolis extract was also investigated *in vivo* using the ovalbumin (OVA)-induced asthma model. Sy et al. [90] demonstrated that propolis extracts can suppress the serum levels of OVA-specific antibody IgE and IgG1 and attenuate the airway inflammation in treated mice, probably by the ability of propolis to modulate cytokine production. These findings suggest that propolis extracts may be a potential novel therapeutic agent for asthma [90].

Park et al. [91] evaluated another ethanolic extract of propolis (EEP) from Korea in an inflammatory animal model of hind paw edema induced by carrageenan. They observed a significant inhibition of the development of paw edema and increased vascular permeability coupled with an excellent analgesic effect in treated animals. They also showed a significant inhibitory effect on granuloma and exudate formation. The authors suggested that the anti-inflammatory effects of propolis observed might be due to its inhibitory effect on prostaglandin production [91].

In fact, Mirzoeva et al. [92] demonstrated the effect of another ethanolic extract of propolis in suppressing the prostaglandin and leukotriene generation by murine peritoneal macrophages *in vitro* and during zymosan-induced acute peritoneal inflammation *in vivo*. Furthermore, the authors described the caffeic acid phenethyl ester (CAPE) as being the most potent modulator of the arachidonic acid cascade among the propolis components examined [92].

Similarly, Borrelli et al. [93] investigated two ethanolic propolis extracts (EPE): with and without the caffeic acid phenethyl ester (CAPE) for their anti-inflammatory activity in rats

using carrageenan foot edema and carrageenan pleurisy models. They observed that only EPE with CAPE and CAPE alone significantly inhibited the carrageenan edema in the rat paw and the number of leukocytes in the pleural exudate in rats, suggesting that the anti-inflammatory activity of propolis is due to CAPE [93].

It is important to say that, despite Brazilian green propolis does not present CAPE in its composition, it presents a wide range of studies describing its beneficial properties such as antiulcerogenic, anti-inflammatory, antimutagenic, antifungal, angiogenesis, antioxidant, and immunomodulatory [14, 94–98]. Different from most European propolis extracts, which present flavonoids as the major component responsible for their effects, the biological activities of Brazilian green propolis are due to its high levels of phenolic acids such as artepillin C [99].

Studies with Brazilian green propolis have showed its role in inhibiting the development of pulmonary cancers [100], an antiviral activity *in vivo* [101], anticancer [102], an anti-inflammatory activity *in vivo* and *in vitro* [12, 16, 87], an antioxidant function in patients with type 2 diabetes mellitus [101, 102], antiherpetic activity [103], and among others [104, 105].

Despite several and growing studies involving the biological effects of Brazilian propolis, the detailed molecular and cellular basis of the action of propolis on immune cells is still unknown.

The administration of green propolis in animals subjected to chronic stress increased the generation of hydrogen peroxide, suggesting a modulation in the macrophage activation [106]. Machado et al. [12] verified an immunomodulatory effect of Brazilian green propolis extracts in acute and chronic inflammation models *in vivo* where the treated animals showed a decrease production of proinflammatory cytokines such as TNF-α and IL-6 and an increase in the IL-10 and TGF-b anti-inflammatory cytokines [12].

Most of the studies reported to date are associated with the immunomodulatory effect presented by propolis extracts with the modulation of the transcription factor NFkB [107–110].

Recently, it has been demonstrated that Brazilian green propolis can also act in a new inflammatory pathway named inflammasome. The inflammasomes are a large molecular platform formed in the cell cytosol in response to stress signals, toxins, and microbial infections. Once activated, the inflammasome induces the molecule caspase-1, which in turn provokes the processing of inflammatory cytokines IL-1β and IL-18. The Brazilian green propolis analyzed in this study (EPP-AF®) was capable of inhibiting the NLRP3 inflammasome and hence significantly reduces the IL-1β secretion in mouse macrophages. Thus, indicating that Brazilian green propolis EPP-AF® extract has a significant role in regulating the inflammasomes [15].

In conclusion, the immunomodulation caused by propolis has been amply demonstrated in recent years, both in the stimulation and suppression of the immune system, making it potentially applicable as an alternative adjuvant therapy or even in the treatment of various diseases.

Table 3 presents a summary of the activities presented in Section 3.

Kind of propolis	Biological properties	References
Brown	Antigenotoxicity	[43, 44]
	Antimicrobial	[38, 41, 44, 45]
	Antioxidant	[42, 75]
Green	Antibacterial	[8–10, 46, 47, 49]
	Anticancer	[102]
	Antifungal	[51–53]
	Anti-inflammatory	[12, 15, 16, 87]
	Antimutagenic,	[96]
	Antioxidant	[27, 42, 75, 76, 103]
	Antiulcerogenic	[94]
	Antiviral	[17, 54, 55]
	Immunomodulatory	[12, 88]
	Inhibition of angiogenesis	[98]
	Preservative	[60]
Red	Antibacterial	[24, 56–59]
	Anticancer	[33]
	Anticariogenic	[24]
	Antifungal	[24, 52, 59]
	Anti-inflammatory	[24, 80]
	Antioxidant	[24, 33]
	Antiproliferative	[24]
	Immunomodulatory	[24]

Table 3. Biological activities presented in Section 3—summary.

4. Safety aspects

4.1. Nonclinical studies

Although propolis has been used for centuries around the world demonstrating to be safe, several scientific studies have been done in order to evaluate propolis safety by oral or topical route. Here we intend to present Brazilian propolis studies done in animals and, the studies found in humans, as clinical trials are not so numerous, despite the clinical trials did not focus on safety, we are presenting them in order to compare the dosages previously used and documented in humans.

Sforcin et al. [111] evaluated some biochemical parameters of animals treated with several different types of propolis aiming to study propolis safety and the differences in the propolis source could interfere in the results. The authors determined total proteins, glucose, urea,

creatinine, triglycerides, cholesterol, cholesterol-HDL, aminotransferases, and lactic dehydrogenase (LDH). The results demonstrated that all parameters were under standard values for the species studied and the propolis sources did not affect the results.

Reis et al. [95] evaluated the safety of propolis standardized extract (EPP-AF®), a Brazilian propolis composition that presents more than 50% of green propolis, by oral route in mice, in an acute model. DL50 was determined to be 3000 mg/kg after 24 h of treatment, and dosages under this value did not demonstrate intoxication signs in the animals. In the subchronic protocol (30 days) done in Wistar rats, there were no differences in the food and water intake, animals' weight and diuresis. Hematological and biochemical analysis did not show statistical differences between the treated (propolis 650 mg/kg) and placebo group. All parameters were in accordance with reference standards for the species studied. Microscopic analysis of all tissues did not show any differences with the placebo group, and it was not possible to detect any lesions, hemorrhages or cells infiltration, demonstrating the safety of oral administration of Brazilian propolis up to 650 mg/kg during 30 days of ingestion.

Mani et al. [112] evaluated the safety of Brazilian propolis in distinct treatments: (i) rats treated with 1, 3, and 6 mg/kg/day during 30 days; (ii) rats treated with 1 mg/kg/day of propolis alcoholic or aqueous during 30 days, and (iii) rats treated with 1 mg/kg/day during 90 and 150 days, demonstrating that all levels of seric cholesterol, HDL-cholesterol, total lipids, triglycerides, aminotransferases (AST), and lactic dehydrogenase (LDH) of propolis treated group were similar to the control group. The authors suggested that Brazilian propolis in the dosages used during the period of treatment were safe (**Table 4**).

Type of study (oral route)	Dosage	Propolis source	Species	Dosage converted to human according FDA guideline (mg/day)*
Biochemical parameters, 150 days [112]	1, 3 and 6 mg/kg	Brazilian	Rats	67.74
Biochemical parameters, 60 days [113]	2000 mg/kg	Iranian	Rats	22,580
Acute safety study [114]	2000 mg/kg	Polish	Rats	22,580
DL50 determination [95]	3000 mg/kg	Brazilian	Mice	17,073
Biochemical parameters—30 days [95]	650 mg/kg	Brazilian	Rats	7338
Acute toxicity parameters**	2500 mg/kg	Brazilian	Wistar rats	28,225
Subchronic study (28 days)**	1000 mg/kg	Brazilian	Wistar rats	11,290
Subchronic study (28 days)**	100, 300 and 1000 mg/kg	Brazilian	Rabbits	22,580

* Conversion considering adult weight around 70 kg.
** Results of our group and not published yet.

Table 4. Safety non-clinical results for propolis administration for oral route.

According to Dobrowolski et al. [114], LD_{50} for different sources of propolis varied from 2 to 7.3 g/kg in mice, suggesting a safe dose for humans of 1.4 and 70 mg/day (when using safety

factor of 1000). In conclusion, considering Brazilian propolis LD_{50} as 17,073 mg/day for humans [95], the application of a safety factor of 10 suggested by FDA guidelines, we would have a safe dose of 1700 mg or 1.7 g/day of propolis for an adult.

4.2. Clinical studies

Khayyal et al. [115] evaluated propolis extract activity in asthmatic patients with oral administration of 260 mg of propolis/day, for 2 months. The results demonstrated reduced night attacks (2.5 attacks/week for 1/week) and improved ventilatory functions, as a consequence of a decrease of TNF-, ICAM-1, IL-6, and IL-8, and an increase of 3· of IL-10, besides a decrease of prostaglandins E2, F2, and leukotriene D4.

Cohen et al. [116] evaluated 430 children aged 1–5 year old. Treated group (*n*=215) received a mixture of echinacea (50 mg/ml), propolis (50 mg/ml), and vitamin C (10 mg/ml), during 12 weeks, and compared to the placebo group. Children aged 1–3 year old received 5.0 ml, 2·/day, orally while children aged 4–5 year old received 7.5 ml. They were benefits in the incidence and severity of respiratory tract infections, with a decrease of 55% in the number of sick children, 50% in the incidence of respiratory diseases, and 60% decrease in the number of days with fever.

Type of study (oral route)	Dosage	Dosage converted to human adult (mg/day)	Reference
Double-blind study with children—prevention to respiratory infections	50 mg/ml—10 ml/day (children 1–3 year old)	2482.27	[116]
Double-blind study with children—prevention to respiratory infections	50 mg/ml—15 ml/day (children 4–5 year old)	2876.71	[116]
Pilot clinical trial with asthmatic volunteers	2–3 tablets/day with 88.4 mg propolis each	265.20	[116]
Pilot clinical trial with healthy volunteers—prophylactic study	500 mg propolis (2 capsules/day)	500.0	[117]
Pilot clinical trial—recurrent stomatitis	500 mg propolis/day	500.0	[118]
Propolis for wound healing	500 mg propolis/day	500.0	[119]
Clinical trial with asthmatic patients	1 sachet with 260 mg propolis/day	260.0	[115]
Pilot clinical trial with Brazilian propolis for treatment of Helicobacter pylori	20 drops, 3× /day	˜350.0	[120]

Table 5. Clinical trials done with propolis administrated for oral route.

Brätter et al. [117] evaluated the oral administration of 500 mg of propolis for 13 days in healthy volunteers focusing on the evaluation of the immune response (TNF-α, IL-6, and IL-8). There was an increased ability in the cytokines secretion, however, without plasmatic levels. Then, prophylactic administration of propolis depends on the immune system reactivity and time, with no adverse effects.

Samet et al. [118] tested the oral administration of 500 mg of propolis in a randomized, placebo-controlled double-blind study, in which it was possible to demonstrate the benefits of propolis treatment in the repeated stomatitis, especially important in cases of resistance to treatment.

Finally, Zedan et al. [119] evaluated the administration of 500 mg of propolis/day in 45 patients aiming to offer an alternative treatment to cutaneous healings. The study compared propolis with echinacea and placebo, and propolis demonstrated to be more efficient than the other groups, especially in usual and superficial healings (**Table 5**).

Jasprica et al. [121] studied the antioxidant effects of propolis (propolis soluble in water and maltodextrin, 0.65 g of propolis, presenting 2.5% of flavonoids, equivalent to 16.25 mg expressed as galangin, Specchiasol, Italy) when administered in healthy volunteers (n=47, women and men), 3 doses/day (total daily dose of 48.75 mg of flavonoids) for 15 and 30 days, with the following parameters under investigation: superoxide dismutase, glutathione peroxidase, and catalase, malondialdehyde, total cholesterol, low- and high-density lipoprotein cholesterol, triglycerides, glucose, uric acid, ferritin and transferrin, and all routine red blood cell parameters. Interestingly, only men with 30 days of treatment presented differences in malondialdehyde (decrease), superoxide dismutase activity (increase), and a few changes in some parameters of red blood cell were detected.

Considering all the previously presented studies, it is possible to suggest that propolis dosages ranging from 260.0 mg to 2.87 g, which have already been used in humans can be considered safe. The biological results observed also varied much. Considering that propolis around the world is largely used as a supplement or functional food, it is reasonable to assume that dosages within this range will probably be safe, since none of the articles suggested any damage or complications for the volunteers. Regarding the antioxidant evaluation proposed by Jasprica et al. [121] in humans and the literature available until now, it is likely that the propolis dosage used was very high for this purpose. Some data previously published suggested that propolis can have a "pro" or "anti" action and, because several *in vitro* and *in vivo* studies demonstrated antioxidant actions at certain does, it is possible that the best result may be achieved using dosages around 500 mg/day successfully, but further investigation is needed.

5. Extraction technologies and innovation products

5.1. Extraction technology

Propolis is a very complex material depending on the vegetation present in the area visited by bees. Besides the propolis source, the extraction process associated with solvents used will definitely provide a completely different extract [20]. It is well established that chemical compounds possess several particularities such as solubility, volatility, partition coefficient oil/water, pKa, and therefore, different solvents will probably extract different compounds [122]. Depending on the temperature and equipment necessary, volatile compounds can be lost and then, it is common to use cold procedures such as maceration or percolation, and when it is necessary to use higher temperatures, it is important to be careful in order to preserve every compound of interest.

Park and Ikegaki [122] studied propolis extracts obtained from water with 96% ethanol solution as solvents. The results demonstrated that propolis extract obtained with 80% ethanol grade showed higher absorption at 290 nm. Using ethanol solution at 60%, higher quantities of isosakuranetin, quercetin, and kaempferol were extracted, while pinocembrin and sakuranetin were better extracted with ethanol solution at 70% and kaempferide, acacetin, and isorhamnetin were most extracted with ethanol solution 80%. More expressive antimicrobial activities were found in propolis extracts from 60 to 80% of ethanol solution extraction and higher antioxidant results came from propolis extract obtained with ethanol 70–80%.

The most common extraction process for propolis to oral administration is the alcoholic (70%) extraction using maceration, percolation and/or turboextraction. The ratio of propolis raw material:extract used is completely variable and usually, in Brazil, 1:3–4, i.e., 1 part of propolis raw material may offer 3 or 4 parts of extract, considering the production of a liquid extract with at least 11%w/v of propolis dry matter. Of course, this ratio may vary and it is completely dependent on the quality of propolis raw material used.

Jorge et al. [46] studied green propolis extracts obtained from four different locations in São Paulo and Minas Gerais States, using the same extraction process, i.e., hydroalcoholic solution 70% with maceration for 30 days. Although all samples evaluated were Brazilian green propolis using the same extract, different results were found for drupanin, baccharin, and artepillin C during the same month, in spite of seasonal differences. Therefore, it is possible to conclude that, using the same floral source, solvent and process extraction, different regions, and seasonal variations also offer a different chemical composition. Interestingly, these differences did not affect the safe of propolis ingestion [111].

Besides maceration, percolation or turboextraction, Trusheva et al. [123] compared ultrasound extraction and microwave-assisted extraction with the maceration process. The careful analysis of the results obtained with each process demonstrated that statistical differences were found for total phenolics and propolis total extractable matter for ultrasound process (30 min) and microwave (2 · 10 s) when compared to maceration extraction. For ultrasound, higher total phenolics were found (52 3%) in comparison to maceration (43 2%) while the reduced total extractable matter (53 3% versus 55%). In turn, microwave offered reduced values for total phenolics (40.4 0.6% versus 43 2%) and expressively higher amounts of the total extractable matter (75% versus 55%). Flavonoids analysis did not show important differences among the procedures evaluated. It is important to consider the time of extraction in each process, since maceration takes around 72 h, and ultrasound was effective with 30 minutes and microwave 2 · 10 s. Another important thing is to define the objective of the extraction: for analytical purposes, it is more practical and cheaper to use ultrasound or microwave, however, for industrial scale, these latter may not be easily implemented.

Propolis water extract was also obtained by some authors [12, 17, 98, 124] using completely different procedures, demonstrating some interesting biological activities that had been previously studied for propolis alcoholic extract, such as anti-inflammatory [12], inhibition of inflammatory angiogenesis [98], and antiviral [17]. Although the demonstration of these interesting results, chemical characterization was poorly explored in the manuscripts, except by Urushisaki et al. [17] that presented the caffeoylquinic derivatives as the most important

compounds of this extraction process; however, the manuscript does not present the extraction process used. De Moura et al. [98] performed the extraction from propolis raw material properly crushed in water maceration (500 ml) with temperature around 70°C (30–60 min), two fractions were obtained from the same propolis raw material, followed by filtration. The filtrate was then lyophilized. A similar procedure was used by others too. Nafady et al. [124] in turn, used an innovative process with -cyclodextrin as encapsulate agent. To obtain this extract, 10 g of crushed propolis was dispersed in 1 l of water containing 10 g of -cyclodextrin previously dissolved. The inconvenient of this procedure is the elevate costs involved in the acquisition of -cyclodextrin besides the limitation of the propolis concentration obtained in the final product. Machado et al. [12] proposed the extraction using hydroalcoholic solvent (70%) as usual, however, the solvent was evaporated and after a hydrolysis step the propolis soft extract was then resuspended in water, in this last case, the obtained extract demonstrated similar chemical results of the alcoholic extract in the moment of preparation.

5.2. Nanoparticles and innovation products

Nowadays, finding natural additives has increased the efforts both to obtain bioactive compounds from natural raw materials and develop stable and functional derivative products. The former mentioned properties attributed to propolis are valuable and find applications in several industries, such as pharmaceutics, agrochemical, and food. The growing interest in propolis has also promoted technological development for the suitable application of propolis.

Propolis in the powder form, for example, exhibits several advantages as increased concentration of propolis dry matter, higher chemical stability of the compounds, and longer preservation of the biological properties. Additionally, the powder form also permits the production of presentations with higher compliance in therapeutics, i.e., sachets, tablets, and capsules. The drying process may also involve the encapsulation of the product resulting in micro/nanoencapsulation systems, which can minimize sensory flavor and odor and control the release of the active compounds.

Propolis dry extract was obtained by Da Silva et al. [125] by employing arabic gum and octenyl succinic anhydride (OSA) starch as carriers by spraydrier. The process allowed obtaining propolis in the powder form with preserved antioxidant activity, stability, and low hygroscopicity. Microencapsulated propolis extract obtained by complex coacervation was reached and presented inhibitory activity against *S. aureus* [126]. Bruschi et al. [127] obtained gelatin microparticles containing propolis extractive solution by spray-drying technique. The microencapsulation by spray-drying technique maintained the activity of propolis against *S. aureus*. In another study, the effect of spray drying parameters on the chemical and biological properties of alcoholic extract of green propolis was investigated [128]. Several parameters of the process demonstrated to influence the polyphenol and flavonoid content, as well as the antioxidant activity, but under an optimized condition, the dried propolis extract showed significant antioxidant activity, with 50% lipid peroxidation inhibition at concentrations ranging from 2.5 to 5.0 mg/ml.

More recently, Marquiafável et al. [129] aimed to develop a propolis dry extract with high propolis (~40%w/w of propolis dry matter) and artepillin C contents by employing a combi-

nation of silicon dioxide with arabic or modified starch and silicon dioxide by spray-drier. They have successfully obtained a standardized propolis extract with high amount of propolis, flavonoid content, expected amounts of artepillin C, and with maintained antibacterial activity, and obtained microparticles with both excipients used. Recently, results of the same group obtained dry extracts of propolis with 70–80% of dry matter; however, the microparticles were not obtained (data not published yet), and then, the odor, color, and taste are not similarly reduced as it is possible to observe when microparticles are obtained (**Figure 10**). Although microparticles were not obtained with 70–80% of propolis dry matter, this extract is the most concentrated one found in the market until now and can be used in several products with very good results, for example soft or hard capsules or tablets.

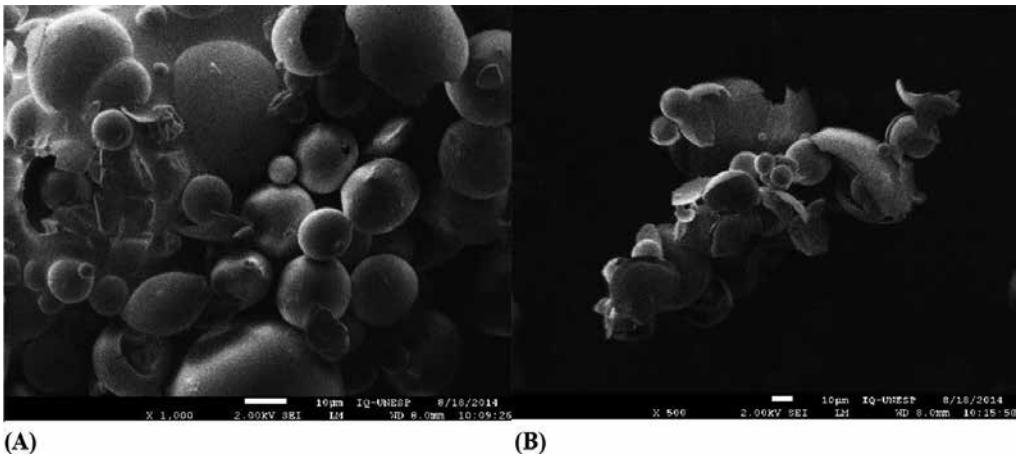

Figure 10. Propolis standardized water extracts of green propolis; C: propolis standardized extract (EPP-AF®).

In general, the propolis powder extracts obtained by spray-drying technique investigated in the literature demonstrated the formation of particles at the micrometer scale, from 1 to 10–20 μm. On the other hand, as nanotechnology can offer new opportunities for propolis application, in another line of research, nanosized particles have been developed. Patil et al. [130] have obtained and characterized silver nanoparticles containing propolis [130]. Propolis nanoparticles have also been obtained employing lipid carriers. Our research group has focused on developing solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) encapsulating propolis. **Figure 11** shows atomic force microscopy (AFM) images of propolis-loaded NLC. Additional studies were also conducted covering NLC surface with chitosan. The chitosan-covered particles presented positive residual surface charge [≈ +40 mV], while the uncoated ones presented negative charges [≈ −30 mV]. Particles were anisometric in shape and approximately 150–200 nm in size. The images demonstrate the particle surface and confirm the nanometric size of the particle. Additionally, no roughness was observed on the particle surfaces.

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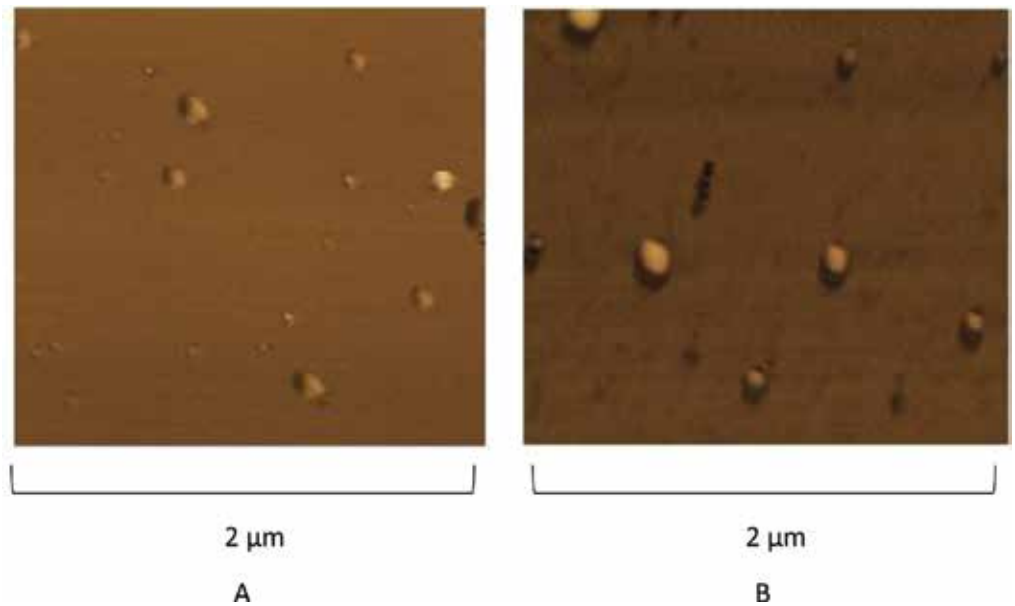

Figure 11. Atomic force microscopy (AFM) images of propolis-NLC (A) and chitosan coated propolis-NLC.

Several important applications can be carried out with innovative propolis extracts. Different presentations of propolis were previously showed as in a liquid presentation without alcohol, usually using propylene glycol or polyethylene glycol, propolis powders in different systems or concentrations, micro or nanoparticles, and others, as soft or hard capsules, with immediate or sustained release systems. Considering the applications, it is possible to formulate capsules, tablets, pills, or others with a specific amount of propolis dry matter, or with a focus on some groups of compounds (total flavonoid or polyphenols) and finally, on a biomarker or a group of these substances such as artepillin C, drupanin, or baccharin, all presentations completely applicable to functional or supplement food, or medicines. Besides oral administration, it is possible to use all of these propolis presentations in topical products, as previously published by Berretta et al. [8] who presented a propolis thermoreversible gel to treat cutaneous lesions or burns, Barud et al. [131] with a propolis biomembrane for the same application, or Berretta et al. [50] that demonstrated the benefits of the application of a propolis mucoadhesive gel in vulvovaginal candidiasis. Several other products can be found in the market and in the literature, such as mouthwashes, toothpastes dental creams, among others.

6. Regulatory affairs

Functional food and natural health products have become an important part of people's daily diet, contributing to the general health of the population and boosting the global food industry. Therefore, its importance is reflected in the interest in regulation of health claims and standards from industry stakeholders and policymakers.

In this chapter, we examine propolis product regulations and policies in many important producing and consuming countries around the world. The goal of this study is to incentive legislators to update the regulation on propolis products in order to improve information available to consumers so they can make better choices and also be provided with healthier and more innovative options.

The regulatory climate worldwide appears to be tending toward propolis classification into the health food products category, although this category also has different names, registration requirements, and allowed claims throughout the world.

Nevertheless, there are still some countries that categorize propolis as a conventional food together with the other bee products, such as honey, royal jelly, and bee pollen. That is the case of Brazil, where the product is regulated by the Ministry of Agriculture with very stringent regulation that limits the product's presentations, information to consumer and does not allow health claims. In 2005, the Brazilian "National Health Surveillance Agency" (ANVISA) published a technical note allowing the registration of propolis as a topical medicine with the claims of anti-inflammatory, antiseptic, and wound healing [132]. The publication of another regulation [133] reinforced the same rules but, due to the very strict rules for medicines, although Brazil is one of the biggest propolis markets, there are no propolis medicines registered to this date.

In the United States, propolis is encompassed together with a wide range of substances by the definition of a dietary supplement in the Dietary Supplement Health Education Act of 1994 (DSHEA) [134]. The use of function claims is also regulated by the above-mentioned regulation that established some special regulatory requirements and procedures for claims of general well-being. These claims are not preapproved by FDA, but the manufacturer must have substantiation that the claim is truthful and not misleading and must submit a notification with the text of the claim to FDA no later than 30 days after marketing the dietary supplement with the claim.

In the European Union (EU), propolis belongs to the food supplement group, regulated by the Directive 2002/46/EC [135], which defines the category as concentrated sources of nutrients or other substances with a nutritional or physiological effect. Since 2006, EU has been engaged in assessing generic health claims to surpass local regulation of member states and after this harmonization product's labels can only bear health claims authorized by the European Food Safety Authority (EFSA) [136], which evaluates scientific data on claims provided by the applicant. Up to this date, there is still no authorized health claim for propolis.

In Australia, all food supplements fall within the category of "complementary medicines" under the *Therapeutic Goods Act 1989* and the supporting Therapeutic Goods Regulations 1990 [137], in which the substances are evaluated according to their level of risk. It includes vitamin, mineral, herbal, aromatherapy, and homeopathic products. A positive list of low-risk substances that may be used has been established and propolis is one of them. It can be used as an active, excipient, or component in all listed medicine formulations. Propolis products can make indications for health maintenance and health enhancement or certain indications for nonserious, self-limiting conditions. It is the manufacturer responsibility to hold evidence to support

any indications as well as any other claims made for the medicine (according to Requirements of section 26A of the Act).

Food supplements in Canada are regarded as "Natural Health Products" under the Natural Health Products Regulations (SOR/2003-196) [138–141] and may contain a wide range of substances, such as vitamins and minerals, herbal remedies, homeopathic medicines, traditional medicines, and probiotics. All products must be safe to use as over-the-counter products and not need a prescription to be sold. Propolis is positive listed to be used orally in multiple pharmaceutical dosage forms as a source of antioxidants for the maintenance of good health and to help relieve sore throat and/or other mouth and throat infections. It can also be used topically to assist in minor wound healing.

Japan is one of the first countries to move toward regulating functional foods. There are lists containing a broad range of substances that are not restricted to medicinal use and can therefore be used in food supplements. Propolis in this scenario can be used as an authorized excipient under the Food Sanitation Act 2010, as regular health food without any claims or as an active of a "Food for Specified Health Uses" (FOSHU) [142] with health claims.

The Republic of Korea defines functional food significantly differently from other countries, restricting functional food to nutraceuticals. They are regulated under the Health Functional Food Act of 2004 [143] and there is a positive list in the Health Functional Food Code with 37 categories. Propolis preparations in all forms are allowed and may include two health claims: antioxidant activity and antimicrobial activity in oral cavity.

The People's Republic of China is another example of an Asian country that uses a product-specific system of registration. The State Food and Drug Administration in China (SFDA) [144] regulates these food supplements as "health foods" and maintains positive and negative lists of substances that may be used in health foods. Propolis is in the positive list. There are 27 categories of health function claims approved by the SFDA, but the regulatory process for achieving approval of these health claims is very strict and expensive, requiring the applicant to conduct nonclinical or even clinical studies through an approved agency in addition to the regular scientific literature review.

With this brief regulatory framework on propolis products, we have presented different policies and regulations around the world and we hope that policymakers can improve the regulatory scenario in the near future in order to accelerate and foster innovation in the sector.

7. Propolis nowadays market and potential

The green propolis has gained preference in the world market since the 1980s, unveiling new horizons for the product. The growing interest of the market for green propolis in the context of international food trade follows the increasing trend in search of healthier habits, which have gained ample space in people's daily lives all over the world.

In the Japanese market, green propolis has a high commercial value: according to SEBRAE, the price of 1 kg of this product in 2010 was around $ 87 and the same amount of honey was

priced at about 3 dollars. In Tokyo's market, this product is even more valued: a bottle of green propolis, in 2010, was sold for around $ 150 [145, 146]. In 2008, it was estimated that in Japan, 700 million dollars a year started to be moved by green propolis [147]. Japan's interest in green propolis is justified not only by consumption: one of the most important examples of its use is as an adjuvant in the treatment of cancer; but also by Japanese research related to the chemical composition and biological activities of this type of propolis, especially studies with artepillin C [16, 147].

The high demand for green propolis, especially from Asian countries, such as Japan, is essential for sustaining the economy that revolves around this product, which is fairly lucrative. Green propolis is produced mainly in the southeastern region, highlighting the state of Minas Gerais, where there are over 8000 beekeepers, which produces more than 35 tons of propolis per year [148]. These data show the importance of production and exports of green propolis, which is one of the pillars of Brazilian apiculture economy.

Red propolis found in the Brazilian state of Alagoas has been internationally certified by the Brazilian National Institute of Industrial Property (INPI) as the only producer of this kind of propolis in the world and most of its compounds were not found in other types of Brazilian propolis, which makes it a singular bee product [24]. Due to that, its commercial value is internationally high. It has been reported that a kilogram of this product can cost around R $ 500. Its importance to the Brazilian economy and to red propolis producer states is immeasurable. Many propolis producers are being qualified and thereby, they are improving their product quality and the production process. Like green propolis, red propolis is also highly exported to Japan due to its chemical composition and biological effects [149].

Brazil is currently the world's third largest producer of propolis, second only to Russia and China [150]. Although it represents 10–15% of world production, Brazil fulfill about 80% of Japanese demand. Minas Gerais State (Brazil) Beekeepers Federation data show that the propolis produced in the Midwest region of the state is considered the best in the world by the Japanese market, where the kilogram of product has jumped from $ 5 to $ 200 in recent years [150].

The propolis production in Brazil is estimated at around 140 tons, and the major part is destined for international market, both in raw form and as finished products. It is estimated that 100 tons are green propolis and 40 tons other types of propolis. About 80% of the green propolis produced in Brazil comes from the Midwest region of Minas Gerais State, close to the source of the São Francisco river at Serra da Canastra, region where are the highest number of producers. Despite the great Brazilian beekeeping potential, the current production is not enough to fulfill a growing global demand. The Brazilian honey bees are Africanized, presenting defensive and disease-resistant features, with no need to use chemical treatments as in other countries, which ensures Brazilian bee products excellent quality and free from contamination.

Many research fronts have been opened in the pursuit of development and adaptation of professional management techniques in the production of green propolis. In addition to the improvement actions and training of producers in beekeeping management practices, a group

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of green propolis producers in the Midwest region of Minas Gerais in the Source of the São Francisco River created an independent association supported by governmental agencies, universities, researchers, and local private institution.

This association aims to establish the technical and scientific cooperation between the scientific community and the beekeepers, aimed at regional development, improving the quality and increasing the amount of green propolis produced in the region. Among the main projects carried out, stand out the training of beekeepers in the professionalization of beekeeping, conservation and cultivation of *B. dracunculifolia* fields, periodical replacement of old to younger queen bees, and others projects. It is believed that the interaction of technical and practical knowledge of beekeepers in conjunction with the application of scientific knowledge by researchers and universities will contribute significantly to a comprehensive training in the professionalization of Brazilian beekeepers to fulfill the goal of maintaining the quality and increase the amount of green propolis produced in the region.

Trade promotion strategies are being constantly designed and implemented by the Brazilian Association of Honey and Propolis Exporters (ABEMEL) and the Brazilian Trade and Investment Promotion Agency (APEX-Brasil) to disseminate Brazilian bee products around the world. The result is the increasing demand from Asia, Europe, and North America countries.

In recent years, Brazil has been prominent on the international scene by winning important prizes at the World Beekeeping Awards of Apimondia, the main world beekeeping event that brings together representatives of over 130 countries and is held every 2 years. In the last editions, Brazil won gold and silver medals in the category honey and gold in the category propolis.

8. Future perspectives

Considering all information presented here, it is easy to imagine the important potential of propolis in the health of the population and in the Brazilian and international market, especially because of the important biological activities and safety demonstrated with scientific reports as "*in vitro*", "*in vivo*," and in some clinical trials. It is possible to generate several innovative products in different fields considering food, cosmetics, and medicines, and this choice obviously will be related to propolis dosages, formulations, and indications.

It is important to consider the investment in more clinical trials aiming to explore the benefits observed with traditional use and in animal studies, in order to refine the dosages and formulations, with regard to the development of medicines. Besides clinical studies, another important area is the improvement in the investments in productivity in field, since the Brazilian propolis available nowadays is not enough to supply all the countries that may be interested in working with this fabulous natural material produced by bees with the support of Brazilian Biodiversity. And finally, the effort of beepers and entities, such as ABEMEL, is crucial to stimulate and support this work.

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Chapter 5

Native Chilean Fruits and the Effects of Their Functional Compounds on Human Health

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Additional information is available at the end of the chapter

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Abstract

In recent years, there has been great interest in the nutraceutical compounds of fruits from native Chilean plant species. In this context, fruits of *Amomyrtus meli* (Meli), *Aristotelia chilensis* (Maqui), *Berberis microphylla* (Calafate), *Luma apiculata* (Arrayán), *Luma chequén* (Chequén), and *Ugni molinae* (Murtilla) growing predominantly in Chilean forests have been studied. This chapter has compiled the existing information about antioxidant activity and antioxidant compound contents of the above mentioned fruit species and their association with the prevention of pathophysiological disorders in humans, such as inflammation, diabetes, and cardiovascular diseases. Results show that the antioxidant compounds of these species, particularly anthocyanins, decrease inflammation as well as the risk of diabetes and cardiovascular diseases. Therefore, consumption of these fruits is a good alternative for preventing cardiovascular and age-related diseases and pathophysiological disorders.

Keywords: *Amomyrtus meli*, *Aristotelia chilensis*, arrayán, antioxidant, *Berberis microphylla*, calafate, cardiovascular, carcinogenesis, chequén, diabetes, inflammatory, *Luma apiculata*, *Luma chequén*, maqui, meli, murtilla, phenolic compounds, *Ugni molinae*

1. Introduction

Chile is a South American country bordered by Perú to the north, Bolivia to the northeast, and Argentina to the east. The continental Chilean territory is a long, narrow strip of land between the Andes in the east and the Pacific Ocean in the west. Its island territories include the Pacific

islands of Juan Fernández, Salas y Gómez, Desventuradas, and Pascua and Easter Island in Oceania [1, 2]. Due to its long surface and geographical location, Chile has a large range of different climates from tropical to polar, creating great diversity in its ecosystems and richness in its biodiversity [1–4]. This promotes better use of natural resources for improving the bioeconomy of the country [1, 3]. Traditionally, native Chilean fruits have been used as medicine by Chilean ethnic groups [5]. Currently, studies into native Chilean fruits have focused on identifying and defining their key compounds with medicinal effects [6]. Native Chilean species with edible fruits include woody or shrub forest species belonging to the Elaecarpaceae, Berberidaceae, and particularly Myrtaceae families (**Table 1**). These families are distributed from Illapel to Tierra del Fuego (**Figure 1**) and are species-rich in antioxidant and nutraceutical compounds with benefits to human health, including *Amomyrtus meli* (Meli) (**Figure 2**), *Aristotelia chilensis* (Maqui) (**Figure 3**), *Berberis microphylla* (Calafate) (**Figure 4**), *Luma apiculata* (Arrayán) (**Figure 5**), *Luma chequén* (Chequén) (**Figure 6**), and *Ugni molinae* (Murtilla) (**Figure 7**) [3, 7–11]. These species inhabit the Valdivian Evergreen Forest (Bosque siempreverde Valdiviano) and the Evergreen Patagonian Forest (Bosque siempreverde Patagónico) together with other Chilean forest species that have not been domesticated. Interestingly, Murtilla is the most domesticated among them [10, 12–16].

Scientific name of plant species	Common name	Family	References
Amomyrtus meli (Phil.) D. Legrand & Kausel.	Meli	Myrtaceae	[39, 54]
Aristotelia chilensis (Mol.) Stuntz.	Maqui	Elaeocarpaceae	
Berberis microphylla G. Forst.	Calafate	Berberidaceae	
Luma apiculata (DC.) Burret.	Arrayán, Arrayán rojo, Luma, Palo colorado, Temu	Myrtaceae	
Luma chequen (Mol.) A. Gray.	Arrayán Blanco, Chequén	Myrtaceae	
Ugni molinae Turcz.	Murtilla	Myrtaceae	

Table 1. Scientific and common name as well as families of the native Chilean fruits.

Scalbert et al. [17] and Lila et al. [18] reported a relationship between consumption of fruits with high antioxidant contents and a reduction in oxidative stress in humans. The richness and abundance of enzymatic and nonenzymatic antioxidants in plant species decrease the oxidative damage, helping to inhibit the formation of free radicals by several mechanisms: (1) inhibiting the initiation of the peroxidation, (2) preventing formation of reactive oxygen species (ROS), and (3) breaking the autoxidation chain reaction in humans [19–22]. ROS are normally produced in humans but are exacerbated under exogenous stresses (ozone, cigarette smoking, air pollutants, etc.) [23–27]. Chemical effectiveness of antioxidants against ROS is due to the protection of biomolecules such as lipids, proteins, and carbohydrates to prevent damage by oxidative stress in biomembranes. For this reason, it is very important that people consume antioxidant-rich fruits or foods to decrease ROS [28]. The human diseases associated with oxidative stress are the inflammatory process, cardiovascular disorders, and carcinogenesis (gastric and colorectal) [29–32]. Therefore, fruits with a high antioxidant power are of great benefit in disease prevention related with the inflammatory process and in particular associated with oxidative stress [6, 33–35].

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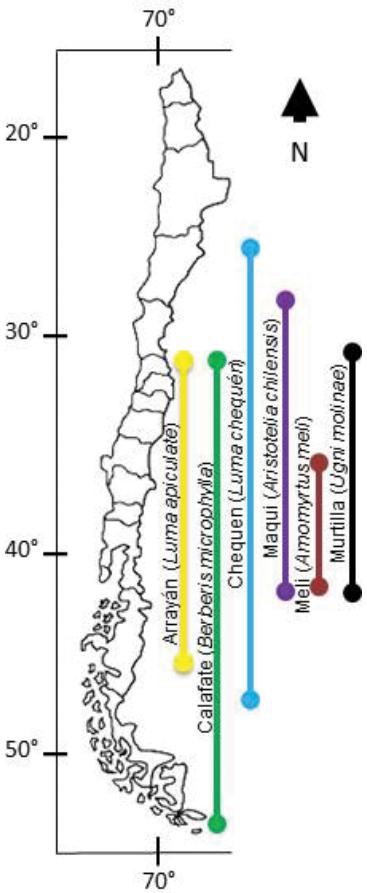

Figure 1. Geographical distribution of native species in the forests of continental Chile.

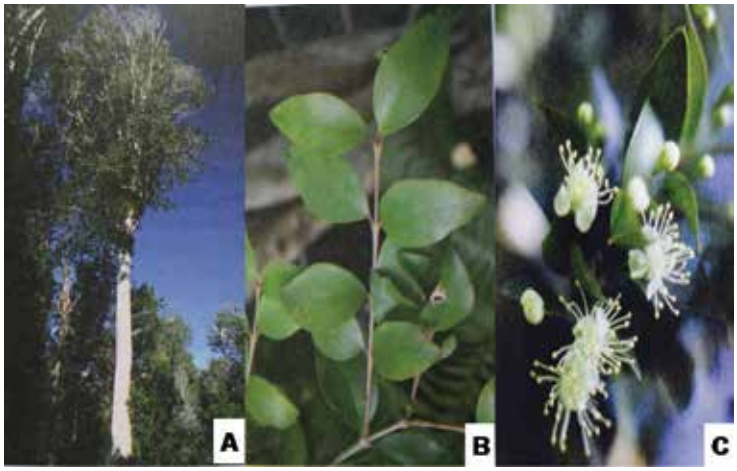

Figure 2. Pictures of *Amomyrtus meli* (Meli): tree (A), leaves (B), and flowers (C). Extracted from Donoso [40].

Figure 3. Pictures of *Aristotelia chilensis* (Maqui): tree (A), leaves (B), and fruits (C).

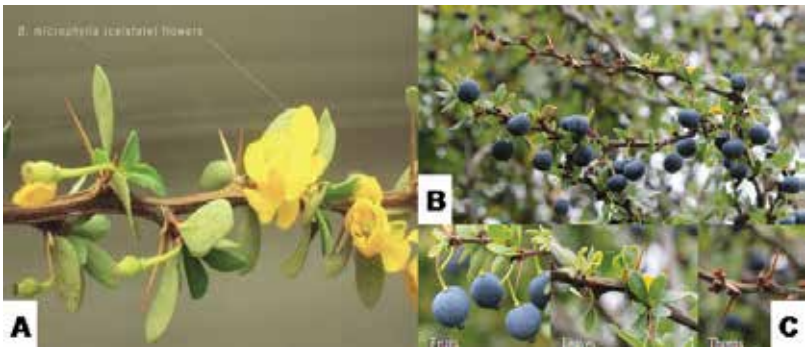

Figure 4. Pictures of *Berberis microphylla* (Calafate): flowers (A), fruits (B) and composed pictures of fruits, leaves, and thorns (C).

Figure 5. Pictures of *Luma apiculata* (Arrayán): tree (A), leaves (B), and fruits (C). The picture was replaced by an our picture.

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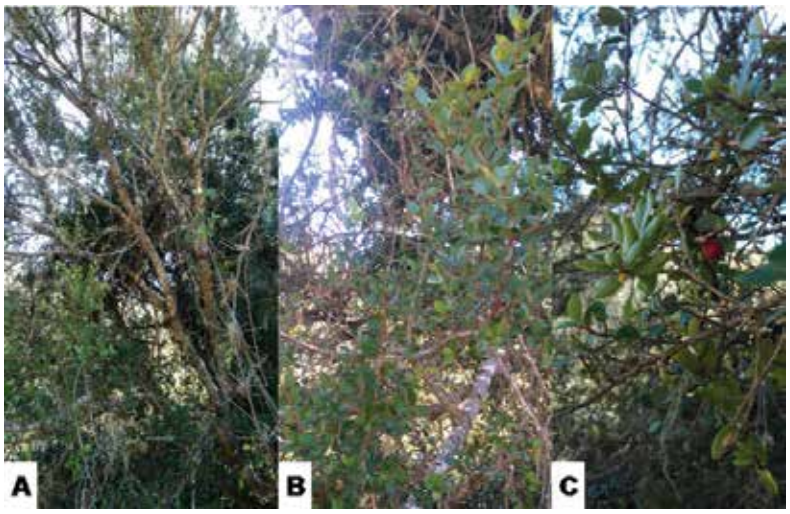

Figure 6. Pictures of *Luma chequen* (Chequén): tree (A), leaves (B), and immature fruits (C).

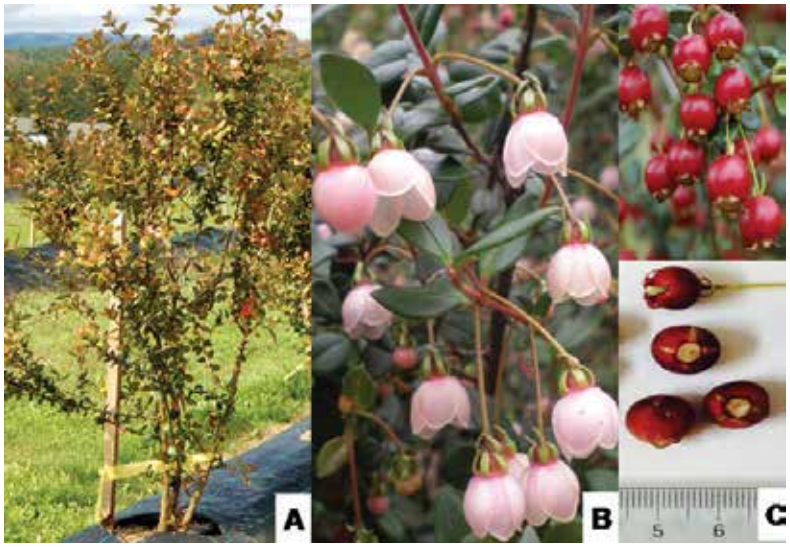

Figure 7. Pictures of *Ugni molinae* (Murtilla): tree (A), flowers (B), and fruits (C).

Regarding native fruits rich in antioxidants and other bioactive compounds, it is important to detect the quality and quantity of the compounds responsible for the health beneficial properties [36–38]. In this context, the aim of this review is to associate the main bioactive compounds, antioxidant activity, and content of individual phenolic compounds of some native Chilean fruits such as Arrayán (**Figure 5**), Calafate (**Figure 4**), Chequén (**Figure 6**), Maqui (**Figure 3**), Meli (**Figure 2**), and Murtilla (**Figure 7**) with the prevention of diseases and pathophysiological disorders in humans.

2. Morphological characterization, geographical distribution, and ethnobotany of native Chilean fruits

2.1. *A. meli* (Meli) (Phil.) D. Legrand & Kausel

A. meli is a deciduous tree endemic to Chile, reaching a height up to 20 m (**Figure 2A**). It has a very distinctive white trunk, which makes it unmistakable among the species of Chilean trees [39]. It grows mostly on moist and shaded sites from the Arauco to Chiloé Regions (**Figure 1**) in the coastal rainforest, growing on the banks of waterways or under the canopy of other larger species [40]. No conservation problems have been reported for this species, although it is present in a few protected areas such as Chiloé National Park, the Valdivian Coast Reserve, and Oncol Park [39]. Despite its palatability, its fruits are not consumed because they have been reported to cause headaches. The infusion of its leaves is recommended as medicinal use for hypertension (**Figure 2B**) [14, 39]. It has great ornamental potential due to its abundant and very fragrant flowering (**Figure 2C**).

2.2. *A. chilensis* (Maqui) (Mol.) Stuntz

A. chilensis is an evergreen species endemic to Chile (**Figure 3A**), and it is distributed from Illapel (Coquimbo Region) to Chiloé (Los Lagos Region) (**Figure 1**) [37]. It grows naturally, forming wild populations named "macales" and can protect against erosion, since it grows rapidly in abandoned, burned, or overexploited soils [41]. The bright green Maqui leaves are oval-lanceolate with a serrated edge (**Figure 3B**) [42]. The fruits are small, purple bright berries about 5 mm of diameter (**Figure 3C**), with two to five small seeds inside and are eaten fresh, in juice or in jam, and also used as food coloring and in wine-making [42, 43]. Maqui fruits have been of great interest to consumers for their high antioxidant activity, mainly due to phenolic compounds (**Figure 3C**) [44–46], which means this species has been considered a "superfruit" [47].

2.3. *B. microphylla* (Calafate) G. Forst

B. microphylla, commonly called Calafate, is a native Chilean plant species belonging to the Berberidaceae family (**Table 1**) [10, 15]. It is an evergreen shrub 2–3 m high with thorns and single yellow flowers (**Figure 4A**). Its fruit (barberry) is a dark blue-purple berry 7–11 mm in size (**Figure 4B** and **C**) [15, 48, 49]. Berberis genus incorporate among 20–60 species in South America, and the majority is commonly known as Michay or Calafate [50, 51]. In Chile, Calafate is distributed from Curicó to Tierra del Fuego at different altitudes and grows under a wide range of ecological conditions (**Figure 1**) [15, 48, 52]. This species is not cultivated and grows in the wild or in small gardens. Its fruits are used to prepare jam, juice, and wine [10, 15, 48, 50].

2.4. *Luma apiculata* (Arrayán) (DC) Burret

L. apiculata or Arrayán is an evergreen tree of the Valdivian Evergreen Forest (**Figure 5A**). Its bark is smooth reddish with whitish parts, reaching heights from 12 to 15 m tall with a crooked trunk around 50 cm in diameter (**Figure 5A**) [14, 40, 53]. The flowers are hermaphrodites and axillary arranged in groups of three or five, which form a berry, a small, black edible fruit

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(1.3–1.5 cm in diameter) (**Figure 5C**) [39, 51]. It is distributed in Chile from the Valparaíso to Aysén Regions (**Figure 1**) on lakeshores and riverbeds and on very moist soils. Trees of this species are conserved in various National Parks, including La Campana, Radal Siete Tazas, Laguna del Laja, Huerquehue, Puyehue, and Chiloé. Arrayán roots have medicinal uses as an anti-hemorrhagic and astringent, while the bark is used to treat herpes and ulcers, and the leaves (**Figure 5B**) to heal wounds, and treat stomach disorders [55].

2.5. *Luma chequen* (Chequén) (Mol.) A. Gray

The Chequén is an endemic tree of the Andean forests in Chile (**Figure 6A**). It is distributed from Coquimbo to Capitán Prat (**Figure 1**) [39]. It grows only in areas of high humidity, on the banks of streams, and in gulches [39]. It is a medium-sized branched shrub, which reaches 9 m in height, with grayish brown bark, and the whole plant gives off a pleasant aroma (**Figure 6A**) [39]. The leaves are oval, wide, and short (**Figure 6B**). The berry fruits are edible and dark purple 1 cm in diameter, ripening in early autumn (**Figure 6C**) [14, 39].

2.6. *Ugni molinae* (Murtilla) Turcz

Natural habitats of Murtilla are forests and coastal mountains from Valparaiso to Aysén (**Figure 1**). It is an evergreen shrub from 1 to 2 m tall (**Figure 7A**) [56, 57]. The dark green leaves are lanceolate, and the flowers are hermaphrodite (**Figure 7B**) [56]. The fruits are bright red globose berries 5–15 mm in diameter with a sweet taste and a strong aroma (**Figure 7C**) [56, 58]. Murtilla is adapted to most soils and is resistant to drought, wind, and cold, but not frost [59]. The traditional uses are for jams, juices, chocolates, and liqueur production (enmurtado or enmutillado).

3. Effect of Chilean fruits on diseases and pathophysiological disorders: beneficial effects on human health

3.1. Bioactive compounds in native Chilean fruits

Native Chilean fruits are naturally rich in phenolic compounds beneficial to human health (**Tables 2** and **3**) [8, 14, 48]. Phenolic compounds are plant secondary metabolites with bioactive properties and are generally involved in the defense against stress conditions in plants [60, 61]. They can be generally characterized by astringency, color, flavor, odor, and oxidative stability [62, 63]. In recent years, the phenolic compounds of wild or domesticated Arrayán, Chequén, Maqui, Meli, and Murtilla have been studied, with the results highlighting the high antioxidant activity of their leaves and fruits [8, 14–16, 48]. The main phenolic compounds in these fruits can be divided into phenolic acids, flavonoids, flavanols, and anthocyanins [63, 64] (**Tables 2** and **3**). Ruiz et al. [48] performed a comparison using total antioxidant activity and Trolox equivalent antioxidant capacity (TEAC) methods among edible fruits of Calafate, Maqui, and Murtilla, showing that Maqui and Calafate had a higher antioxidant activity with 88.1 and 74.5 Trolox equivalent (TE) g^{-1} of FW, respectively, followed by Murtilla with 11.7 TE g^{-1} FW. Afterward, this was confirmed by Dai and Mumper [65], who determined antioxidant activity in Maqui and Murtilla fruits with one of the most

commonly used methods, that is, the 2,2-diphenylpicrylhydrazyl (DPPH) assay. The antioxidant activity was higher in Maqui (399.8) than in Murtilla (82.9) in milligrams of crude extract per Liter^{-1} (mg of crude extract L^{-1}). This method is based on the measurement of the antioxidant compounds able to scavenge the stable free radical DPPH. It is a simple, rapid, and inexpensive method [66]. Usually, the results are expressed as milligrams of a sample that bleached 50% of the DPPH solution (IC_{50}) [67]. Therefore, low IC_{50} values show a high antioxidant activity. In this antioxidant assay, Brand-Williams et al. [68] reported that to reach IC_{50} only 0.0016 g L^{-1} of Maqui fruit is necessary; meanwhile, for "Blueberries" (*Vaccinium corymbosum*), "Strawberries" (*Fragaria ananassa*), and "Raspberries" (*Rubus idaeus*) an average of 0.03 g L^{-1} of fruits is needed. The result of IC_{50} for Maqui fruits was confirmed by Fredes et al. [69] and Céspedes et al. [70], who reported IC_{50} values of 0.0012 and 0.0019 g L^{-1} by DPPH assay, respectively. This means that Maqui fruits exhibited the highest antioxidant activity compared with other berries cultivated in Chile. Additionally, Dai and Mumper [65] compared phenolic compounds of leaves and fruits, showing that in Maqui leaves, concentrations were 200% and in Murtilla, 50% higher than in other fruits. Another commonly used method to determine total antioxidants is the oxygen-radical absorbing capacity (ORAC), which measures the antioxidant values as TE and includes both inhibition time and extent of oxidation inhibition [71, 72]. With this method, Prior et al. [73] reported that fruits of wild Calafate have 25 and 150% higher antioxidant activity than Maqui and Murtilla, respectively.

Phenolic compounds profile	**Arrayán (*L. apiculata*)**	**Calafate (*B. microphylla*)**	**Chequén (*L. chequen*)**	**Maqui (*A. chilensis*)**	**Meli (*A. meli*)**	**Murtilla (*U. molinae*)**
Caffeic acid	ND	+	ND	ND	ND	ND
Catechin	ND	ND	ND	+	ND	+
Chlorogenic acid	+	+	+	ND	+	+
Cinnamicbenzenepropenoic acid	ND	ND	ND	ND	ND	+
Coumaric acid	ND	+	ND	ND	ND	ND
Dimethoxy-quercetin	ND	ND	ND	+	ND	ND
Ellagic acid	ND	ND	ND	+	ND	+
Epigallocatechin gallate	+	ND	ND	ND	ND	ND
Ferulic acid	ND	+	ND	ND	ND	ND
Feruloyl-quinic acid	+	+	+	ND	+	+
Furosinin	ND	ND	+	ND	ND	ND
Gallic acid	ND	+	ND	+	ND	+
Hyperoside	+	+	+	ND	+	+
Isoquercitrin	+	+	+	ND	+	+
Isorhamnetin	ND	+	+	ND	ND	ND
Isorhamnetin-3-rutinoside-7-glucoside	ND	+	ND	ND	ND	ND

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Phenolic compounds profile	Arrayán (*L. apiculata*)	Calafate (*B. microphylla*)	Chequén (*L. chequen*)	Maqui (*A. chilensis*)	Meli (*A. meli*)	Murtilla (*U. molinae*)
Isorhamnetin-3-galactoside	ND	+	ND	ND	ND	ND
Isorhamnetin-3-glucoside	ND	+	ND	ND	ND	ND
Isorhamnetin-3-rutinoside	ND	+	ND	ND	ND	ND
Isorhamnetin-3-(600-acetyl)-hexoside	ND	+	ND	ND	ND	ND
Isorhamnetin-3-malonylgalactoside	ND	+	ND	ND	ND	ND
Isorhamnetin-3-malonylglucoside	ND	+	ND	ND	ND	ND
Kaempferol	ND	+	ND	ND	ND	+
Kaempferol-deoxyhexoside	ND	+	ND	ND	ND	ND
Myricetin	+	+	+	+	+	+
Myricetin-3-rutinoside-7-glucoside	ND	+	ND	ND	ND	ND
Myricetin-3-glucoside	ND	+	ND	+	ND	ND
Myricetin-3-rutinoside	ND	+	ND	ND	ND	ND
Myricetin-3-O-rhamnose	+	ND	ND	ND	ND	ND
Myricetin-3-galactoside	ND	+	ND	ND	ND	ND
Neochlorogenic acid	+	+	+	ND	+	+
Protocatechuic	ND	ND	ND	+	ND	ND
Protocatechuic 3,4-Dihydroxybenzoic	ND	ND	ND	ND	ND	+
p-coumaric acid	ND	ND	ND	+	ND	+
Quercetin	+	+	+	+	+	+
Quercetin-3-rutinoside-7-glucoside	ND	+	ND	ND	ND	ND
Quercetin-3-galactoside	ND	+	ND	+	ND	ND
Quercetin-3-rutinoside	ND	+	ND	ND	ND	ND
Quercetin-3-glucoside	ND	+	ND	+	ND	ND
Quercetin-3-malonylgalactoside	ND	+	ND	ND	ND	ND
Quercetin-3-malonylglucoside	ND	+	ND	ND	ND	ND
Quercetin-3-rhamnoside	ND	+	ND	ND	ND	ND
Quercetin-galloyl-hexoside	ND	ND	ND	+	ND	ND
Quercetin-3-(600-acetyl)-hexoside 1	ND	+	ND	ND	ND	ND
Quercetin-3-(600-acetyl)-hexoside 2	ND	+	ND	ND	ND	ND

Phenolic compounds profile	Arrayán (*L. apiculata*)	Calafate (*B. microphylla*)	Chequén (*L. chequen*)	Maqui (*A. chilensis*)	Meli (*A. meli*)	Murtilla (*U. molinae*)
Quercetin-3-rhamnoside	ND	+	ND	ND	ND	ND
Quercetin-3-O-(6″-O-galloyl)-hexose	+	ND	ND	ND	ND	ND
Quercetin-3-O-glucose (isoquercitrin)	+	ND	ND	ND	ND	ND
Rutin	+	+	+	ND	+	+
Rutin hydrate	ND	ND	ND	+	ND	ND
Vanillic 4-Hydroxy-3-methoxybenzoic acid	ND	ND	ND	ND	ND	+
Unknown quinic acid derivative	+	ND	+	ND	ND	ND
Unknown gallotannin	ND	ND	+	ND	ND	ND
References	[8, 11, 14]	[14, 15, 48]	[8, 14]	[48, 81]	[14]	[14, 74, 79]

+, presence of compounds in fruits.
ND, not detected.

Table 2. Identification of phenolic compounds in native Chilean fruits.

Anthocyanin profile	Arrayán (*L. apiculata*)	Calafate (*B. microphylla*)	Chequén (*L. chequen*)	Maqui (*A. chilensis*)	Meli (*A. meli*)	Murtilla (*U. molinae*)
Delphinidin-3-glucoside	ND	+	ND	+	ND	+
Cyanidin-3-glucoside	ND	+	ND	+	ND	+
Petunidin-3-glucoside	ND	+	ND	ND	ND	ND
Peonidin-3-glucoside	ND	+	ND	ND	ND	+
Malvidin-3-glucoside	ND	+	ND	ND	ND	ND
Delphinidin-3-rutinoside	ND	+	ND	ND	ND	ND
Cyanidin-3-rutinoside	ND	+	ND	ND	ND	ND
Petunidin-3-rutinoside	ND	+	ND	ND	ND	ND
Peonidin-3-rutinoside	ND	+	ND	ND	ND	ND
Malvidin-3-rutinoside	ND	+	ND	ND	ND	ND

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Anthocyanin profile	**Arrayán (*L. apiculata*)**	**Calafate (*B. microphylla*)**	**Chequén (*L. chequen*)**	**Maqui (*A. chilensis*)**	**Meli (*A. meli*)**	**Murtilla (*U. molinae*)**
Delphinidin-3-sambubioside	ND	ND	ND	+	ND	ND
Cyanidin-3-sambubioside	ND	ND	ND	+	ND	ND
Delphinidin-3-rutinoside-5-glucoside	ND	+	ND	ND	ND	ND
Petunidin-3-rutinoside-5-glucoside	ND	+	ND	ND	ND	ND
Malvidin-3-rutinoside-5-glucoside	ND	+	ND	ND	ND	ND
Delphinidin-3-sambubioside-5-glucoside	ND	ND	ND	+	ND	ND
Cyanidin-3-sambubioside-5-glucoside	ND	ND	ND	+	ND	ND
Delphinidin-3,5-dihexoside	ND	+	ND	ND	ND	ND
Cyanidin-3,5-dihexoside	ND	+	ND	ND	ND	ND
Petunidin-3,5-dihexoside	ND	+	ND	ND	ND	ND
Peonidin-3,5-dihexoside	ND	+	ND	ND	ND	ND
Malvidin-3,5-dihexoside	ND	+	ND	ND	ND	ND
Delphinidin-3,5-diglucoside	ND	ND	ND	+	ND	ND
Cyanidin-3,5-diglucoside	ND	ND	ND	+	ND	ND
Delphinidin-3-*O*-arabinoside	ND	+	ND	ND	ND	ND
Peonidin-3-*O*-arabinoside	ND	+	ND	ND	ND	+
Peonidin-3-*O*-di hexoside	ND	+	ND	ND	ND	+
Cyanidin-3-*O*-di-hexoside	+	ND	ND	ND	ND	ND
Cyanidin-3-*O*-galactoside	ND	ND	+	ND	+	ND

Anthocyanin profile	**Arrayán (*L. apiculata*)**	**Calafate (*B. microphylla*)**	**Chequén (*L. chequen*)**	**Maqui (*A. chilensis*)**	**Meli (*A. meli*)**	**Murtilla (*U. molinae*)**
Petunidin-3-*O*-galactoside	+	+	+	ND	+	+
Malvidin-3-*O*-galactoside	+	ND	ND	ND	ND	ND
Delphinidin-3-*O*-glucoside	ND	+	ND	ND	ND	ND
Cyanidin-3-*O*-glucoside	+	ND	+	ND	+	ND
Petunidin-3-*O*-glucoside	+	ND	+	ND	+	+
Peonidin-3-*O*-glucoside	+	+	+	ND	ND	+
Malvidin-3-*O*-glucoside	+	+	+	ND	+	ND
Cyanidin-3-*O*-rutinose	ND	ND	ND	ND	ND	+
Petunidin-3-*O*-rutinoside	ND	+	ND	ND	ND	+
Cyanidin-3-*O*-(6-succinoyl)-glucoside	ND	+	ND	ND	ND	+
Malvidin-3-*O*-(6-coumaroyl) glucoside	ND	+	ND	ND	ND	ND
Petunidin-3-*O*-(6-acetyl) glucoside	ND	+	ND	ND	ND	ND
Malvidin-3-*O*-(6-acetyl) galactoside	+	+	ND	ND	ND	ND
References	[7, 8, 10]	[7, 8, 10, 14, 48, 78]	[8, 10, 14]	[38, 46, 48, 75, 78, 80, 81]	[8, 14]	[10, 14, 48, 79]

+: presence of compounds in fruits.
ND, not detected.

Table 3. Identification of anthocyanins in native Chilean fruits.

Genotype, environmental factors, and geographical location are among the main causes for the differences in the antioxidant capacity in native Chilean fruits, since in all studies, fruits were collected in different locations. Mariangel et al. [15] analyzed Calafate fruit antioxidant capacity by DPPH method from different sites in southern Chile (Mañihuales and El Blanco; Aysén and Temuco and Lonquimay; Araucanía). They found that more southern provenances (Mañihuales and El Blanco) showed a higher antioxidant capacity—9.4 and 7.5 TE g^{-1} of DW, respectively—than northernmost provenances (Temuco and Lonquimay) with 5.2 and 3.3 TE

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g^{-1} DW, respectively. This suggests that growth conditions have a direct influence on the content of nutraceutical compounds in fruits, as total phenols in Calafate also showed the same trend: 16.1 mg gallic acid equivalent (GAE) g^{-1} DW (Lonquimay) and 34.6 mg GAE g^{-1} DW (Mañihuales). Thus, fruits from the southernmost region exhibited higher levels of total phenols.

Several types of phenolic compounds have been reported in native Chilean "superfruits," including caffeic acid, ferulic acid, gallic acid, myricetin, *p*-coumaric acid, and others (**Tables 2** and **3**) [8, 14, 15, 47, 48, 74]. In native berries, total phenolics have been analyzed by Ruiz et al. [48] using the Folin-Ciocalteu method. The results showed a higher total phenol content for Maqui (97 μmol GAE g^{-1} FW) followed by Calafate (87 μmol GAE g^{-1} FW) and Murtilla (32 μmol GAE g^{-1} FW). However, no statistically significant differences were found between Maqui and Calafate. Afterward, Brito et al. [14] found higher values for Calafate (65 mg GAE g^{-1} DW) than for Arrayán (27 mg GAE g^{-1} DW), Meli (17 mg GAE g^{-1} DW), Murtilla (9 mg GAE g^{-1} DW), and Chequén (5 mg GAE g^{-1} DW). In addition, these studies have recognized anthocyanins as the most important compounds in native Chilean fruits (**Table 3**) [10, 14, 47, 48, 75–80].

Anthocyanins in Calafate fruits from the Aysén and Magallanes Regions, analyzed by HPLC-DAD, showed total anthocyanin concentrations between 14 and 26 μmol g^{-1} FW, corresponding to the highest values in fruits from the Aysén Region [48]. Comparable anthocyanin values as in Calafate were found in Maqui (16 and 20 μmol g^{-1} FW), whereas the lowest values were found in Murtilla (mean 0.2 μmol g^{-1} FW) and in Blueberry (2.0 μmol g^{-1} FW). The lowest results in Murtilla could be explained by the weaker coloration (rose) of their fruits compared with the black and blue-purple color of the other analyzed fruits [48]. In this context, Mariangel et al. [15] reported differences among Calafate fruits collected in different sites of the Araucanía (Temuco and Lonquimay) and Aysén Regions (Mañihuales and El Blanco). Higher values of cyanidin were found in El Blanco (0.6 mg g^{-1} DW), followed by Temuco (0.2 mg g^{-1} DW), Mañihuales (0.1 mg g^{-1} DW), and Lonquimay (0.06 mg g^{-1} DW). These results suggest that anthocyanin concentrations vary depending on the different agro-characteristics of the growth areas and the fruit-ripening time. Brito et al. [14] reported anthocyanin contents (in mg cyaniding 3-O-glucoside g^{-1} of DW) with higher values in Calafate fruits (51.6) than in Arrayán (15.2), Meli (13), Murtilla (6.85), and finally Chequén (1). Therefore, it is reported that there is higher anthocyanin content in fruits of Calafate and Maqui compared with other native berries [7, 14, 48, 81].

Due to the difficulty of having fresh fruits rich in antioxidants for consumption out of season, it is very important to know the effect of fruit preservation techniques on the content of bioactive compounds. Among the common techniques in use (convective hot-air, freeze drying, and direct cold), native Chilean fruits have demonstrated minor variation in the concentration of phenolic compounds and antioxidant activity compared with the fresh fruits [46, 82]. The effect of freeze-drying and direct cold on the content of bioactive compounds in native Chilean fruits are the least studied of these techniques, and therefore, they should be explored because cold storage in the postharvest period of these berries may produce fewer changes in the antioxidant levels.

3.2. Oxidative stress and antioxidant response in human pathophysiological disorders

Under normal conditions, the human body produces free radicals and other reactive oxygen species (ROS) [29, 83]. ROS are molecules characterized by an unpaired electron in an atomic orbital, being highly unstable, such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical [83]. In the human body, the ROS molecules are produced in the mitochondria, peroxisomes, in inflammation, phagocytosis processes, and ischemia. They are exacerbated by exposure to stress conditions such as ozone, cigarette smoking, air pollutants, and industrial chemicals, among others [84–86]. The ROS have a high affinity for organic molecules (proteins, carbohydrates, and lipids), causing oxidative stress, damage in biomembranes, and altering body homeostasis [87]. The body counteracts the damage induced by ROS by activating antioxidant systems, which are stable molecules able to donate an electron to the free radical, neutralizing it and reducing ROS damage [88, 89]. The human metabolism produces enzymatic antioxidants such as superoxide dismutase, catalase, xanthine oxidase, lipogenase, and cyclooxygenase, and nonenzymatic compounds such as glutathione, ubiquinol, and uric acid [90]. However, it is also necessary to supply other antioxidants in the diet to strengthen the antioxidant capacity. Among them, vitamin E (α-tocopherol), vitamin C (ascorbic acid), and B-carotene are important. These antioxidants reduce the process of lipid peroxidation, preventing or decreasing oxidative reactions and cell damage [91, 92]. In tissues injured by infection, heat, hypertoxia trauma, and toxin-enhanced oxidative stress, processes are induced in the short-term [36]. To counteract the oxidative damage in tissues, transport of antioxidant enzymes (e.g., xanthine oxidase, lipogenase, and cyclooxygenase) and activation of phagocytes related to the release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation take place [36]. However, when the conditions of oxidative damage are higher than antioxidant defense responses, a critical imbalance between free radical generation and antioxidant defenses occurs. This imbalance in the human body induces complications such as diabetes mellitus, onset, promotion or progression of cancer, and neurodegenerative damage such as Parkinson's disease, among others [93–95]. Epidemiological evidence suggests that diets high in antioxidants reduce the incidence of heart disease, cancer, and neurological disorders, altering the inflammatory process common in these diseases [96–100]. Despite the wealth of information that relates to the consumption of fruits in general with the prevention of diseases linked to oxidative stress, studies into native Chilean fruits are limited.

3.3. Native Chilean fruits as a source of antioxidants

Antioxidant compounds in Chilean berries, such as phenolic acids, flavonoids, flavanols, anthocyanins, and procyanidins, among others, have been widely studied for their highly protective effect on human health, particularly with respect to age-related diseases and pathophysiological disorders related to oxidative stresses [6, 11, 37, 69, 82, 86, 101–104]. Antioxidants have an important role as anti-inflammatory or cancer chemopreventive compounds and against degenerative disorders, decreasing the risk of oxidative stress [6, 99, 105]. Furthermore, antho-

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cyanins in berry fruits have a positive effect on human health, related to their capacity to act as antioxidants, and the protective effect in chronic diseases such as diabetes, cardiovascular diseases, and different type of cancers [6, 10, 14, 64, 106, 107]. Regarding the health beneficial properties of fruits, it is important to study different aspects: the species, the composition of bioactive compounds, their antioxidant capacity, and the capacity of different compounds to modulate the transcription factors of enzymes that induce inflammatory diseases [108, 109]. In this context, the traditional use of the plants is also important. Folk medicine in Chile has used leaves and fruits of Arrayán, Calafate, Chequén, Maqui, Meli, and Murtilla to treat throat pain, ulcers, inflammation, and kidney pain disorders [5, 41, 43]. Interestingly, the richness, abundance, and diversity of bioactive compounds in berries of the Chilean native species have shown effects against some pathophysiological disorders (inflammation, diabetes, and cardiovascular) as reported by Fredes et al. [69], Reyes-Farias et al. [6], Alonso [37], Wellen and Hotamisligil [101], Glass and Witztum [104], Lipfert et al. [86], Fuentes et al. [11], and Genskowsky et al. [82].

Inflammation is a defensive mechanism of the organism to specific noxa (factor producing damage), involving different cellular or humoral agents. Its purpose is to restore the body homeostasis, eliminating the noxae. When the inflammatory process becomes self-perpetuating, it ceases to be beneficial and becomes harmful [110–113]. Cell membrane damage activates the phospholipase A2, favoring the synthesis of arachidonic acid, which serves as a substrate for the formation of lipoxygenase and promotes the oxygenase cycle and leukotriene synthesis, which in turn induce the synthesis of prostaglandins and thromboxanes [114]. This promotes neutrophil chemotaxis, ultimately phagocytizing the damaged cell [115]. In addition, leukotriene favors vascular permeability, facilitating the influx of neutrophils to the injured tissue. In general, berries are important inhibitors of inflammatory processes due to the phenolic compounds like anthocyanins present in their fruits [116–121]. This has been supported by reports about moderate consumption of raspberry, strawberry, and bilberry (*Vaccinium myrtillus*) juices and green and black tea that can help prevent the development of early atherosclerosis [118]. Consumption of lingonberry (*Vaccinium vitis-idaea*) juice for 10 weeks has an anti-inflammatory effect on salt-induced hypertension in rat models, probably due to high polyphenol concentrations in the juice [119]. In wild blueberries, the fruits have provided *in vivo* evidence of the improvement or the prevention of metabolic disturbances associated with developing obesity, particularly a systemic low-grade inflammation and hypertension in mice [120]. In the case of strawberry, its tannins (as enriched extract or as pure compounds) are able to act on gastric epithelial cells, thereby inhibiting the inflammatory response [121]. More detailed information about the berries and their anti-inflammatory properties has been reported by Yang and Kortesniemi [122] and Joseph et al. [123]. Studies with native berries such as Maqui and Calafate fruits have given evidence that the compounds of these fruits can be considered a good anti-inflammatory agent [116, 124]. The anti-inflammatory activity has been shown *in viv*o in the ears of mice and *in vitro* using macrophages and guinea pigs [125–127]. Rouanet et al. [118] also showed that leaf extracts of these species have anti-inflammatory activity in mice, showing that leaf extracts containing quercetin and kaempferol can reduce inflammation.

Diabetes has been recognized as one of the most important chronic diseases in the world [128]. According to the International Diabetes Federation (IDF), 400 million people worldwide had diabetes in 2013, and it could reach 642 million by 2040 [128]. In 2013 alone, US$548 billion was spent on diabetes management [128]. Therefore, it is a great challenge to find alternatives to reduce this global epidemic. Type II diabetes comprises disease groups of diverse etiology characterized by the presence of chronic hyperglycemia, altering the secretion and action of insulin, as well as alterations in the metabolism of carbohydrates, proteins, and lipids [128, 129]. Type II diabetes represents over 90% of diabetes cases, and its etiology involves both genetic and environmental factors [130]. In the last few years, a gradual increase in incidences has been reported, inducing metabolic alterations and cardiovascular complications [129–131]. Early treatment with dietary and/or pharmacological hygienic measures in patients with prediabetic states can reduce the incidence of diabetes [132]. However, clearly there is a genetic predisposition that is favored by some factors such as obesity or a sedentary lifestyle [133–135]. In this sense, the adipose tissue sets free inflammatory mediators such as interleukins, tumor necrosis factor (TNF-alpha), or free fatty acids, which increase insulin resistance and oxidative stress [130, 136, 137]. Most of the reports about this disease comprise the effects of commercial berries [138]. Thus, preclinical and clinical studies have suggested that consumption of commercial berries has health benefits with preventive effects on diabetes, improving insulin resistance [122, 139–142]. Studies in obesity-prone rats with a diet containing 2% (wt/wt) freeze-dried powder of highbush blueberry reduced the phenotypes of metabolic syndrome, affecting the gene transcripts of the peroxisome proliferator-activated receptors in adipose and muscle tissues involved in fat and glucose metabolism [143]. This may be due to fibers and/or polyphenols present in the berry fruits. Powder from lingonberries did not change the insulin curve in humans, when consumed together with added glucose [139]. Mursu et al. [140] reported that the intake of berries in the diet may reduce risk of type 2 diabetes in Finnish men. In addition, consumption of blackcurrant (*Ribes nigrum*) extract in amounts roughly equivalent to 100 g in fruit drinks with low sugar and administered immediately before a high-carbohydrate meal reduced postprandial glycemia, insulinemia, and incretin secretion, being beneficial to human health [141]. Further information about the link between berries and their anti-diabetic properties is available in Yang and Kortesniemi [122] and Tsuda [142]. Nevertheless, there are more limited reports in native Chilean fruits in the form of *in vitro* and *in vivo* studies. In this context, Dai and Mumper [65] reported that in *in vitro* studies, Maqui fruits have hypoglycemic effects that inhibit α-amylases and α-glucosidases, enzymes involved in the carbohydrate metabolism. In line with this evidence, the inhibition of both enzymes by Maqui fruits was also confirmed by Schreckinger et al. [144]. Using *in vivo* assays, Fredes et al. [78] reported the anti-diabetic properties of Maqui fruits in mice. They showed that oral administration of a standardized anthocyanin-rich formulation from Maqui fruits decreased blood glucose in obese hyperglycemic mice. It has been suggested that Maqui fruits could act by inhibiting sodium glucose cotransporter in the small intestine [145]. However, more studies are needed to confirm this physiological mechanism involved in reducing blood glucose. The results of *in vitro* studies of the biological effects of phenolic compounds are questioned due to the limited bioavailability and absorption of these compounds in the human body [146].

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Cardiovascular diseases are a heterogeneous group of pathologies, the common substrate of which is the alteration of different arteries of the body regardless of caliber of arteries [147, 148]. In particular, these supply the brain, heart, lower limbs, and aorta [149]. The cascade of events for atherosclerosis are proliferation of smooth muscle cells, recruitment of inflammatory cells, and lipid deposits within the blood vessel walls, forming plaques of atheroma [149, 150]. This formation prevents normal tissue irrigation, inducing ischemia reduction or loss of blood flow in a tissue [150]. If the atheromatous plaque ruptures, the body in an attempt to repair activated coagulation cascade induces the formation of a platelet plug, which totally or partially obstructs the flow distally [151]. Therefore, the tissues supplied by the artery suffer hypoxia or anoxia due to the induction of tissue necrosis [149, 152, 153]. Plant antioxidants have been shown to reduce cellular oxidative damage and to protect against cardiovascular diseases [154]. Berry consumption has been known to benefit human health with preventive effects on cardiovascular diseases. Thus, Erlund et al. [155] reported that the consumption of moderate amounts of berries such as bilberries, nectar of lingonberries, blackcurrant, strawberry puree, cold-pressed chokeberry (*Aronia melanocarpa*), and raspberry juice resulted in favorable changes in platelet function, high density lipoprotein (HDL) cholesterol, and blood pressure in male and female volunteers, concluding that the berries may play a role in the prevention of cardiovascular disease. Afterward, Basu et al. [156] deepened the evidence about berry-rich diet that controls the risk of chronic diseases among them the cardiovascular risk. Interestingly, Oudot et al. [157] reported that a high salt diet (8% NaCl) with 2 g/day berries can prevent the cardiac alterations independently of changes in systolic pressure in rats. This was verified by Yang and Kortesniemi [122] and Huang et al. [158], who highlighted that berries are an essential fruit group in heart-healthy diets as a supplementary option to better prevent and control cardiovascular disease in humans. More details are available in Zhu et al. [159] and Rodriguez-Mateos et al. [160]. In the case of native Chilean fruits, the preclinical and clinical studies are more limited, although a recent interest in the study of these berries as a beneficial food to protect the heart is growing. Fredes et al. [69] found that Maqui fruits can significantly reduce the cardiac injury produced by ischemia-reperfusion (I/R) in rat heart *in vivo*. The I/R injury occurs after a myocardial ischemia, and it is known to generate free radicals, heart injury, and necrosis [69, 161, 162]. Therefore, the high level of phenolic compounds and antioxidant activity of this endemic berry can scavenge free radicals produced by I/R and protect the heart [69]. Similarly, concentrated Maqui juice has a high capacity for the oxidation of low-density lipoproteins, which is considered one of the first steps in the development of atherosclerosis [163]. In this sense, Fuentes et al. [11] suggested that Arrayán fruit extracts could protect endothelium-dependent vasodilation (measure to probe endothelial function in different pathophysiological disorders), which is impaired by high glucose [11]. Consequently, they consider that the extract may have an important use in the prevention of vascular damage induced by high glucose. In addition, Falkenberg et al. [164] showed an inhibition of "platelet aggregation" (induced by adenosine diphosphate and collagen) in sheep and human blood through the application of Arrayán and Chequén extracts, which was confirmed by the inhibition of platelet surface activation markers. Afterward, research showed that Murtilla fruits demonstrated vasodilator activity in the aortic rings [165]. Another important damage parameter used as a marker for the risk of developing heart disease is the

low-density lipoproteins (LDL). Maqui extracts may reduce oxidative modifications of LDL in overweight people and smokers [124].

Finally, all the properties mentioned of the native Chilean fruits place them in the category of "superfruits" due to their excellent biological effects on human health. Thus, these "superfruits" could be used as nutraceuticals and functional foods with potential use in the human health industry.

4. Conclusion

We conclude that Arrayán, Chequén, Maqui, Meli, and Murtilla fruits are rich in antioxidant compounds with high antioxidant power, such as anthocyanins. The results show that the consumption of native Chilean fruits reduces the risk of pathophysiological disorders such as inflammation, diabetes, and cardiovascular disease due to their bioactive compounds. Therefore, these berries have a potential for increased use in the functional food and nutraceutical industries.

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Chapter 6

Chilean Endemic/Native Plant Resources as Functional and Superfoods

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Additional information is available at the end of the chapter

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Abstract

The current consumer demand for foods or food supplements with "super properties" is being covered by previously under-exploited ethnic products. The endemic flora from multiple continents serve as source of plant foods such as cereals or tropical fruits. Chile, one of the top five plant biodiversity hotspots on the planet, is a promising source of functional foods with little scientific and commercial research. The aim of this chapter is to summarize the findings related to the antioxidant and antibacterial potential of native/endemic plants and plant-derived compounds from Chile. Resources of these compounds may be found in honey, bee pollen, and berry-like fruits. These products, unknown to many parts outside the country, not only have the advantage of their functional properties but also possess denomination of origin, which gives added value and allows them to be used as food additives such as natural colorants, antioxidants, antibacterials, and antifungals. In the coming years, many of these products will be more commercially known and many of these plant species will be selected and improved, as have happened with products such as tofu or blueberries.

Keywords: biodiversity, endemism, berries, honey, bee pollen, antioxidant, antibacterial

1. Introduction

The latest food consumer trends point beyond fulfilling the function of providing nutrients to the body. It is intended that foods provide compounds capable of reducing the likelihood of developing diseases, improving or complementing the functions of the body, and even increasing life expectancy. A search for new food sources of these "healthy compounds" is

underway to meet the needs of today's consumers. New analytical methods are being used with known foods to demonstrate properties they always had, but properties that had not been properly tested because of technological limitations; new foods or derived food compounds are also being found.

The diversity of food we know is derived from the biodiversity of plant and animal species we know. However, there are still many plant species which have not been explored or whose potential is just beginning to come to light. Many of these plants have been used by aboriginal groups around the world since ancient times. These species, which only grow in specific geographic locations (endemism), are rarely objects of scientific study or for industrial or commercial scaling.

Chile is one of the top five hotspots of plant biodiversity on the planet; here it is possible to find new food and food-derived resources of interesting compounds in the poorly explored flora. In addition, the biodiversity of plant species found in Chile have a high degree of endemism, indicating that they do not grow elsewhere. Leaves, stems, roots, or fruits can be sources of antioxidants and/or antibacterial compounds. Among the plant species with potential are the non-fruiting tree specimens such as quillay and ulmo; within fruit tree species, we may find maqui, murta, calafate, and others that are less known. All these products have high contents of polyphenolics, which have high antioxidant and antibacterial properties.

Polyphenolics are secondary metabolites from plants that have been associated with several healthy benefits such as the prevention of cancer, cardiovascular, inflammatory, and neurodegenerative diseases [1–3]; they are also associated with bioactive properties such as antioxidant and antibacterial properties [4–7].

Each phenolic/flavonoid compound has different antioxidant/antibacterial potency depending on its action mechanism. Phenolic compounds alter the permeability of bacterial cell membranes, which may result in the uncoupling of oxidative phosphorylation, the inhibition of active transport, and the loss of pool metabolites due to cytoplasmic membrane damage [8, 9]. Other authors explain the antibacterial activity of phenolics by the presence of more number of hydroxyl groups that may form hydrogen bonds with enzymes, altering their metabolism and also the lipid solubility and the degree of steric hindrance [10, 11]. In the case of flavonoids, antibacterial activity has been associated with its capacity to form complex bonds with proteins through non-specific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, it may inactivate microbial adhesins, enzymes, and cell envelope transport proteins. Lipophilic flavonoids may also disrupt microbial membranes [12, 13].

2. Effect of endemic/native Chilean plants on the functional activity of honeybee products

Honey has been recognized for many centuries as a healthy food, because of its positive effects such as healing [14], anti-inflammatory [15], antibacterial [16–20], and antioxidant [20–24]

properties; and prebiotic capacity [24–28]. Meanwhile, the pollen has also been recognized by health claims. Scientific studies have been shown that bee pollen acts as an anti-anemic, tonic and restorative, hormonal and intestinal regulator, vasoprotector, hepatoprotective and detoxifying agent, and antioxidant and antibacterial [29, 30]. All these properties vary with the botanical and geographical origin (**Table 1**).

Honey	Phenolic compound	References
Heather	Benzoic acid, phenyl acetic acid	[31, 32]
Heather	Mandelic acid, B-phenyllactic acid	[32]
Honeydew	Protocatechuic acid	[32]
Rape	Hydrocinnamic acid	[32]
Buckwheet	4-hydroxybenzoic acid	[32]
Honeydew	Protocatechuic acid	[33]
Chestnut	Ferulic acid, p-coumaric acid	[33]
Chestnut	4-hydroxibenzoic acid, 4-hydroxyphenyllactic acid, phenylacetic acid	[34]
Heather	B-phenyllactic acid, benzoic acid, phenyl acetic acid	[34]
Sunflower	p-coumaric acid, phenyllactic acid, caffeic acid	[34]
Lime	3-hydroxybenzoic acid	[34]
Lavander	Caffeic acid, gallic acid	[34]
Strawberry	Homogentisic acid	[35]
Heather	Ellagic acid,abscisic acid	[36, 37]
Eucaliptus	Abscisic acid, ellagic acid	[38]
Citrus	Hesperetin	[39]
Rosemary	Kaempferol	[40]
Sunflower	Quercentin	[36, 37]
Eucaliptus	Myricetin, tricetin, luteolin, quercentin	[38, 41]
Manuka	Methylglyoxal	[42]
Heather	p-hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic. p-coumaric, ferulic, m-coumaric, o-coumaric, ellagic, cinnamic acids	[43]
Lavander	Gallic, vanillic, chlorogenic, p-coumaric, ferulic, m-coumaric, cinnamic acids	[43]
Black locust	p-hydroxybenzoic, vanillic, p-coumaric, ferulic, trans-cinnamic acids. Vanillin, pinobanksin, apigenin, kaempherol, pinocembrin, crysina, acacetin	[44]

Honey	Phenolic compound	References
Acacia	Abscisic acid, p-hydroxybenzoic, vanillic, p-coumaric, Ferulic, trans-cinnamic acids. Vanillin, pinobanksin, apigenin, kaempherol, pinocembrin, crysina, acacetin	[44]
Rosemary	Pinobanksin, quercetin, luteolin, 8-methoxykaempferol, kaempferol, apigenin, isohamnetin. quercetin 3,3′-dimethyl ether, pinocembrin, quercetin 7,3′-dimethyl ether, quercetin 3,7-dimethylether, chrysin, galangin, tectochrysin	[40]
Eucalyptus	Quercentin, luteolin, myricetin	[45]
Lotus	Quercentin, luteolin, myricetin	[45]
Buckwheat	3-hydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, ferrulic acid, p-coumaric acid, rosmarinic acid, ellagic acid, myricetin, quercetin, kaempferol, chrysin, galangin	[45]
Sage	Myricetin, quercetin, luteolin, kaempferol, apigenin, isorhamnetin, chrysin, galangin, abscisic acid, caffeic acid, p-coumaric acid	[46]
Robinia	Myricetin, quercetin, luteolin, kaempferol, apigenin, chrysin, galangin	[47]
Eucalyptus	Myricetin, tricetin, quercertin, luteolin, quercertin-3-methyl ether, kaempferol, pinobanksin, chrysin, pinocembrin	[41]
Quillay	Chlorogenic, caffeic, coumaric, syringic, p-coumaric, vanillic and salicylic acids. Naringenin, quercetin, kaempferol	[48]
Ulmo	p-coumaric, ferulic, chlorogenic, caffeic, sinapic, syringic and salicylic acid Kaempferol luteolin	[49]

Table 1. Different polyphenolic compounds found in honeys with several botanical origins.

2.1. Honeys

Chilean honey has shown biological activity against bacteria and fungi. *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Vibrio cholerae* have been inhibited by hydroalcoholic extracts derived from honey [49, 50]. Meanwhile, *Candida albicans* has also shown sensitivity to Chilean honey. Chilean honey even has higher antimicrobial activity than Manuka honey, which has a standard antioxidant and antimicrobial activity potential [51]. The antimicrobial activity of honey is probably the result of the total number of active compounds and not the presence of any one of them (i.e., phenolics and flavonoids). This activity may be the result of synergism between flavonoids and phenolic compounds or between phenolic compounds and terpenes. Some phenolic compounds and flavonoids are present only in certain unifloral honeys. These results have allowed for the identification and certification of these honeys. References [48, 52, 53] identified chlorogenic, caffeic, coumaric, syringic, p-coumaric, vanillic and salicylic acids, naringenin, quercetin and kaempferol in the unifloral honey of Quillay (*Quillaja saponaria*). In the same report, [52] found p-coumaric, ferulic, and salicylic acids in the endemic unifloral honey of Ulmo (*Eucryphia cordifolia*). Pinobanksin and kaempferol are typically identified in Chilean honeys.

Other more recent Chilean honeys currently being studied are Avellano honey (*Gevuina avellana* Molina), Tiaca honey (*Caldcluvia paniculata* (Cav.) D. Don), and Corontillo honey (*Escallonia pulverulenta*), which have shown antibacterial and antioxidant properties [50].

2.2. Bee pollen

Bee pollen provides important ingredients to the human diet, such as carbohydrates, protein, fat, and other components in lesser amount such as minerals. Carbohydrates are mainly polysaccharides such as starch and sugars and represent between 13 and 55 g per 100 g of sample. With regard to protein content, bee pollen provides all essential amino acids to the human diet and their percentages vary between 10 and 40% of the test sample [55–63]. Referring to fats, a study reveals that 3% of the total lipids are free fatty acids and about half of them are omega-3 unsaturated oleic, linoleic (omega-6), and linolenic acids (omega-3) [55]. With reference to the mineral content, bee pollen contains potassium, phosphorus, calcium, magnesium, iron, copper, zinc, and selenium in amounts that satisfy the daily recommended intake per person [64].

Bee pollen classification	Total phenolics	FRAP	DPPH	β-Carotene	Licopene	Total flavonoids	References
Pollen multiflora	–	–	–	17.05–489.20 μg/g	–	–	[63]
Pollen multiflora	–	–	–	1–20 mg/100 g	–	1293–8243 mg/100 g	[23]
Pollen multiflora	–	–	–	–	–	530–3258 mg/100 g	[71]
Pollen multiflora	–	0.25–5.35 mM Fe^{2+}/g	0.27–2.8 mmol Trolox/g	–	–	–	[55]
Pollen multiflora	4.4–16.4 mg GAE/g	0.255–5.355 mM Fe^{2+}/g	0.274–2.814 mmol Trolox/g	–	–	2.8–13.6 mg eq quercetin/g	[74]
Pollen multiflora	817,33-138367 mg tannin/kg	-	47,97-86,25 % of inhibition	-	-	-	[75]
Quillaja saponaria	18.15 mg GAE/g	34.48 mM Fe^{2+}/g	2.97 mg ascorbic acid/g	0 μg/g	0 μg/g	–	[54, 76]
Azara petiolaris	16.43 mg GAE/g	14.58 mM Fe^{2+}/g	2.86 mg ascorbic acid/g	13.60 μg/g	60.40 μg/g	–	
Puya chilensis	11.83 mg GAE/g	28.24 mM Fe^{2+}/g	2.87 mg ascorbic acid/g	4.60 μg/g	14.70 μg/g	–	
Cryptocarya alba	11.74 mg GAE/g	28.32 mM Fe^{2+}/g	3.06 mg ascorbic acid/g	0 μg/g	0 μg/g	–	
Colliguaja odorifera	11.50 mg GAE/g	46.39 mM Fe^{2+}/g	2.87 mg ascorbic acid/g	7.10 μg/g	7.30 μg/g	–	
Schinus polygamus	7.12 mg GAE/g	24.85 mM Fe^{2+}/g	2.93 mg ascorbic acid/g	25.40 μg/g	28.80 μg/g	–	

Table 2. Main antioxidant parameters and pigments presented in bee pollen from different resources.

Several reports demonstrate the health benefits of bee pollen. Scientific studies have shown that bee pollen acts as an anti-anemic, tonic and restorative, hormone regulator, intestinal regulator, vasoprotector, and hepatoprotective, detoxifying, and antioxidant agent [28, 29, 65]. However, very few studies have identified the phenolic compounds of Chilean bee pollen. The information on bee pollen production for food applications and some reports concerning their antimicrobial and antioxidant activity [54, 66, 67].

Phenolic acids, flavonoids, and pigments such as β-carotene are mainly responsible for the healthy properties such as antioxidant and antibacterial properties exhibited by bee pollen [68–70]. The phenolic acids and flavonoid glycosides are present in the nectar of flowers visited by bees, which are hydrolyzed and transferred to bee pollen. The number and variety of phenolic acids and flavonoids are highly variable, since beekeepers mix bee pollen with different botanical origins from different plant species [22, 71]. A major flavonoid found in bee pollen is rutin [72]. The main group of pigments that compose bee pollen are carotenoids, especially β-carotene, whose concentration also depends on the botanical origin of the sample [63]. The β-carotene content is about 17% of total carotenoids. In some cases, it may contain 20 times less carotenoids that some foods [73]. In Chilean bee pollen, the carotenoid content varies with the botanical origin (**Table 2**).

The type and concentration of the polyphenolic compound influence the antibacterial and antioxidant activity exhibited by bee pollen. The most important polyphenolic compounds related to these activities are vanillic acid, protocatechuic acid, gallic acid, p-coumaric acid, hesperidin, rutin, kaempferol, apigenin, luteolin, quercetin, and isorhamnetin [70]. Bee pollen rich in these compounds has shown activity against specific pathogens such as *S. aureus*, which causes skin infections; *E. coli*, which causes diarrhea [67, 77], *Streptococcus pyogenes*, which causes acute bacterial pharyngitis [78], *P. aeruginosa*, which produces tissue damage and affects the immune system [79] and *S. pyogenes*, which causes skin wounds [16]. Another important study demonstrated the inhibition activity against *Salmonella* spp., as shown **Figure 1** [66].

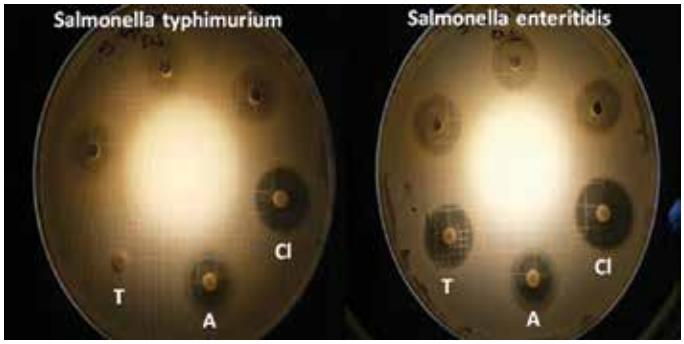

Figure 1. Antibacterial activity of Chilean multiflora bee pollen hydrophilic extracts evaluated by inhibition zone diameter against *Salmonella typhimurium* and *Salmonella enteriditis*. Tetracycline (T), ampicillin (A) and chloramphenicol (Cl) were used as controls.

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3. Endemic/native berries

Chile is the main exporter of berries in the Southern Hemisphere and the fifth berry exporter worldwide because of its comparative advantages: geographic isolation of the country (desert in the north, the Pacific Ocean, the Andes mountains, and the Patagonian ice), which makes it an island from the health point of view, decreasing the incidence of pests and diseases; the Mediterranean climate is beneficial to obtain optimal raw material and production and in a counter-season and phased production [80, 81]. Maqui, murta, and others recently explored are included in the list of actual and future production (**Figures 2** and **3**).

Figure 2. *Luma apiculata* or "arrayán" fruits. These berry-like fruits have higher antioxidant activity than blueberries. Many unknown Chilean endemic/native fruits are potential functional foods.

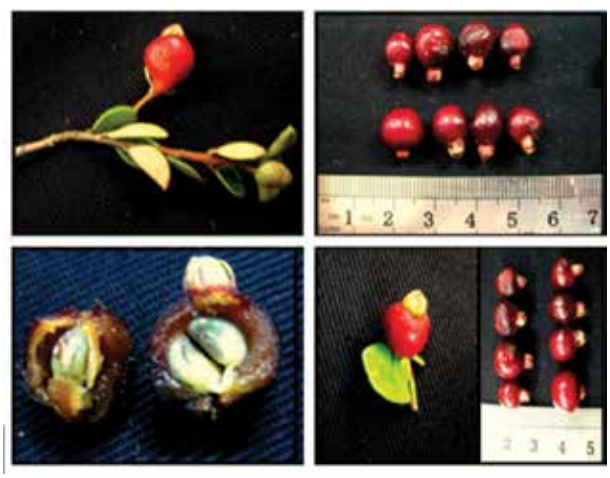

Figure 3. *Myrceugenia obtusa* or "Rarán" fruits. These berrys have antioxidant and antibacterial activities (Orellana et al., 2017).

3.1. "Maqui" (*Aristotelia chilensis*)

Maqui is a berry with antioxidant and antihemolytic properties [82, 83], and it limits adipogenesis and inflammatory pathways in vitro [84, 85], protects against oxidative stress by reducing lipid peroxidation [86], inhibits LDL oxidation in vitro and protects human endothelial cells against oxidative stress [87] and has cardioprotective [88] and gastroprotective properties [89]. These healthy effects are produced by anthocyanins and many other bioactive compounds such as flavonoids, coumarins, phenolic acid (i.e. gallic, gentisic, sinapic, hydroxybenzoic, vanillic acids, makonine, 8-oxo-9 dehydrohobartine and 8-oxo-9 dehydromakomakine [90–93] present in the fruits. Recently, Maqui has been used to design new functional foods such as drinks and cakes with antioxidant properties for in vivo and clinical trials [94–96].

3.2. "Murta" or "murtilla" (*Ugni molinae*)

Murta fruits are berries which have a rich chemical composition of bioactive compounds associated with health properties [97]. They have shown analgesic *in vitro* activity [98], protective capacity against oxidative damage of human erythrocytes [99], antimicrobial activity [100], antioxidant activity [101, 102], and α-glucosidase/α-amylase inhibition [102] as the main beneficial effects.

3.3. Other berries and berry-like fruits

"Calafate" (taxonomically described as *Berberis buxifolia* and also *Berberis microphylla*) fruits are berries that are scarcely studied. However, the available information is very interesting and indicates its potential as an antioxidant, which may be related to its high anthocyanin and hydroxycinnamic acid levels [103, 104]. Most recently, exploratory studies have revealed new native/endemic berry-like fruits such as *Luma apiculata*, *Ribes punctatum*, *Ribes magellanicum*, *Ribes cucullatum* and *Ribes tribolum* [105, 106]. *Ribes* spp., *Rubus* spp., *Gaultheria* spp., and *Berberis* spp., among others, as promising crops of functional foods or food additives/supplements such as natural colorants (**Table 3**). Some other non-scientific studies have been related with functional properties of several non-fruiting plants with anticoagulant, antithrombin, and analgesic properties and related health effects [107].

Common name	Scientific name	Attributed properties	Description	References
Chaura	*Gaultheria pumila*	Antioxidant (antocyanin content)	The fruit is a berry, white or pink. ovoid shaped, 6 mm to 12 mm in diameter	[108]
Chaura	*Gaultheria mucronata*	Antioxidant	The fruit is a berry, between 6 and 9 mm in diameter, plum-shaped, passing from white to pink and finally to dark purple when ripe	[104, 109]
Chaura	*Gaultheria antarctica*	Antioxidant	The fruit is a berry, white or pink, ovoid shaped, 6 mm to 10 mm in diameter	[110]

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Common name	Scientific name	Attributed properties	Description	References
Uva de cordillera, calafatillo	*Berberis empetrifolia*	Antioxidant	The fruit is a globose, blue-black,about 7 mm in diameter	[109, 110]
Calafate, chelia	*Berberis ilicifolia*	Antioxidant	Fruits are blue-black berries about 1 cm long, with four to six seeds, 5–6 mm in diameter	[110]
Calafate	*Berberis microphylla*	Antioxidant, antibacterial	The fruit is a spherical blue-black berry, about 1 cm. in diameter, and contains six angular seeds	[110–113]
Calafate	*Berberis buxifolia*	Antioxidant (anthocyanin content)	The fruit is a globose, blue-black, about 7–10 mm in diameter	[109, 114, 115]
Michay, mechay	*Berberis darwinii*	In vitro evidence for Alzheimer's disease therapy	The fruit is a globose, blue-black, about 7–10 mm in diameter	[116]
Copihue, Chilean bell national flower	*Lapageria rosae*	Antioxidant	The fruits are red berries, ovoid, between 3 and 6 cm long, with a thick skin containing numerous seeds	[117, 118]
Chilco, Chilca, Palo blanco	*Fuchsia magellanica*	Hypotensive and diuretic effect, antioxidant activity, significant inhibitory activity against B-glucuronidase enzyme	Fruit is a black berry, about 8–10 mm diameter	[104, 119–122]
Peumo	*Cryptocarya alba*	Significant inhibitory activity against B-glucuronidase enzyme, free radical scavenging activity, antibacterial activity	Red fruit with one large seed	[119, 123–125]
Daudapo, Huarapo, Zarapito, Té de la turba, naurapo, mirteola	*Myrteola nummularia*	Antioxidant (higher antioxidant content than blueberries), it may reduce colon cancer risk, source of natural colorant as anthocyanin	The fruit is up to 1 cm in diameter, it has a soft juicy flesh and a delicious slightly aromatic flavor	[104, 109, 126–128]
Copihuelo, Copihue chilote, Copihuelo, Coicopiu,	*Philesia buxifolia, Philesia magellanica*	Antioxidant	The fruit is a yellowish green ovoid berry, size up to 13 mm long	[121, 129]
Queule, keule,	*Gomortega nítida, Gomortega keule*	Antioxidant	The fruit is a drupe, yellow, about 34–45 mm (1.3–1.8 in) in diameter, usually with 1–2 seeds	[130]
Cauchao (from Luma or red	*Amomyrtus luma*	Antioxidant, inhibit	The fruit is a black to purplish-black berry when ripe, with about 1–1.5 cm in	[121, 131, 132]

Common name	Scientific name	Attributed properties	Description	References
luma tree)		platelet aggregation (anticoagulant effect), antibacterial	diameter, generally with 3 seeds, about 3–4.5 mm	
Cauchao (from Meli or White Luma tree)	*Amomyrtus meli*	Antioxidant, antibacterial	The fruit is a black or purplish black Berry, 5–8 mm in diameter, generally with 3 seeds, about 3–4.5 mm	[112, 113, 132]
Chequén, Arrayán blanco,	*Luma chequen*	Antioxidant	The berry-like fruit (drupe) is a dark purple, about 1 cm in diameter	[112, 113, 133]
Arrayán	*Luma apiculata*	Antioxidant, antibacterial, inhibit platelet aggregation (anticoagulant effect)	Berry rounded black fruit, about 1.3–1.5 cm. diameter, containing three seeds	[105, 112, 125, 131, 133]
Chilean strawberry, wild strawberry	*Fragaria chiloensis*	Antioxidant, free radical scavenging activity, anticancer cell Proliferation properties (human lung epithelial cancer cells	The fruit is whitish or pale pink	[134–139]
Chañar, chañal	*Geoffroea decorticans*	Antioxidant, antinoceptive, anti-inflammatory activities; antitussive and expectorant significant effect, antibacterial	The berry-like fruit is a drupe, ovoid, red-brown when ripe, about 1.7–3.5 cm to 1.5 cm. The pulp is white-yellowish and has 1 or 2 seeds	[140–142]
Maqui	*Aristotelia chilensis*	Inhibidor de la enzima xantina oxidasa (sintomatología de la gota); antimicrobial activity (wound treatment); in vitro and in vivo antidiabetic effects, antibacterial activity, cardioprotective effects, antioxidant	The fruit is a small fleshy edible berry (green when unripe and purple black when ripe), about 5 mm, with 2–4 seeds	[82, 83, 86, 88, 90, 103, 143–146]
Murtilla de Magallanes, brecillo, uvilla	*Empetrum rubrum*	Antioxidant	Globose and fleshy fruit, about –8 mm in diameter, dark red	[109]
Murta, murtilla, Murta blanca, Tautau	*Ugni molinae*	Antioxidant, vasodilator activity, antibacterial	The fruit is a bright red berry, around 5–15 mm in diameter	[83, 99, 103, 112, 113, 147–149]
Zarzaparrilla, parrilla, uvilla, mulul, milul, Chilean currant	*Ribes punctatum*, *Ribes cucullatum*, *Ribes magellanicum*,	Antioxidant, cytoprotective effect in human gastric cells	Fruits are red, black, or green	[104, 106]
Zarzaparrilla, parrilla, Chilean currant	*Ribes trilobum*	Antioxidant, cytoprotective effect in human gastric cells	The fruit is initially green and becomes glossy black when ripe	[106]

Common name	Scientific name	Attributed properties	Description	References
Zarzaparrilla, parrilla, Chilean currant	*Ribes valdivianum*	Antioxidant	Purple-black berry-like fruit	[150]
Zarzaparrilla, Miñe-miñe, strawberry of Magallanes, wild raspberry	*Rubus geoides*	Antioxidant, cytoprotective effect in human gastric cells	Berry-like fruit	[111, 151]

Table 3. Main functional properties of native/endemic berries and berry-like fruits.

4. Conclusions and future trends

In spite of the endemism, there are promising bee hive–derived products obtained from Chilean plants, as well as Chilean plant products in general. We are convinced that the main exponents of functional foods and super foods are in nature, which is where we have to explore to find them. However, they should be used and exploited in a sustainable way.

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Chapter 7

Açaí (*Euterpe oleracea*) and Bacaba (*Oenocarpus bacaba*) as Functional Food

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Abstract

This chapter reviews two oleaginous fruits that are widely consumed by people in the Amazon region: Bacaba (*Oenocarpus bacaba*) and Açaí (*Euter*pe *oleracea*). Besides their food and the folk medicinal uses, studies suggest that substances present in both berries have antioxidative effects, antimicrobial, and therapeutic properties such as hypocholesterolemic and neuroprotection effects. These therapeutic effects are related to phenolic compounds, anthocyanins, and fatty acids, which can prevent serious problems such as coronary heart disease, hypertension, and depression. The use of supercritical fluid technology is described as a technique to obtain the best extracts of bacaba and açaí, as well as their valuable constituents. Indubitably, this technology is a great tool for human health and all with the advantage of presenting nontoxic solvents such as carbon dioxide or water. Açaí and bacaba fruits represent not only food but also a source of compounds that can work in both prevention and treatment of diseases.

Keywords: Amazon, açaí, bacaba, bioactive compounds, antioxidants, functional food

1. Introduction

The Brazilian Amazon represents one of the richest biomes found in the world. It presents many sources of plants, including native ones not yet explored, but that have potential for use. The economic importance that the aromatic plants represent to the Amazon region is

associated with the application of their vegetable oils and aromas in technological and industrial processes. Because of this, there is a greater investment in such plants extraction sector, causing an expansion of the domestic and international markets.

Because of this biodiversity, there is a wide variety of oleaginous species, as is the case of andiroba (*Carapa guianensis*), tucumã (*Astrocaryum vulgare*), buriti (*Mauritia flexuosa*), palm (*Elaeis guineensis, Jacq*), açaí (*Euterpe oleracea*), and bacaba (*Oenocarpus bacaba*). These species experimentally have a high yield in vegetable oils, with the potential for production of biologically active natural products, the so-called bioactive compounds, which have a high value added. Among these, the fat-soluble vitamins carotenoids (provitamin A), tocopherols (provitamin E and antioxidant), dyes, and flavonoids (anthocyanins, which are dyes with antioxidant effects) can be highlighted.

The characteristics of the Amazon region are conducive to the proliferation of palm trees, among which there are the oleaginous ones that are commercially cultivated with already fully established management technology, as is the case of açaí and bacaba, which can be considered new "superfruits." The consumption of these fruits pulps has been increasing, mainly due to the benefits that are being showed by scientific papers. Açaí, for example, has a high economic potential, mainly due to its use in the preparation of açaí beverages, which are exported all over the world as an energetic drink [1].

Besides the folk use as a drink, studies suggest that substances present in both berries have therapeutic properties such as hypocholesterolemic and neuroprotection effects. These therapeutic effects are related to fatty acids, which can prevent serious problems such as coronary heart disease, hypertension, and depression [2, 3]. The presence of phenolic compounds in their composition also gives them properties such as antimicrobial and antioxidant effects [4, 5].

Another group of compounds with significant presence in açaí and bacaba is anthocyanins. Anthocyanins are plant-derived compounds belonging to the flavonoids subgroup of phenolic compounds. Besides antioxidative properties, anthocyanins are the focus of studies for application on humans against diseases such as cancer and Alzheimer's [6–8].

Among the various methods of obtaining natural extracts, the process of supercritical fluid extraction has become appropriate and of great interest to the food industry, pharmaceutical, and cosmetic technology. It provides the obtainment of products free of residual solvents and with superior quality, while preserving the organoleptic properties of the material. The most used solvent in the supercritical technology is carbon dioxide (CO_2), which is inert, nontoxic, has a high solubility, and allows performing low-temperature processes, which are perfect for the extraction of thermosensible compounds, as is the case of, for example, anthocyanins.

2. Açaí and bacaba as functional food

The food industry has high expectations in food products that meet the consumers' demand for a healthy lifestyle. In this context, functional food plays a specific role, which is not only to

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satisfy hunger but also to provide humans the necessary nutrients. It also prevents nutrition-related diseases and increases their physical and mental well-being [9].

In Brazil, there are two kinds of functional food: açaí and bacaba (see **Figure 1**), which are oleaginous fruits, present black-violet color, and are from typical palm trees in the Amazon region. They belong to the *Arecaceae* family and when processed with water, form an emulsion. Both are commercially exploited for the production of foods and beverages. The juices of bacaba and açaí are considered tasty and much appreciated by the Amazonian population. In the period between harvests of açaí, from December to April, bacaba has the highest sales potential, in a relay system [10, 11].

The functional quality of bacaba oil was analyzed by Pinto [12] through the determination of atherogenicity index (AI) and thrombogenicity index (TI) proposed by Ulbricht and Southgate [13] and hypocholesterolemic/hypercholesterolemic ratio (h/H) suggested by Santos-Silva et al. [14]. The results of AI, TI, and h/H were satisfactory. Although the values of AI and TI were low, h/H was high in levels that show bacaba oil could be regarded as cardioprotective, suggesting the direct consumption of it in the form of table oil, similar to olive oil, or in encapsulated form as a phytopharmaco. In the same study, bacaba oil was used for coating iron oxide for the synthesis of Fe_3O_4 magnetic nanoparticles (MNP). The results showed that the oil well replaced the oleic acid, with the formation of MNP with morphological and desirable

Figure 1. Bacaba (**a**) and açaí (**b**) berries.

magnetic characteristics. MNP have therapeutic features, being used as drug carriers in the treatment of cancer by magnetic induction, reducing collateral effects to patients.

Açaí, being a source of fibers and rich in antioxidants, has considerable potential for nutritional applications and in the health field as a functional food or dietary supplement [15]. The work conducted by Barbosa et al. [2] evaluated the effect of a diet with daily consumption of açaí pulp in the prevention of oxidative damage by measuring the activity of antioxidant enzymes and the use of protein biomarkers in healthy women. The results showed that the açaí intake increased the activity of catalase, an intracellular enzyme which is also known as hydroperoxidase, able to decompose the hydrogen peroxide (H_2O_2), which is associated with various pathologies connected to oxidative stress; the results also showed an increase in total antioxidant capacity and a reduction in the production of reactive oxygen species. These studies reveal the antioxidant effect of açaí, increasing the understanding of its beneficial health properties.

The antioxidants found in açaí and bacaba are necessary to prevent the formation and oppose the actions of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are continuously formed in the human body. Mechanisms of free radicals such as these are related to various human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurodegenerative diseases. Many components of the diet such as carotenoids and plant pigments are suggested as important antioxidants; however, the interest in phenolic compounds of plants, particularly flavonoids, is also increasing. Thus, diets based on functional foods rich in antioxidants are important for the maintenance of human health [16–19].

3. Chemical composition of açaí and bacaba

The nutritional properties of Amazonian palm trees are related to the composition of fatty acids and phytochemical compounds, the so-called bioactive compounds. Açaí and bacaba are some of the species of fruits that have become quite attractive, not only for lipid content they present, but also for their composition of bioactive compounds.

The fatty acids present in fruit species such as these are considered one of the most important constituents in living organisms due to their structural role in cell membranes and as metabolic energy sources [20]. Those considered essential to life are known as essential unsaturated fatty acids and must be supplied by food. The main representatives are omega-9 (ω-9), omega-6 (ω-6), and omega-3 (ω-3). Of these groups, the α-linolenic acid (n-3), the linoleic and arachidonic acids (n-6), and the oleic acid (n-9) can be highlighted [21]. The vegetable oils, such as bacaba and açaí, are good sources of these components and fat-soluble vitamins such as vitamins A, D, E, and K [22].

According to Martin et al. [23], the availability of ω-3 and ω-6 fatty acids in the human species depends on the food supply, and moreover, it is important to know what are the sources capable of supplying these needs. **Table 1** shows some sources of monounsaturated and polyunsaturated fatty acids of fruits that come from palm trees and are considered as dietary sources of fatty acids.

Batista et al. [8] obtained the fatty acids profile of lyophilized açaí pulp extracts obtained by extraction with supercritical CO_2, as shown in **Table 2**.

Fruits that come from palm trees	Part of the fruit analyzed	(C12:0) lauric (%)	(C14:0) myristic (%)	(C16:0) palmitic (%)	(C18:1) oleic (%)	(C18:2) linoleic (%)	(C18:3) linolenic (%)
Babaçu (*Orbignya phalerata* Martius)[1]	Kernel	44.0	17.0	8.0	14.0	2.0	–
Buriti (*Mauritia flexuosa L.f.)*[2]	Mesocarp	–	–	18.0	73.5	2.7	2.1
Dendê (palm) (*Elaeis olifera)*[3]	Endocarp	47.9	16.1	8.4	16.2	2.7	Traces
Pupunha (*Bactris gasipaes*)[4]	Mesocarp	–	–	35.20	51.7	4.9	1.2
Tucumã (*Astrocaryum vulgare*)[5]	Epicarp + mesocarp	–	0.10	24.6	65.1	2.6	0.2
Bacaba (*Oenocarpus bacaba*)[6]	Mesocarp	0.18	0.59	32.27	40.82	9.78	1.93
Bacaba (*Oenocarpus bacaba*)[7]	Mesocarp	–	–	30.6	47.3	20.6	–
Patauá (*Jessenia bataua*)[5]	Mesocarp	–	0.10	13.3	76.7	3.9	0.1
Açaí (*Euterpe oleracea*)[3]	Mesocarp	–	–	25.9	54.9	11.5	1.1

Sources: [1]Lima et al. [24], [2]Tavares et al. [25], [3]Rogez. [26], [4]Yuyama et al. [27], [5]Rodrigues et al. [28], [6]Montúfar et al. [29], [7]Santos et al. [30].

Table 1. Content of the main fatty acids present in palm tree fruits consumed in the human diet.

Foods rich in fatty acids, such as bacaba and açaí, can play an important role in human food base, because the linolenic, linoleic, and oleic acids present in these raw materials are considered functional and exhibit inflammation-reducing and immunity-increasing properties in the human body, as demonstrated by Wallace et al. [31], Schwab and Serhan [32], Serhan et al. [33], and Calder [34].

In addition to fatty acids, various bioactive compounds can be found in these fruits. Yamaguchi et al. [1] report that about 90 substances have been found in açaí, of which approximately 31% consist of flavonoids, followed by 23% of phenolic compounds, 11% of lignoids, and 9% of anthocyanins. These are compounds that are correlated with high biological activity.

Content of fatty acids in % g/100mg									
Fatty	**50°C**	**50°C**	**50°C**	**60°C**	**60°C**	**60°C**	**70°C**	**70°C**	**70°C**
Acid	**150 bar**	**220 bar**	**350 bar**	**190 bar**	**270 bar**	**420 bar**	**220 bar**	**320 bar**	**490 bar**
C8:0	0.69	1.26	0.83	0.77	1.58	0.40	0.33	2.27	0.02
C10:0		0.03	0.02	0.02	0.04	0.03	–	–	–
C12:0	0.07	0.17	0.17	0.13	0.19	0.25	0.07	0.33	0.14
C13:0	–	–	–	–	–	–	0.02	0.21	–
C14:0	0.13	0.24	0.16	0.19	0.21	0.30	0.13	0.42	0.18
C15:0	–	–	–	–	–	–	–	–	–
C16:0	28.15	30.91	23.47	26.29	29.20	28.58	25.41	90.86	27.81
C16:1	4.95	0.03	5.49	6.14	7.08	6.83	4.16	0.08	5.81
C17:0	–	0.04	0.14	0.03	–	–	0.05	0.19	0.03
C18:0	1.05	1.25	1.02	0.80	1.14	1.16	1.43	5.35	1.33
C18:1	64.86	65.81	52.73	50.78	60.42	62.41	55.71	0.23	64.65
C18:2	–	–	15.54	14.80	–	–	12.59	–	–
C18:3	–	–	–	–	–	–	–	–	–
C20:0	0.08	–	–	–	0.10	–	–	–	–
C22:0	–	0.22	0.38	–	–	–	0.04	–	–
SFA	30.18	34.15	26.22	28.25	32.48	30.74	27.53	99.67	29.53
MUFA	69.81	65.84	58.23	56.93	67.51	69.25	59.87	0.31	70.46
PUFA	–	–	15.54	14.80	–		12.59	–	–
S/U	0.43	0.52	0.35	0.39	0.48	0.44	0.38	321.52	0.42

C8:0 (caprylic acid); C10:0 (capric acid); C12:0 (lauric acid); C13:0 (tridecanoic acid); C14:0 (myristic acid); C15:0 (pentadecanoic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C17:0 (margaric acid); C18:0 (stearic acid); C18:1 (oleic acid); C18:2 (linoleic acid); C18:3 (linolenic acid); C20:0 (arachidic acid); C22:0 (behenic acid); SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids).

Table 2. Content of fatty acids in açaí pulp extracts obtained by extraction with supercritical CO_2.

Of these components, anthocyanins have received great attention due to their potential benefits in preventing chronic diseases, including cancer and Alzheimer [8]. They are glycosides of anthocyanins and have, at their core, the flavylium cation. They belong to the group of flavonoids and subgroup of phenolic compounds. These compounds are responsible for defining the color of a variety of vegetables, including purple color in açaí [1]. They are hydrophilic, stable at acid pH, sensitive to light exposure, elevated temperatures, and presence of O_2.

To obtain bioactive substances such as anthocyanins, different extraction techniques have been developed with the aim of reducing the extraction time and the solvent consumption, increasing the extraction yield and improving the quality of the extracts. Among these

techniques are included: ultrasound assisted extraction, microwave assisted extraction, supercritical fluid extraction, and accelerated solvent extraction [35].

The choice of a method for extracting anthocyanins depends largely on the purpose of extraction and the nature of the constituent molecules of these compounds [36]. Therefore, as these pigments are very soluble in water, they are easily extracted by polar solvents. Their extraction typically involves the use of aqueous acidified solutions of ethanol, methanol, or acetone [37]. However, these solvents have also used limitations such as lower extraction efficiency compared to other solvents, as well as a lower durability of their extracts [38, 39].

References	Application	Anthocyanins quantification
Finco et al. [40]	Characterization and analysis of total phenolic compounds and total flavonoids of bacaba extract (*Oenocarpus bacaba* Mart.) by HPLC-DAD-MS	The total content of monomeric anthocyanin was evaluated by a differential pH method described by Sellappan et al. [41]. The anthocyanin cyanidin-3-glucoside was used as pattern
Gouvêa et al. [42]	Isolation of anthocyanins patterns (cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside) of lyophilized açaí (*Euterpe oleracea* Mart.) by HPLC	The isolation of anthocyanins was carried out by HPLC. The anthocyanin identification in the lyophilized açaí was done by mass spectrometry. They used the anthocyanins patterns: cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside
Santos et al. [43]	This study evaluated the encapsulation of anthocyanin extract obtained from jabuticaba (*Myrciaria cauliflora*) using supercritical CO_2 as solvent and ethanol as co-solvent	In the extraction of jabuticaba anthocyanin, supercritical CO_2 was used together with the co-solvent ethanol in certain conditions of pressure, temperature, and flow ratio
Paes et al. [44]	Extraction of anthocyanins and phenolic compounds of blueberry (*Vaccinium myrtillus* L.) using supercritical CO_2 and water and ethanol as co-solvents	HPLC and mass spectrometry. Pelargonidin was used as pattern for the identification of anthocyanins
Neves et al. [45]	The objective of this study was to follow the physicochemical and functional alterations of açaí and bacaba pulps processed by hand	For the determination of total anthocyanins, the method of Francis [46] was used
Novello et al. [47]	This study aimed to evaluate the influence of organic solvents on the extraction of anthocyanins from açaí. The anthocyanins, the fatty acids profile, and the antioxidant activity of the extract were analyzed by HPLC	The anthocyanins were determined by spectrophotometry using differential pH method described by Giusti and Wrolstad [48]. The identification and quantification of anthocyanins of lyophilized açaí extract were performed by HPLC-DAD. The identified anthocyanins were cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside
Batista et al. [8]	This study determined the phenolic compounds and anthocyanins of lyophilized açaí pulp after extraction with supercritical CO_2	The anthocyanins were determined by UV-visible spectrophotometry using the Folin-Ciocalteu reagent, according to the method described by Singleton and Rossi [49]

Table 3. Overview of anthocyanin extraction applications.

The main anthocyanins found in açaí are cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside. In bacaba, it is cyanidin-3-glucoside. This information is presented in **Table 3**, as well as an overview of some anthocyanin extraction applications of açaí, bacaba, and other raw materials. Their chemical structures are presented in **Figure 2**.

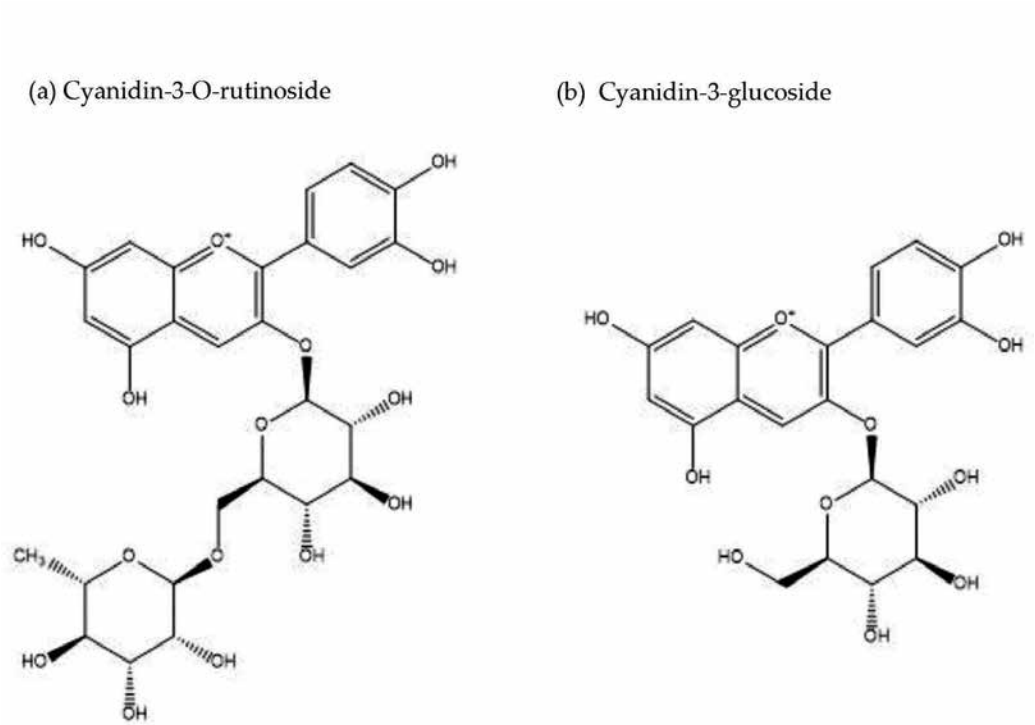

Figure 2. Chemical structures of the main anthocyanins found in açaí and bacaba (**a**): 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-chromeniumyl6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside; (**b**): 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-chromeniumyl β-D-glucopyranoside [nomenclatures according to IUPAC].

In addition to anthocyanins, other bioactive compounds have been identified in açaí and bacaba. Pacheco-Palencia et al. [50] analyzed two species of açaí and identified several flavones, including homoorientin, orientin, deoxyhexose taxifolin, and isovitexin; flavanol derivatives, including (+)—catechin, (−)—epicatechin, procyanidin dimers and trimers, and phenolic acids such as protocatechuic, p-hydroxybenzoic, vanillic, syringic, and ferulic. Phenolic compounds are also reported to be potentially protective against cardiovascular disease and cancer [51]. Also, large amounts of phenolic compounds such as phenolic acids, flavanols, and flavonols can be found, which act as cofactors to improve the biological action of anthocyanins [52].

Santos et al. [53] evaluated the content of bioactive compounds and total antioxidant capacity of native fruits of the Amazon palm trees, including the species *O. bacaba*. Their results showed a high content of total polyphenols, presence of carotenoids, higher levels of anthocyanins, and antioxidant capacity in the bacaba extracts. In the study of Finco et al. [40], the phenolic classes: C-glycoside, flavonoid, C-hexoside, C-glycosylflavone, isorhamnetin hexoside, quercetin hexoside, quercetin diglycoside, quercetin glycoside, and isorhamnetin glycoside, were identified.

4. Methods for obtaining vegetable oils

The economic importance that aromatic plants have in the Amazon region is associated with the application of their vegetable oils and use of their aromas in technological and industrial processes. Because of this, there is a greater investment in such plants extraction sector, causing an expansion of the domestic and international markets.

The soil and climate of the Amazon region are conducive to the proliferation of palm trees, among which there are the oleaginous ones cultivated with commercial purpose. This is the case of açaí and bacaba, whose extraction already constitutes a significant economic activity in the state of Pará-Brazil. There are other native palm trees in the region that provide oleaginous fruits rich in provitamins A and E, yet poorly explored, such as pupunha (*Guilielmaspeciosa*) and tucumã (*Astrocaryumvulgare*). These and other vegetable raw materials present in their composition have a high content of lipids, with significant potential for extraction.

Extraction is a unit operation widely used in the food industry and can be used for the production of coffee, sugar, caffeine extraction, vegetable oils, flavorings, and essential oils [54]. Obtaining these extracts may be accomplished by different methods such as mechanical pressing extraction, solvent extraction, supercritical fluids extraction, or others, depending on their content [55–57].

4.1. Mechanical pressing extraction

The extraction by mechanical pressing is one of the oldest methods of obtaining oil and fats from seed and fruits. For this kind of extraction, the packaged material enters through a feed shaft in the press. The press consists of a basket formed of spaced rectangular steel bars, through blades, whose thickness varies depending on the raw material. In the center of the basket, there is a screw that rotates and moves the material forward, compressing it at the same time. The pressure is regulated via an outlet cone [58, 59].

Souza et al. [60] and Pighinelli et al. [61] report that although the mechanical pressing extraction is less efficient than other methods, it is a more workable system on a small scale, for not being dependent on facilities and safety that are characteristics of the solvent processing, besides being fast, easy to handle and presents low cost of installation and maintenance.

One of the disadvantages of the mechanical pressing method is its low oil yield recovery: even in the most efficient presses, there is still a range of 3–5% of remaining oil in the cake. This residual oil present in the cake can be recovered by a two-step process: pre-extraction (with the screw-press) and solvent extraction, thus maximizing efficiency. Furthermore, the solvent extraction is recommended only in raw materials with <25% of fat content [62–69].

4.2. Solvent extraction

This type of extraction occurs by partitioning a solute between two immiscible or partially miscible phases. The mass transfer occurs from the solutes in the food matrix to the solvent. First, the solute is dissolved in the solvent, then the penetration of the particle solution in the

food surface occurs, and finally the solution is dispersed in the solvent. According to Ghosh [64], solvent extraction can be classified into four types depending on the phase of the matrix: (i) solid-liquid extraction; (ii) liquid-liquid extraction; (iii) vapor extraction; and (iv) supercritical fluids extraction.

The solvent choice is of fundamental importance in the aspects that aim at efficiency, economy, and preservation of the physicochemical and nutritional characteristics of oils. In conventional extraction, some solvents used for obtaining oils from plants are hexane, n-hexane, pentane, ethanol, and petroleum ether [59, 63, 65–70].

In the solvent extraction, there can be a reduction in the product quality because of the several steps necessary to recover the solvent, elevated temperature, long periods of thermal exposure, high oxygen concentration, and extraction of other compounds considered undesirable [63, 71].

4.3. Supercritical fluids extraction

The supercritical fluids (SCFs) extraction is a unit operation by contact that is based on the balance and on the physicochemical properties of the SCFs, being dependent on operating conditions such as temperature, pressure, solvent flow, the material morphology, prior treatment of the porous solid matrix, and the physical properties of the packed bed, such as porosity, distribution and particle size, initial content of solute in the solid matrix, and the fixed bed height [72].

The SCFs present intermediate characteristics between liquids and gases. The diffusion coefficient (DC) of SCFs is high and close to the gases DC, thus increasing the diffusivity when they are in the liquid state, providing a rapid and efficient mass transfer. The density of SCFs is greater than that of a gas, having a higher solvating power due to the high compressibility. Furthermore, they exhibit low viscosity and the absence of surface tension, which promote greater penetration into the solid matrix [73, 74].

Carbon dioxide (CO_2) is widely used as SCF due to having low critical temperature and pressure (73.74 bar and 304.12 K, respectively), besides being: nontoxic, nonflammable, odorless, and easily separated from the extract. Due to its low critical temperature, it is possible to use it to extract reactive and thermosensitive compounds. CO_2 is suitable for extracting apolar compounds, but when polar organic solvents such as ethyl acetate, ethanol, or methanol are added, the polarity is modified, being possible to extract other compounds. These aggregate solvents are called co-solvents [75].

Batista et al. [8] obtained açaí extracts fractions with supercritical CO_2 and analyzed the allelopathic effects of these extracts on two species of invasive plants: *Mimosa pudica* and *Senna obtusifolia*. They observed that depending on the operating conditions of temperature and pressure used, the pattern of phytotoxic responses can change: in some cases, the effect may be stimulatory to seed development. Studies on allelopathy have direct influence on human health, because the use of chemicals such as pesticides, which can cause diseases such as cancer, can be avoided [76–78]. However, other studies must be conducted to isolate the specific metabolites for each role assigned to the açaí.

Pinto [12] also obtained bacaba extracts fractions with supercritical CO_2 at different conditions of temperature and pressure. In his work, bacaba is mentioned as a rich source of natural antioxidants and dyes. However, there is a need for further studies to elucidate bacaba's behavior in different processes.

4.4. Other extraction methods

The methods of soxhlet, hydrodistillation, solid-liquid, and ultrasound-assisted extraction do not present a performance as good as the one presented by the extraction with supercritical fluids: it has a high selectivity, low or no organic solvent consumption, operates at temperature close to room, no request for subsequent purification steps, and reduces post-processing costs as there is no longer need to eliminate solvent extracts [75, 79, 80].

5. Anthocyanins extraction by SFE

Anthocyanins are the most abundant flavonoid constituents of fruits and vegetables. Their use into food and/or medical fields has proven to be a technological challenge since these compounds have low stability and are susceptible to degradation through factors such as the presence of light, pH, temperatures usually higher than 60–80°C, the presence of sulfite, ascorbic acid, enzymes (such as glycosidases and phenolases), among other factors [43, 81, 82].

In the literature, the recovery of phytochemicals from solid wastes has been reported using conventional and alternative technologies. According to Paes et al. [44], conventional methods are Soxhlet extraction, maceration extraction, extraction by infusion and vapor distillation. Alternative techniques such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) eventually assisted with ultrasound are also reported.

Supercritical fluids processes have proved to be an excellent alternative to extract natural pigments due to the use of environmentally friendly solvents, such as carbon dioxide. According to Vatai et al. [83], extractions with supercritical CO_2 result in non-deteriorated reactions, due to low process temperatures. The CO_2 is readily available, relatively cheap, and accepted as a solvent in the food industry. SFE with CO_2 is an excellent isolation method for natural materials and gives an alternative to replace the nonpolar organic solvents, which can be a source of food contamination.

Supercritical fluid extraction (SFE) using carbon dioxide (CO_2) has been applied for the pretreatment of natural materials, as shown in the works of Paula et al. [84], Ghafoor et al. [85], and Floris et al. [86]. Operating conditions (temperatures varying from 40 to 50°C and pressures above 200 bar) and the use of co-solvents such as ethanol and water were used in their studies as modifiers to obtain the maximum extract yield. According to Seabra et al. [87], even though the choice of the appropriate polar solvent is a key factor for the success of the anthocyanin extraction procedure, its influence on the extract's characteristics is not always clear, due to the diverse structure and composition of plant materials and also the relation material-solvent.

6. Conclusion

Açaí (*Euterpe oleracea*) and bacaba (*Oenocarpus bacaba*) are highly consumed fruits in Amazon that come from common palm trees and have remarkable properties. There are many benefits that help increasing their role in the growing market for nutraceuticals. Their extracts have a range of bioactive and polyphenolic components with antioxidant properties that make them new "superfruits"; however, further studies still need to be conducted in order to elucidate all the roles that these fruits can play. Açaí and bacaba represent not only food, but also a real source of health for humans.

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Section 3

Functional Food: Value Addition, Health & Safety Aspects

Chapter 8

Phytochemicals in Fruits and Vegetables

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Additional information is available at the end of the chapter

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Abstract

Fruits and vegetables are the most important sources of phytochemicals. Phytochemicals use for both human diets and natural antimicrobial agents in food preservation. Their benefits for health are mainly due to high antioxidant activity. Antimicrobials of plant origin are known as secondary metabolites that could play a role not only individually or jointly against food-borne pathogens but also contribute to food flavor. Phytochemicals have a strong effect on control and prevention of natural spoilage processes and growth of microorganisms, including pathogens causing food safety issues. Microorganisms are always associated with harvested plants and slaughtered animals, the basic unprocessed materials of the food industry. Since foods consumed by humans undergo several processing treatments, it is important to understand the effect of such treatments on the phytochemical composition of foods.

Keywords: phytochemicals, food preservatives, food spoilage, food phenolics

1. Introduction

Fruits and vegetables are consumed as fresh or processed and known to be among the most important sources of phytochemicals for the human diet. About 200,000 phytochemicals are known so far and 20,000 of them have been identified as originating from fruits, vegetables and grains [1]. Phytochemicals has many health effects as antioxidants against many diseases or antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic, or anti-inflammatory effects [2]. Phytochemicals are used for various purposes such as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides and food additives [1]. Their chemical structures composed of phytochemicals such as phenolics, alkaloids, saponins and terpenoids [1]. These compounds are known as secondary metabolites having various identifiable structures, although a benzene ring with one or more hydroxyl groups is a common feature. They are commonly classified as flavonoids (anthocyanins, flavan-3-ols,

flavonols, proanthocyanidins or flavones, non-hydrolyzable tannins, isoflavones and flavanones) and non-flavonoids (hydroxycinnamic, hydroxybenzoic acid, hydrolyzable tannins, benzoic acids and stilbenes) [3]. Sugars, acids and polysaccharides are an important source of phytochemicals, secondary metabolites of plants also known as their antioxidant activity and other properties [4]. Lately, there are many investigations on plant "antimicrobial," "antiviral," or "antibacterial" effects [1]. In addition, phytochemicals are some of the most important natural preservation structures to reduce and inhibit pathogenic microorganism growth and preserve the overall quality of food products [5]. These antimicrobials can protect food products, extending the shelf life naturally [5]. Chilling, fermentation, freezing, acidification, nutrient restriction, water activity reduction, synthetic antimicrobials and pasteurization have been used in food preservation technology and phytochemicals such as flavonoids, polyphenols, anthocyanins and carotenoids are also used to preserve and control microbial spoilage in foods traditionally [6]. In general, food antimicrobials can be classified as natural and synthetic substances depending on their origin. Synthetic antimicrobials are found in fruits naturally such as benzoic acid in cranberries, tartaric acid in grapes, sorbic acid in rowanberries, malic acid in apples and citric acid in lemons [6]. Secondary metabolites are in close contact through sophisticated communication involving metabolic attacks by plants on their pathogens [7]. Fruits and vegetables have phenolics which are biologically active compounds. Fruits and vegetables have a special phytochemical group which protect plants from their environment stress such as pollution, pathogens, or various abiotic stresses [2]. Even if secondary metabolites having different structures, they can have similar functions. First, plant-defensive metabolites include phytoalexins biosynthesized to respond to biotic and abiotic stresses with the effect of both protecting the plant and controlling the pathogen growth [7]. Secondly, most of these metabolites are responsible for the organoleptic and qualitative properties of foods originating from such plants. For example, anthocyanins constitute a pigment group responsible for the color of a great variety of fruits, flowers and leaves [8] and flavan-3-ols are polyphenols involved in the bitterness and astringency of tea, grapes and wine [9, 10]. Thirdly, these compounds are unique sources of industrial material in the form of food additives, pharmaceuticals and flavors [11]. Finally, they are considered to be beneficial for health, mainly due to their antioxidant activity. Many studies have suggested that a high intake of polyphenol-rich foods may have cardiovascular benefits and provides some level of cancer chemopreventive activities and beneficial effects against other less prevalent but devastating illnesses, such as urinary bladder dysfunctions and Alzheimer's disease [12]. Furthermore, food scientists and nutrition specialists suggest that phytochemicals offer many health benefits when consumed as part of the usual human diet [13].

2. Commonly used methods of treating plant foods

Many fresh fruits especially small berries and vegetables are highly perishable after harvest. During the harvest, bruising can reduce shelf life, influencing both color and texture of fresh products. The freshness of fruits and vegetables can be maintained in storage through reduction of temperature and/or oxygen levels, increase in carbon dioxide levels, use of modified atmosphere packaging or edible coatings, or treatment with gamma irradiation or high

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pressure. These can also be combined with treatments of 1-MCP, ozone and ultraviolet (UV) irradiation to further prevent losses. One of the most basic treatments used to lengthen the shelf life of fresh commodities during storage is to store in a low temperature and high relative humidity conditions. It has been known and used to extend the shelf life of fruits and vegetables since antiquity [14]. Moreover, exposure to low temperature during storage optimizes produce appearance and has the additional benefit of protecting nonappearance quality attributes, such as texture, nutrition, aroma and flavor [14]. There are many chemical and natural preservative treatments used to reduce postharvest losses and extend the shelf life of fresh commodities. Using plant extracts with known antimicrobial properties can be of great importance in food preservation. There are some chemical substances in plants that produce a definite action on the microbiological, chemical and sensory quality of foods and these phytochemicals have been grouped in several categories including polyphenols, flavonoids, tannins, alkaloids, terpenoids, isothiocyanates, lectins, polypeptides, or their oxygen-substituted derivatives [6]. On the other hand, alternative sources of natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. The use of natural antimicrobials as phytochemicals is organic acids, essential oils, or plant extracts and could be a good alternative to ensure food safety [6]. To inactivate or inhibit the growth of spoilage and pathogenic microorganisms during preservation of food, there are several processing techniques used including the use of chemical preservatives and synthetic antimicrobials [5]. However, these techniques have not been considered natural antimicrobial agents in food preservation. But, naturally derived compounds in plant extracts can be good control agents for pathogenic microorganisms. The use of synthetic chemicals is increasingly restricted in many countries. Nowadays, the recent trend has been for the use of natural preservatives due to the adverse health effects of synthetic ones. The alternative methods preserve foods and reduce pest and pathogen injury, with the use of resistant varieties or integrated cropping strategies in which plant secondary metabolites may improve crop protection [6]. The major goals of such natural antimicrobials are to protect the food from food poisoning and spoilage microorganisms that cause off-odors, off-flavors and discoloration quality losses [6]. Antimicrobials are called traditional when they have been used for many years and many countries approve them for inclusion in foods. Although many synthetic antimicrobials are found naturally (benzoic acid, sorbic acid, citric acid, malic acid, tartaric acid), the perception of natural has become important for many consumers. The safety and shelf life of food ingredients can also be improved by application of novel technologies to avoid or delay microbial growth like packaging in modified atmosphere, nonthermal treatments, activated films, irradiation, etc. [6]. The use of fruits and vegetables as a source of certain phytochemicals, such as ascorbic acid (AA), carotenoids, phenols and flavonoids, has not only health-promoting effects but also widely used to restrict oxidation-induced degenerative changes in cell physiology and aging [15] and is well known due to their significant impact on the food industry [16]. Both glucosinolates and leaf surface waxes are important phytochemicals that also play an important role in protecting plants from pest and pathogen injury [17]. These factors that positively affect plant protection also minimize crop damage by pests and pathogens. B-Carotene, lycopene, lutein and zeaxanthin are known to exhibit antioxidant activity. Increasing oxidative stress results in produce losing keeping quality, not only in terms of

microbial contamination, excessive softening and browning but also in terms of significant depletion of phytochemicals, such as phenolics, flavonoids, ascorbic acid and carotenoids. The addition of AA minimizes oxidative deterioration in processed fruits and vegetables. Exogenous treatment of AA in minimally processed fruits and vegetables reduces or stops enzymatic browning and oxidation-susceptible degenerative changes such as the deterioration of carotenoids, phenolics and flavonoids [16].

Phenolics and their metabolites are common constituents of fruits and vegetables that play an important role as to provide astringency and aroma constituents [15]. Polyphenolic compounds are important as food preservatives that inactivate free radicals giving them an important role fighting against pathogenicity, infestation and photooxidation [1, 15]. In general, antibacterial activity of phenolic acids is stronger against Gram-positive bacteria than Gram-negative bacteria [1]. The main problems for such antimicrobials are food-poisoning microorganisms and spoilage microorganisms that are metabolic end products causing off-odors, off-flavors, texture problems and discoloration of food [1]. Phytochemicals, such as phenolic compounds, are of great importance as antithrombotic, anticarcinogenic and anti-inflammatory agents. However, due to the possible negative effects of synthetic antioxidants, food industries prefer natural ones and can be used as food additives or pharmaceutical supplements [13]. First of all, they protect plants from biotic and abiotic stress factors. Indeed, such phenolic compounds are only induced when stress factors are present and so-called phytoalexins are specifically involved in defense mechanisms and are synthesized after pathogen or predator attack or injury [18].

3. Phytochemical and secondary metabolites present in plant foods

Secondary metabolites present in plant foods, such as alkaloids, phenolic compounds (flavonoids, isoflavonoids and anthocyanins) and terpenoids, have gained importance because of their antioxidant, antiviral, antibacterial and anticancer effects [19]. These phytochemicals are mixtures of several components, including phytophenolics in herbs and spices, phenolics, flavonoids and acids in fruits and glucosinolates in cruciferous vegetables (mustard) [5]. As shown in **Table 1**, *Rubus* (cloudberry and raspberry) extracts have antimicrobial effects against food spoilage and poisoning bacteria [21]. In general, antioxidant compounds have important protection effects from fruit insects and microbial organisms during storage [23]. Secondary metabolites are very important in medicine and agricultural science due to the activity of chemotherapeutic compounds or pesticides. Phenolics and flavonoids provide very important defense mechanisms against postharvest diseases [23]. For example, walnut seed coats contain gallic acid which is a phenolic compound that prevents aflatoxin biosynthesis by *Aspergillus flavus* (**Table 1**) [23]. Therefore, polyphenol compounds have antiviral activities to some various types of viruses [23]. One of the other very important phytochemical groups are flavonoids which have antiallergenic, antiviral, antifungal and anti-inflammatory activity. It is abundant in most of the fruits and vegetables such as apples, grapes, lemons, tomatoes, onions, lettuce, broccoli, etc. [23]. Flavonoids are also known as one of the largest groups of natural phenolic compounds in plants [27]. These natural compounds have important effects against a variety of microorganisms [27]. In addition, flavonoids either have protective effects from microbial attacks or respond as phytoalexins against them. Volatiles are phytochemicals that are either inhibitory or stimulatory to

fungal growth. Acetaldehyde, a volatile compound that occurs in fruit during ripening, has a fungicidal effect on postharvest pathogens [23]. As shown in **Table 1**, "Isabella" (*Vitis labrusca* L.) grape variety volatiles have a strong effect on *B. cinerea* development [19].

Name plant	Effects	References
The cranberry juice	Inhibition of *E. coli*	Howell et al. [20]
Some berry extracts (*Rubus*)	Food spoilage and poisoning bacteria	Rauha et al. [21]
Grape seed or rosemary extracts	Food preservatives	Blasa et al. [22]
Riesling grape	Gram-positive food-borne pathogens	Tajkarimi and Ibrahim [5]
Flavonoids in plant tissue	Antimicrobial	Saxena et al. [15]
Flavonoids	Antiallergenic, antiviral, and antifungal	Ippolito and Nigro [23]
Bergamot fruit extract	*Saccharomyces cerevisiae*	Mandalari et al. [24]
Citrus species and grape	*Fusarium oxysporum*	Okwu et al. [25]
Glucosinolates and leaf surface	Pest and pathogen injury	Björkman et al. [17]
The "Isabella" (*Vitis labrusca* L.) grape	(*B. cinerea*)	Makkar et al. [19]
The walnut seed coats (gallic acid)	Aflatoxin	Ippolito and Nigro [23]
Pomegranate fruit	*P. italicum*, *R. stolonifer* and *B. cinerea*	Tehranifar et al. [26]

Table 1. The effects of fruit and vegetable extracts on food pathogens.

4. Phytochemicals as natural preservatives and antimicrobials

Natural preservatives derived from plant extracts such as phytochemicals and essential oils are used against fungal development in many fruits and vegetables after harvest [28]. The efficiency of an antimicrobial treatment depends on many factors, such as type, genus, species and strain of the main microorganism, in addition to environmental factors such as pH, water activity, temperature, atmospheric composition and an initial microbial load of the food materials [6]. Therefore, other important subject to know is type of the microorganism(s) owing to usually combinations of antimicrobials are more effective than adding just one. The natural antimicrobial preservative activity is not clear since there are many influencing factors, one of the most important being the interaction between phytochemicals and growth of microorganisms [6]. Processing of foods containing phytochemicals is expected to result in some changes in their phytochemical content. Phytochemicals present in many food stuffs are lost by heat processing such as sterilization, pasteurization and dehydration [6].

Many investigations have evaluated phytochemical effects on antifungal activity. The potential use of plant extracts as natural antimicrobial agents in food preservation forms the basis for many applications such as grape seed or rosemary extracts that have been used as food preservatives [22]. Researchers reported that grape extracts of Riesling *Vitis vinifera* L. grapes showed strong preservative effects against some of the Gram-positive food-borne pathogens [5]. The alkaloids, steroids, tannins, flavonoids, saponins and gly-

cosides which were secondary metabolites showed various biological activities and act in plant defense mechanisms. Flavonoids usually occur as glycosides and aglycones in plant tissue which have significant antioxidant properties and antimicrobial and insect-repellent properties as well [15]. Flavonoids and their antimicrobial effect are useful as a food preservative to extend the shelf life and safety of foods. Flavonoids play important roles in biological activities, including antiallergenic, antiviral and antifungal effects [23]. It is also present in various common fruits and vegetables (apples, grapes, lemons, tomatoes, onions, lettuce and broccoli). The following flavonoids are antifungal agents in plants: isoflavonoids, flavans flavanones. However, the antifungal activity of flavonoid compounds plays an important role between plant-microorganism and host plant's defensive systems [8]. Saponin and flavonoids are found in fruits and vegetables and in general they form a soapy lather after extracted from parts of plants [5]. Mandalari et al. [24] reported that Bergamot fruit extract which is rich in flavonoid has an effective on the yeast *Saccharomyces cerevisiae* (**Table 1**).

Okwu et al. [25] also showed that the antifungal activity of both citrus species and grape has an important effect against *Fusarium oxysporum* (**Table 1**). Thiosulfinates come from hydrolysis products of garlic and onion. They have a strong potential of producing antimicrobial effects against pathogenic microorganisms [5]. Broccoli, Brussels' sprouts, cabbage mustard and horseradish have glucosinolates that also have a wide range of antibacterial effects. Moreover, olive leaves (*Olea europaea*) are rich in phenolic compounds, with demonstrated strong antimicrobial effects and can be potentially used in food processing [5]. Al-Zoreky [29] reported that phenolics and flavonoids present in pomegranate fruit peels demonstrated strong antimicrobial activity against some food pathogen microorganisms. In addition, *Psidium guajava* has phenolic, flavonoid, carotenoid, terpenoid and triterpenes that demonstrated strong antimicrobial activity [30]. Salas et al. [31] reported that flavonoids extracted from citrus species, not only naringin, hesperidin and neohesperidin but also enzymatically modified derivatives of these compounds, have strong antifungal activity [31]. The limonoid compounds have important antibacterial and antiviral activity as shown in **Table 1** [1]. Vikram et al. [32] reported that seeds of grapefruits have significant inhibitory effect on pathogenic *Escherichia coli* O157:H7. Black raspberry and Chardonnay seed extracts have also antibacterial activity to inhibiting growth of some food pathogen microorganisms [33]. Tehranifar et al. [26] reported that high percentage of phenolic content in the peel and seed of pomegranate fruit has high antifungal activity especially on postharvest fungi (*Penicillium italicum*, *Rhizopus stolonifer* and *Botrytis cinerea*). Another study showed that berry extracts exhibit selective inhibitory properties against intestinal bacteria [1]. Recently, antifungal activity has been found in all tissue types of strawberry fruit due to the phenolic compounds that inhibit the growth of fungi [23].

5. Other beneficial properties of plant phytochemicals

In the last decade, the results of many research have shown the positive effects of phytochemicals in human health. There is a strong correlation of antioxidant consumption

with lower risk of many diseases such as cardiovascular cancer, diabetes and hypertension diseases as well as other medical conditions [34, 35]. Fruits and vegetables have phenolic compounds, pigments and natural antioxidants; these compounds protect many diseases like cancer and heart disease [36]. The importance of antioxidant effects on cardiovascular diseases and cancer is especially important [23] and these antioxidants can be found in various fruits, vegetables and herbs. Phenolics as flavonoids have an important effects such as antimicrobial, anti-inflammatory, antioxidant, antiviral, antiallergic, anticancer, antiulcer, antidiabetic, antiplasmodial, antihypertensive, anticonvulsant and all reducing risks for severe human diseases [27]. Antioxidants in fruits and vegetables have defensive effects and are three main groups: vitamins, phenolics and carotenoids [35]. Vitamin C (L-ascorbic acid, AA) and the oxidized form (dehydroascorbic acid, DHAA), carotenoids and phenolic compounds prevent cardiovascular disease, cancer and cataracts which are associated with the oxidative damage of lipids, DNA and proteins [4]. Moreover, some carotenoids also have antioxidant activity (AOA) and shown beneficial effects on the reduction of cardiovascular diseases [4]. The fruits and vegetables that have phytochemicals are also not only low in fat and saturated fat, cholesterol and calories but also are rich in potassium and sodium, fiber, folic acid and AA [34]. One of the most important flavonols is quercetin, which is higher in onion (red and yellow), broccoli, kale, French beans, apple, red grapes and cherries. Quercetin is anticarcinogenic and inhibits low-density lipoprotein (LDL) oxidation activities [34].

6. Conclusion

In a conclusion, potentially a great number of phytochemicals including some of the vitamins, flavonoids, terpenoids, carotenoids, phenolics, phytoestrogens, minerals and antioxidants in plant materials are used as alternative preservative agents for controlling postharvest physiological disorders or microbial pathogen injuries of both fresh fruit and vegetables in the food industry. Many publications have focused on the potential protective nature of these natural phytochemical compounds against fungal and bacterial attacks. Moreover, these natural compounds have become interesting candidates not only for plant protection but also human and animal health protection from fungal and bacterial diseases because of their lower toxicity or absence of toxicity.

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Chapter 9

Foods with Functional Properties and Their Potential Uses in Human Health

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Additional information is available at the end of the chapter

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Abstract

Vegetables and fruits have been a part of human diet since ancient times; nevertheless, as countries develop, its population's feeding habits change and tend to have a diet poor in vegetables and fruits, with well-known consequences. Several food plant products with massive consumption and within the reach of the population are products such as artichoke, leek, hot chili pepper, coriander, kiwifruit, sweet orange, highbush blueberry, and maracuyá to name a few. They have many beneficial properties principally by its content of phytochemicals with high impact on human health, beyond nutritional support. The phytochemicals are bioactive compounds such as vitamins, carotenoids, phenolic acid, and flavonoids, which contribute to antioxidant capacity and as a whole prevent chronic nontransmissible diseases such as: diabetes, high blood pressure, high cholesterol in blood, cardiovascular risks, among others. This relationship between food plant for human consumption and its impacts on human health is discussed in this chapter, highlighting coriander and kiwifruit by its wide range of benefits.

Keywords: phytochemicals, antioxidant capacity, polyphenols, chronical diseases, healthy feeding habits, life style

1. Introduction

Foods are neither inherently good nor bad. Rather, good or bad eating habits, as well as other factors such as smoking and physical activity, all influence human health. If we desire a healthy lifestyle and wish to avoid chronic nontransmissible disorders such as diabetes, high

levels of cholesterol, cardiovascular diseases, etc., foods, especially those that are functional, are only a part of the solution [1].

Despite a wealth of information, there is no universal definition about what constitutes a functional food. However, there is consensus concerning central concepts, which are associated with their benefit for human health beyond their traditional nutrients [2]. Along the same lines, the importance of phytochemicals as a class of biologically active metabolites in plants is accepted [3]. When discussing "potential use for human health" to refer to a particular plant, preliminary evidence on its outstanding phytochemical content must already exist, which means it can be used in the future as a source to investigate more profoundly its beneficial implications in human health.

Some processes such as cooking alter the content and composition of phytochemicals present in vegetables, reducing their concentrations by thermal degradation or augmenting their concentration with respect to the raw material. However, these effects are varying with the cooking method and type of phytochemical [4]. These, together with the growing consumption of fiber, are the principal reasons to recommend the regular intake of fresh vegetables [5].

Functional foods may be plant or animal products, that are fresh, semi-processed or processed, but in this chapter we will refer mainly to fresh plants and their properties beyond their nutritional characteristics. In addition, we will also discuss the existence of several common horticultural and fruit plants that are widely available and consumed by the human population, whose functional properties have yet to be systematized and categorized. Vegetables with high functional interest such as artichoke, leek, hot chili pepper, and coriander, as well as fruit plants such as kiwifruit, sweet orange, and highbush blueberry are considered [6–9]. Within the species discussed in this chapter, only artichokes must be consumed cooked, whilst the others may be eaten fresh.

We believe that the updated information about plants with characteristics as functional foods responds to a need of the population and scientists to learn more about healthy habits and how consumption of natural foods can improve their quality of life.

2. Horticultural species with functional properties and their potential use in human health

The properties and features of some horticultural species that have beneficial effects on human health are mentioned here. It is important to note that the artichoke, leek, hot chili pepper, and coriander are considered in this section, because these species are widely consumed by the human population.

2.1. Artichoke (*Cynara cardunculus* var. scolymus and *Cynara scolymus*)

These species belong to the *Asteraceae* family (**Figure 1**) are native to the Mediterranean region [10] and grow in many parts of the world [11]. *Cynara scolymus* is cultivated due to its large immature flower heads, which have special functional and nutraceutical characteristics, and a

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high antioxidant capacity, with more than 9000 μmol trolox equivalents (TE) 100 g^{-1} FW [12]. This antioxidant capacity is due to its high content of total polyphenols like caffeoylquinic acids and flavonoids [7]. These polyphenols are present in flower heads, with values ranging from 4.8 to 29.8 mg g^{-1} FW in different Italian varieties [7, 13, 14]. It is important to mention that these values are not only dependent on the genetic background, as the interaction genotype-environment also has an influence [7]. Therefore, antioxidant content may be affected by agricultural practices, because in different locations, the same variety may have different antioxidant capacities.

Figure 1. Immature flower head of artichoke.

Choleretic and hypocholesterolemic activities, due to the presence of chlorogenic acid, cynarin, and lutein, have been reported in clinical studies, which demonstrated the effect of leaf extracts on the inhibition of the biosynthesis of cholesterol in rat hepatocytes [15]. Furthermore, these extracts prevented necrosis in rat hepatocytes provoked by hydroperoxides indicated for treatment of dyspepsia, or dyskinesia of the bile ducts, as well as disorders in the assimilation of fats in humans [16–18]. The nutraceutical and therapeutic actions of several metabolites are summarized in **Table 1**. The positive effects of ingesting *C. cardunculus* flower heads have been widely demonstrated; nevertheless, artichoke leaves and external parts of bracts may be used in industrial processes to obtain functional metabolites for use in human health.

Species	Molecule	Part of plant	Specific function for human health	References
Cynara cardunculus L. var. scolymus L. / *Cynara scolymus*	Caffeoylquinic acids, flavonoids, and cynarin	Flower head and leaves	Antioxidant capacity	[7, 12–14]
	Chlorogenic acid and cynarin	Flower head and leaves	Choleretic, hepatoprotective	[168, 169, 170]
	Luteolin	Flower head and leaves	Hypocholesterolemic	[171]
	Inulin	Flower head, stem and leaves	Stimulates the intestinal flora, hypocholesterolemic	[170]
Allium ampeloprasum Var. porrum / *Allium porrum*	Polyphenols	White part and green leaves	Antioxidant capacity	[28]
	Ascorbate	White part and green leaves	Growth and tissue repair, antioxidant	[28, 30]
	Steroidal saponines	White part	Antiinflammatory, gastroprotective and antiulcerogenis	[31, 32]
	Methanolic extracts	White part and green leaves	Antimicrobial	[35]
	Steroidal saponines/ polyphenols	White part	Cytotoxic activity: a potential anticancer agent	[33, 35]
	Hydroalcoholic extracts	White part and green leaves	Hypolipidemic/ hypocholesterolemic	[172, 173]
Capsicum annuum L.	Polyphenols	Fruit	Antioxidant capacity	[37, 174]
	Dietary fiber	Fruit	Anticarcinogenic, prevents diabetes, hypocholesterolemic	[9, 175]
Coriandrum sativum L.	Essential oil, aqueous, methanolic and ethanolic extracts	Fruit and leaves	Prevention of several chronic degenerative disorders	[8, 44, 48]
	Essential oils	Fruit and leaves	Anticancer activity	[176, 177]
	Extracts	Seeds	Hypocholesterolemic	[51]
	Extracts	Seeds	Antidiabetic	[49]
	Diethyl ether and aqueous extracts	Fruit, seeds and leaves	Anxiolytic, sedatives, antidepressant	[51, 54, 56]
	Aqueous, ethanolic and chloroformic extracts	Aerial parts	Analgesic	[55]
	Ethanolic extract	Root	Antimicrobial	[48]
	Extracts	Aerial parts	Antioxidant activity	[47]
	Hydroalcoholic extract	Aerial parts	Anticonvulsant	[15]
	Ethanolic extract	Seeds	Cognitive effects (improves learning in the long term)	[53]

Table 1. Selected plant species and their compounds that are beneficial for human health.

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Inositol and inulin are soluble carbohydrates, which are present in external bracts of artichokes. In the case of inositol (chiro-scyllo- and myo-inositol), values fluctuate from 6.7 to 9.3 mg g^{-1} DW while for inulin they fluctuate from 69.8 to 114.6 mg g^{-1} DW [16]. These values are higher than the ones reported by Hernández-Hernández et al. [17] in edible bracts of artichoke. Regarding the beneficial properties, inositols have been used in treatments against diabetes mellitus [18]. In this sense, Crawford et al. [19] reported that inositol prevents diabetes mellitus in pregnant women and concluded that myo-inositol shows promising results by preventing the onset of the disease. Furthermore, inulin has been associated with some beneficial functional properties, as it can be a good source of carbohydrates and fiber, associated with positive effects in the prevention of colon cancer [20, 21]. In addition, prebiotic properties and effects on the absorption of calcium have been reported [20, 21]. In this sense, research on mineral absorption of calcium and magnesium concluded that inulin can reduce risk for osteoporosis by increasing their absorption [22].

2.2. Leek (*Allium ampeloprasum* var. porrum or *Allium porrum*)

It has been reported that this species has a substantial nutraceutical and functional properties (see **Table 1**) and is cultivated in Asia, America, and Europe, especially in the Mediterranean region [23, 24]. *A. ampeloprasum*, belongs to subgenus *Allium* section *Allium* (**Figure 2**), is considered a "complex species" due to different ploidy levels and genome constitution, and diploid, tetraploid, and octoploid accessions have been described [25, 26]. In fact, Hirschegger et al. [27] suggested that molecular evidence could be used to consider this species as a tetraploid horticultural group together with other *Allium* genus species. This condition is particularly important in accounting for its nutritional, functional, and nutraceutical characteristics [28].

Figure 2. Green leaves and white part of leek.

The principal beneficial properties of the *Allium* genus are mainly due to the presence of many sulfur compounds containing bioactive constituents, including: dimethyl disulfide, methyl propenyl disulfide, propyl propenyl disulfide, dimethyl trisulfide, methyl propyl trisulfide, methyl propenyltrisulfide, S-methyl cysteine sulfoxide, S-propyl cysteine sulfoxide, S-propenyl cysteine sulfoxide, and N-(γ-glutamyl)-S-(E-1-propenyl) cysteine [6].

In the evaluation of 30 leek cultivars, the content of total phenols varied from 5 to 15 mg gallic acid equivalents (GAE) g^{-1} DW for whole plants [28]. Other studies reported values of 5.5–6.0 mg GAE g^{-1} DW in whole leeks [29]. These differences in the total phenolic content could be attributed to the genetic variability of this species and agricultural systems [28]. Moreover, in the same study, the oxygen radical absorbance capacity (ORAC) was evaluated, where the green leaves possessed 82–135 μmol TE g^{-1} DW, whereas the white part contained just 27–88 μmol TE g^{-1} DW. Additionally, Vandekinderen et al. [30] determined the total vitamin C content (ascorbic acid + dehydroascorbic acid) whose values reached 9.65 mg 100 g^{-1} FW in whole leeks. Bernaert et al. [28] reported 5.54 mg ascorbic acid (AA) g^{-1} DW in whole leeks and higher values in green leaves than in white parts (2.77–8.52 mg AA g^{-1} DW and 0.89–3.55 mg AA g^{-1} DW, respectively). Values of polyphenols, AA, and antioxidant activity may be influenced by the season of year, genetic characteristics, and biotic and abiotic factors during vegetative growth, as well as agricultural practices.

The antiinflammatory, gastroprotective, and cytotoxic activities of organosulfate compounds, saponins, particularly steroidal saponines, have been well documented in *A. ampeloprasum* var. porrum [31–33].

Another property that is exclusive to the *Allium* genus is their antimicrobial activity (see **Table 1**). This has been reported since ancient times, and leeks have been used to treat wounds and respiratory diseases, as well as acting as an antibacterial agent due to the presence of alliin-containing structures [34, 35]. Polyphenols of methanolic extracts of green leaves and white parts of *A. porrum* are potent against Gram-positive (*Staphylococcys aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and Gram-negative (*Klebsiella pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *Proteus vulgaris* ATCC 13315 and *Proteus mirabilis* ATCC 14153) bacteria, as well as fungal species (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) [28]. These authors affirm a negative effect of methanolic extracts of *A. porrumon* Hep2c, L2OB, and RD cell cultures. This cytotoxic activity could indicate a future use of these natural biological compounds in human health.

2.3. Hot chili pepper (*Capsicum annuum* L. var. longum)

The fruit of this species (**Figure 3**), immature or mature and leaves, contains at least two groups of bioactive compounds of significance for human health, polyphenols and carotenoids. The polyphenol content is variable but reaches over 20 mg GAE g^{-1} DW in mature and dried fruits [36], and 40 mg GAE g^{-1} DW in leaves [37]. The polyphenols of fruits have a total antioxidant capacity of 26.6–44.4 μmol TE g^{-1} DW, depending on the variety [36].

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According to Serrano et al. [38], the small intestine has around 25% of bioavailability of total polyphenols.

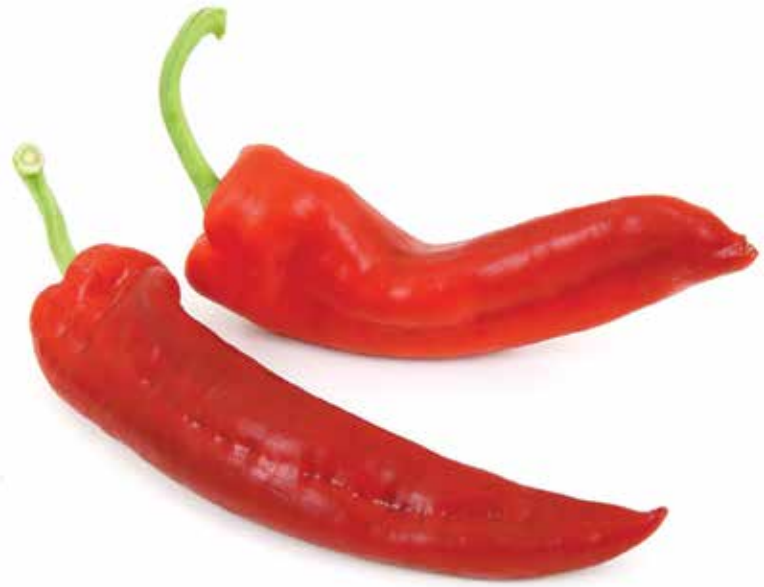

Figure 3. Immature fruit of red hot chili pepper.

Regarding the total carotenoids present in different varieties of red hot chili peppers, Hervert-Hernández et al. [36] indicated values from 87.6 to 373.3 mg 100 g^{-1} DW. In addition, the same authors determined that the bioavailability of chili carotenoids in the small intestine ranges from 20 to 50% of the total content, depending on the variety [36]. In addition, *C. annuum* is also a good source of vitamin C (up to 26.5 mg g^{-1} DW) [39]. Minerals, mainly potassium and magnesium, as well as dietary fiber, reducing sugars (around 20% DW), and antimicrobial activity useful for functional food production are also characteristic of this species [9]. Regarding the antimicrobial activity, De et al. [40] identified three pathogens that are susceptible to ethyl alcohol extracts of chili: *Bacillus subtilis* ATCC6633 (minimum inhibitory concentration (MIC) 10–25 mg mL^{-1}), *Escherichia coli* ATCC10536 (MIC 25–50% mg mL^{-1}), and *Saccharomyces cerevisiae* ATCC 9763 (MIC 2–5% mg mL^{-1}). In addition, an antimicrobial activity in *Staphylococcus aureus* ATCC14154, *Escherichia coli* ATCC-1698, *Pseudomonas aeruginosa* ATCC-23993, *Candida albicans* ATCC-14053, and *Sarcina lutea* (Collection of Microbiology Laboratory of Chemical Engineering Department, Institut Teknologi Bandung) has also been reported using ethanolic extracts of chili [41]. These authors indicate that capsaicin may be one of the main responsibles of microorganism inhibition. Likewise, Huang et al. [42] affirm that developments of *Staphylococcus aureus*, *Saccharomyces cerevisiae*, and *Aspergillus niger* were negatively affected by capsaicin.

2.4. Coriander (*Coriandrum sativum* L.)

This species of the *Apiaceae* family (**Figure 4**) is usually cultivated throughout the year in diverse edafoclimatic areas [43]. It is native to Italy and is currently propagated in several Mediterranean regions of Europe, as well as in America and Asia [44]. It is consumed principally fresh, either alone or in salads. This species has multiple human health benefits and has considerable potential as a functional horticulture species (see **Table 1**). *C. sativum* contains essential oils in seeds and in the pericarp whose content and composition appear to be dependent on biological and geographical traits [35]. The oil content is approximately 1% of seed weight of which linalool is the major component (73%) [44–46]. In the stem and immature leaves, the most important compounds are essential oils, flavonoids (quercetin, kaempferol, and acacetin), phenolic acids (vanillic acid, p-coumaric acid, syringic acid, p-OH benzoic acid, cis-ferulic acid, and trans-ferulic acid), and polyphenols [8]. The principal phytochemicals are β-carotene (5.1 mg g^{-1} DW), AA (1.16 mg 100 g^{-1} FW), total phenolics (2.05 mg GAE 100 g^{-1} DW), and antioxidants (1.12 mg GAE 100 g^{-1} DW) [47].

Figure 4. Stem and immature leaves of coriander.

According to Kumar et al. [48], ethanolic extracts of fresh coriander roots contain alkaloids, flavonoids, terpenoids, sterols, carbohydrates, saponins, and phenolic compounds. This extract and its fractions possess significant antibiotic activity against *Staphylococcus aureus, Bacillus cereus, Salmonella typhi*, and *Klebsiella*.

The antihyperglycemic (antidiabetic) activity of coriander has been studied by several authors. Deepa and Anuradha [49] analyzed the effects of coriander seed extracts in rats, which showed decreases by 44% in blood glucose and by 58% in glycosylated hemoglobin levels with respect to untreated rats. At the same time, the insulin level in plasma increased to 40%. They also reported beneficial effects in kidney and pancreas. Moreover, *C. sativum* seeds have hypocholesterolemic properties in rats, increasing hydroxymethyl glutaryl CoA (HMG-CoA) reductase and plasma lecithin cholesterol acyl transferase activities [50, 51].

Additional properties such as analgesic, anticonvulsive, anxiolytic, sedative, antidepressant, and cognitive effects of coriander have been tested *in vivo* in mice. In many cases, its effect was comparable with equivalent standardized doses of the typical drugs used to treat these diseases, elegantly demonstrating the beneficial properties of coriander in human health [15, 52–56].

3. Fruit species with functional properties and their potential use in human health

Fruits, in addition to horticultural species, constitute a group of foods for humans with important functional characteristics. In this chapter, we consider kiwifruit, sweet orange, and highbush blueberry given their extensive geographical distribution, consumption, and richness in biocompounds with nutraceutical properties.

3.1. Kiwifruit (*Actinidia deliciosa* [A. Chev] C.F Liang et A.R Ferguson/*Actidinia chinensis* [Planch])

This species originated in Asia [56] and belongs to the *Actinidiaceae* family (**Figure 5**). The *Actinidia* genus includes 66 species, but only four are cultivated for fruit production. Of these, *A. deliciosa* and *A. chinensis* are the most accepted by consumers worldwide [58, 59]. Here, we will refer to both species indistinctly. Kiwifruits have multiple sensory, nutritional, and phytochemical properties and are rich in dietary fiber, acids, phenols, and vitamins [57, 60] (see **Table 2**). These contribute to antioxidant activity, which varies with the variety and the part of fruit consumed. For example, Soquetta et al. [57] found higher values of antioxidant activity measured by the ferric reducing ability of the plasma (FRAP) method as well as carotenoids, flavonoids, and vitamin C in flour of the Monty variety compared to the Bruno variety. Moreover, the same authors found the highest content of these compounds in flours from kiwifruit skin compared to flour from kiwi fruit bagasse, reporting values from 59 to 189 mg AA in 100 g of kiwifruit flour, almost double that found in oranges and strawberries [61], and 200–1200 mg GAE 100 g^{-1} for phenolic compounds. D'Evoli et al. [60] indicated that in the total fresh kiwifruit, the content of oxalic acid was 8 mg 100 g^{-1} FW, while citric and malic acid contents were 1.2 and 0.24 g 100 g^{-1} FW, respectively. Furthermore, the same authors indicated that kiwifruits contain 90 mg GAE 100 g^{-1} FW of the total polyphenols, 0.2 mg 100 g^{-1} FW of lutein, and 0.06 mg 100 g^{-1} FW of β-carotene, in addition to α-tocopherol, γ-tocopherol, and γ-tocotrienol which represent 0.9, 0.04, and 0.12 mg 100 g^{-1} FW, respectively. All these compounds give kiwifruit

strong antioxidant properties that contribute to protect cells against oxidative damage [62]. Its antioxidant capacity (ORAC) varies from 0.06 to 1.4 μmol TE 100 g^{-1} FW of fruit, depending on the hydrophilic or lipophilic fraction used in the analysis [60]. Lee et al. [63], using the same method but expressed as vitamin C equivalents (VCE), reported values from 595.7 to 2662.7 VCE 100 g^{-1} FW. Clinical studies indicate that the uptake of vitamin C derived from kiwifruit reaches 40% in humans, similar to the rate of synthetic vitamin C uptake [64, 65]. This was tested by Vissers et al. [66] in a mouse model, where they found highly effective delivery to tissues. Together with vitamin C uptake, several other nutrients and beneficial phytochemicals are consumed, with synergistic effects, among them, of iron (Fe). A clinical study with women [67, 68] indicated that a breakfast fortified with Fe, when consumed with kiwifruit, can improve Fe content in women with low Fe stores; this may be related with the high values of AA, lutein, and zeaxanthin of kiwifruit.

Figure 5. Fruits of *Actinidia deliciosa* commonly name kiwifruit.

The dietary fiber content of kiwifruit is 2–3.39 g 100 g^{-1} FW [60, 69] of which 25–30% is found in the flour skin and fruit bagasse [57]. These values are higher than several of the widely consumed fruits such as orange, apple, banana, strawberries, and blueberries [61]. Thus, kiwifruits contain sufficient fiber thus improving digestive performance, ameliorating digestive transit, alleviating constipation, and irritable bowel syndrome [60, 70–72]. Studies performed in rat have reported that kiwifruits improve digestion of the principal proteins of beef muscle, soy protein, gelatin, and gluten [73]. This is related with its content of actinidin, a very active proteolytic enzyme, which acts in concert with the gastric and intestinal proteases, pepsin, and pancreatin, generating an increment in protein digestion in the gastric and intestinal tracts [74, 75].

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Species	Molecule	Part of plant	Specific function for human health	References
Actinidia deliciosa (A. Chev) C.F Liang et A.R Ferguson/*actidinia chinensis* Planch.	Dietary fiber	Fruit and flour skin and bagasse of fruit	Alleviate constipation/ laxative	[57, 60, 71]
	Actinidin	Whole fruit	Increment on protein digestion	[73 – 75]
	Polyphenols, flavonoids Vitamin C, E, lutein, zeaxanthin and other phytochemicals	Fruit and flour skin and bagasse of fruit	Antioxidant activity/ reduces cellular damage from oxidative stress	[57, 62, 71, 86]
	Vitamin C	Fruit	Positive synergic effect with nutrients and phytochemicals	[61]
	Essential amino acids, linolenic acid, folic acid, vitamins A, B6, B12, C, and E, minerals like Zn, Cu, Fe, and Se.	Fruit	Strengthen the immune system	[82, 84]
	Phytosterols and ursolic acid/independent or synergic actions of polyphenols, vitamin C, and E	Fruit	Improve lipid profile in men/reduction of risk of cardiovascular diseases	[61, 76, 77, 79, 86]
	Unidentified	Fruit	Antihypertensive	[79, 80]
	Unidentified	Fruit	Antithrombotic	[78]
	Vitamin C and inositol and other unidentified compounds	Fruit	Antiinflammatory activities	[71, 178]
	Phytosterols and ursolic acid	Fruit	Inhibit carcinogenesis processes	[86]
	Fiber	Fruit	Antiirritable bowel syndrome	[72]
Citrus sinensis (L.) Osbeck	Unidentified	Juice and ethanolic peel extract	Antihyperglycemic activity	[89, 104, 105]
	Flavonoids and other polyphenols	Juice	Antihypercholesterolemic activity	[107, 110, 111]
	Flavonoids other than anthocyanins	Juice	Antithrombotic	[112]
	Flavonoids and other polyphenols	Juice	Cardioprotective	[108, 111]
	Flavones	Juice	Enhanced antioxidant defense system	[114]

Species	Molecule	Part of plant	Specific function for human health	References
	Unidentified	Juice	Antiinflammatory activity	[119, 120]
	Flavones, flavonoids and flavonols	Juice	Microbial activity	[101, 122]
Vaccinium corymbosum L.	Unidentified	Juice	Hypoglycemic activity	[135]
	Unidentified	Juice	Antiinflammatory	[140]
	Interaction phenolic compounds	Fruit and leaf aqueous extract	Antimicrobial	[138]
	Anthocyanins and other phytochemicals	Fruit	Modulation of vascular function	[178, 179]
	Anthocyanins and other polyphenols	Extract hydroalcoholic of fruit	Cytotoxic activity	[142]
	Antioxidant action	Fruit	Antiatherogenic effect/hypocholesterolemic	[144]

Table 2. Selected fruit species and their compounds that are beneficial for human health.

Kiwifruits possess hypocholesterolaemic activity in hypercholesterolemic men [61]. This property may be related with the expression of the *Taq1B* gene in response to the consumption of kiwifruits, which modulates the content of lipids in blood plasma and has been associated with a reduced risk of cardiovascular diseases [76, 77]. However, other researchers did not find the same effect on cholesterol levels, but concluded that the consumption of two or three fruits per day can reduce levels of blood triglycerides by 15%, compared with the control [78]. Similar clinical studies have demonstrated that kiwifruits (two or three per day) can reduce blood pressure in male smokers [79], possibly related with 11% reduction in angiotensin-converting enzyme (ACE) activity. This finding is considered relevant because it is very difficult modulate hypertension by diet [79, 80]. In addition, a clinical study revealed that daily consumption of kiwifruit produces a reduction of 15% in platelet aggregation, which can be understood as antithrombotic activity [79, 81]. Nonetheless, Brevik et al. [81] discussed this effect because it may be influenced for the rate of kiwifruit consumption.

Additionally, Hunter et al. [82] and Skinner [83] affirm that kiwifruits have an important function in the modulation of the immune system. In this context, Hunter et al. [62] indicate that kiwifruit contribute significantly to lessening upper respiratory tract infections, head congestion, and sore throats in older individuals. Even though, there is a large source of variation in immune function, the nutrient status of this fruit is crucial. The most important phytochemicals present in kiwifruit include essential amino acids, linolenic acid, folic acid, vitamins A, B6, B12, C, and E, and minerals such as zinc, copper, iron, and selenium [84]. Given the type and content of phytochemicals, beneficial immune effects are not unexpected [82], although the mechanisms and the specific molecules underlying these effects are unknown. Moreover,

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preliminary studies under *in vitro* and *ex vivo* conditions found a protective effect of kiwifruit over oxidative damage of DNA, which may be interpreted as inhibition of the carcinogenesis process [85, 86]. Subsequently, Collins et al. [87] determined that kiwifruit consumption could protect against oxidative DNA damage protection in humans and *ex vivo* by both increasing the antioxidant status in the plasma, and stimulating DNA repair.

3.2. Sweet orange (*Citrus sinensis* (L.) Osbeck.)

The sweet orange is one of most economically important fruits in worldwide [88, 89]. It is believed that the *Citrus* genus is native to Asia, specifically from Southern China (Yunnan), which may be the origin and point of distribution of several contemporaneous *Citrus* species [90]. *Citrus sinensis* (L.) Osbeck belongs to the *Rutaceae* family and is believed to be a backcross hybrid between pummelo and mandarin (**Figure 6**) [91, 92]. The sweet orange species have several cultivated varieties, some of which are mentioned by Grosso et al. [93], but in this chapter we will discuss this species without distinguishing between varieties.

Figure 6. Fruits of *Citrus sinensis* commonly name sweet orange.

The sweet orange harbors several interesting phytochemical compounds that play an important role in human health (see **Table 2**). These include vitamins and polyphenols such as hesperidin, gallic acid, sinapic acid, caffeic acid, p-hydroxybenzoic acid, vanillic acid, narirutin,

naringin, p-cumaric, and ferulic acid [93–96]. Hesperidin is the major polyphenol of sweet oranges, accounting for over 77% of the flavonol content [98–100]. These compounds are present in the edible fruit, juice, and/or peel, and here we concentrate on the juice and the edible fresh fruit, due to their direct implications in human health. As a functional food, Letaief et al. [97] determined that the AA content in orange juice fluctuates from 551 to 614 mg L^{-1}, total phenolics range from 413 to 417 mg GAE L^{-1}, and flavonoids from 25 to 60 mg catechin equivalents (CE) g^{-1} DW. Roussos [94] measured total phenols (964–1215 mg TAE L^{-1}). The percentage of antioxidant activity of the juice, evaluated by the DPPH method, fluctuated from 36.4% to 56.6% [94, 97]. The antioxidant activity of sweet orange juice is dependent on the state of maturity of the fruit. Indeed, Adu et al. [101] noted higher levels of antioxidant activity in fruits of 3–6 months (over 80%) than in fruits of 10–12 months (around 70%). Fiber and amino acids are also important in juice. In this sense, Aschoff et al. [96] informed 1.4 g 100 g^{-1} of dietary fiber, and Roussos [94] mentioned that juice contains 18 amino acids (included the essentials amino acids), especially proline, arginine, asparagine, glycine, serine, and γ-aminobutyric acid. Other authors have determined some of these and other phytochemicals in homogenate orange segments without peel, where Aschoff et al. [96] reported 36.2 mg 100 g^{-1} FW of AA, 271.5 μg100 g^{-1} FW of carotenoids, and 13.6 g 100 g^{-1} FW of dietary fiber. Recently, Molan et al. [102] evaluated some compounds and properties of sweet orange seeds, such as total polyphenols (10.9–39.4 mg GAE g^{-1} DW) and antioxidant activity (around 50%) by the DPPH method.

It has been reported that to maintain sufficient antioxidant protection, an estimated average consumption of 60 and 75 mg d^{-1} of vitamin C is required for young women and men, respectively; however, it is suggested an increase of 35 mg d^{-1} for smokers [103]. This is important because orange consumption provides other phytochemicals with multiple benefits to human health. Several clinical studies confirm this assertion. For example, sweet orange juice also harbors antidiabetic activity, as determined in rats by metabolome analysis [104]. This agrees with research performed by Kumar and Bhaskar [105] in rats, using ethanolic orange peel extract, where blood glucose decreased around 60% with respect to the control after 3 weeks of treatment, similar to the drug, glibenclamide. Furthermore, Mallick and Khan [89] suggest a combination of juice of *C. sinensis* and *C. paradisi* in order to reduce the level of glucose and improve the insulin level in the plasma of diabetic rats.

Hypocholesterolemic activity was demonstrated in women with aerobic exercise and a consumption of 500 mL of sweet orange juice daily [106]. These authors found a 15% decrease of low-density lipoprotein (LDL-C) in serum and an 18% increase of high-density lipoprotein (HDL-L), whereas the ratio LDL/HDL-cholesterol decreased by 27%. Furthermore, they also noted an improved performance during physical activity, by a reduction of blood lactate. Moreover, a long-term study (twelve months) showed that consumption of orange juice (480 mL daily) triggered reductions of 11% in total cholesterol, 18% in LDL-cholesterol, 12% in apolipoprotein B, and 12% in the LDL/HDL ratio in comparison to nonconsumers [107]. In addition, an increase in antiatherogenic activity levels with the consumption of sweet orange juice was found [108–110]. Recently, it was informed that in rats, antihyperlipidemic activity is due to phytochemical compounds like flavonoids and other polyphenols with antioxidant capacity present in the juice of sweet oranges [111]. Therefore, the juice of sweet oranges may play an important cardioprotective role by preventing thrombosis [111]. In humans, orange

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juice intake also decreases procoagulant activity, possibly due to flavonoids, like anthocyanins, or other juice components [112].

Another feature of sweet orange juice that supports its cardioprotective role is its effect on diastolic blood pressure, which was significantly lower in men after the daily consumption of 500 mL orange juice for 4 weeks, and an enhancement of endothelium-dependent microvascular reactivity [113]. These authors also suggest that hesperidin could be related to the beneficial effect of orange juice in cardioprotection. Likewise, Rangel-Huerta et al. [114] related the reduction of blood pressure in obese adults with the consumption of at least 300 mg flavanones over 12 weeks. On the contrary, Schär et al. [115] found a relatively high flavanone and phenolic metabolite content in plasma, but no effects were observed on blood pressure and cardiovascular risk biomarkers. Additionally, Giordano et al. [116] reported that a daily intake of 1 L of orange juice for 4 weeks was not effective in reducing cellular markers associated with cardiovascular risks. Nevertheless, in general, more evidence of positive rather than neutral or negative effects on cardiovascular risk of sweet orange juice consumption exists. In fact, risk factors are mainly associated with metabolic syndromes such as cholesterol, blood pressure, and blood coagulation, and frequent intake of orange juice may be a useful delaying strategy [117].

The antiinflammatory activity of sweet orange juice has been reported by Mohanty et al. [118] where glucose induced an acute increase in ROS and inflammation, and orange juice intake prevented meal-induced oxidative and inflammatory stress [119]. Recent studies in rats revealed the positive effect of orange juice over histological and biochemical changes related with a progress in colonic oxidative status [120]. Besides, the antimicrobial activity of sweet orange juice has been reported by several authors. Recently, Adu et al. [101] indicated an inhibitory effect of orange juice from fruits at different stages of development against Gram-positive and Gram-negative bacteria and fungi, like *B. subtilis* NCTC 10073, *C. albicans* ATCC 10231, *E. coli* ATCC 25922, *P. vulgaris* NCTC 4175, *Pseudomonas aeruginosa* ATCC 27853, and *S. aureus* ATCC. Similar results on bacteria and fungi were found by Javed et al. [121] using essential oils of orange peel. This positive effect appears to be related with flavones, flavonoids, and flavonols [122].

3.3. Highbush blueberry (*Vaccinium corymbosum* L.)

The highbush blueberry is a species that belongs to the *Ericaceae* family (**Figure 7**) [123] exhibiting a high level of morphological diversity [124]. It is native to eastern United States and was domesticated during the twentieth century [125, 126]. Its distribution and consumption is extensive due to the human health benefits (antioxidant and mineral characteristics) of fruits and leaves [123] (see **Table 2**). This fruit has a wide range of phenolic compounds, especially flavonols, such as quercetin, as well as anthocyanins [127]. Some values of the main phytochemicals that contribute to antioxidant capacity are: phenolic compounds (261–585 mg g^{-1} FW), flavonoids (50 mg g^{-1} FW), and anthocyanins (25–495 mg g^{-1} FW) [128–130].

The antioxidant activity is higher in wild blueberry species, and part of this activity is conserved in cultivated varieties [131]. The total antioxidant activity of blueberry species ranges from 15.88 to 18.41 μmol Fe^{2+} kg^{-1} FW, using the FRAP reagent [130]. Contreras et al. [132] showed values near to 80% of antioxidant capacity measured by the DPPH method under *in vitro* conditions. The same authors affirmed that antioxidant capacity is related with the content of chlorogenic acid, myricetin, syringic acid, and rutin.

Figure 7. Fruits of *Vaccinium corymbosum* commonly name highbush blueberry.

Plasma antioxidant capacity (PAC) is considered a biomarker for antioxidant status of humans. In this context, Fernández-Panchon et al. [129] indicated that PAC increased following consumption of some foods rich in phenols, which could be related with *in vivo* bioactivity, and its consequent positive effects for human health. More specific biological properties of blueberry have been described, such as anticarcinogenic, antidiabetic, antiinflammatory, antimicrobial, and reducing cholesterol, among other activities [133–135]. The hypoglycemic activity of blueberry is mentioned by several authors. Aktan et al. [135] reported a severe case of hypoglycemia in a patient of 75 years old, who had diagnosed but untreated prediabetes. Just before the episode, this patient consumed about 500 mL juice of blueberry and *Laurocerasus officinalis*, which is also considered hypoglycemic. Similarly, Cheplick et al. [136] affirmed from *in vitro* studies that blueberry fruit has potential for diet-based management of hyperglycemia, especially in the early stages of disease.

Blueberry extracts also have antimicrobial activity, which have interest considering that many microorganisms are pathogenic to humans. In this line, a significant effect of extracts on *Listeria monocytogenes* and *Salmonella enteritidis* was found under laboratory conditions by Shen et al. [137]. In the same conditions (laboratory), other extracts from dried fruits and leaves were tested on contaminant/pathogenic microorganisms. The findings indicate good results in the inhibition of development of *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 27853, *K. pneumoniae* ATCC10031, *Acetobacter baumanii* ATCC 19609, *S. enteritidis* ATCC 3076, *Salmonella typhimurium* KCCM 11862, *Enterococcus faecium* LGM 11423, *Listeria innocua* NCTC 11286, *Bacillus cereu* ATCC 11778, and *P. aeruginosa* ATCC 27853 [134, 138, 139].

Zhong et al. [140] tested homogenized fresh blueberry juice as a therapy of juvenile idiopathic arthritis. The combined therapy of blueberry juice and etanercept (the typical drug used to treat this condition), improved the therapeutic effect of etanercept in patients with this pathology. Samad et al. [141] confirmed the antiinflammatory activity of extracts of blueberry in an *in vitro* study.

Yi et al. [133] studied the effect of phenolic compounds over colon cancer cell proliferation. Results indicated that these phytochemicals could inhibit the carcinogenic cells. Massarotto

et al. [142] demonstrated that anthocyanins and other phenolic compounds have cytotoxic activity, as tested in tumoral cell lines under *in vitro* conditions; thus, blueberry extracts could be useful for future treatment of cancer, as a natural cytotoxic agent. In this context, Tsuda et al. [143] obtained similar results with human leukemia cells and ethanolic extracts of several berries that include blueberry fruits. In all cases, the induction of apoptosis in the cancerous cells may be the mechanism triggered by blueberry.

The antilipidemic and antiatherogenic actions of blueberry have been reported by several authors. Coban et al. [144] indicated that the fresh fruit is food supplements that generate a positive effect over aorta and liver of hypercholesterolemic Guinea pigs [144]. In this respect, Cutler et al. [145] confirmed that berries are a special source of phytochemicals (anthocyanins and other phenolic compounds) and can be exploited as natural phytochemicals to contribute toward the amelioration of several chronic diseases, including those derived from alterations in the lipid profile in vascular systems.

3.4. Maracuyá (*Passiflora edulis* Sims)

Maracuyá (*Passiflora edulis* Sims) is a species that belongs to the *Passifloraceae* family (**Figure 8**) [146, 147] which is native to Brazil, South America. Nevertheless, some authors report that its real origin is Australia and is called *Passiflora edulis* forma flavicarpa [148, 149]. Variability studies have been carried out in South America, mainly in Colombia, as this region is particularly rich in this genus, although a low variability has been reported [150, 151]. Both scientific names *Passiflora edulis* Sims and *Passiflora edulis* forma are indistinctly considered in this chapter. Talcott et al. [152] identified several phenolic acids such as galacturonic acid, p-hydroxybenzoic acid, syringic acid, caffeic acid, p-coumaric acid, tryptophan, flavonoid glycoside, sinapic acid, ferulic acid, o-coumaric acid, and syringic acid in this species. Some compounds such as tryptophan, sinapic acid, and p-coumaric acid are in higher quantity with 733, 626, and 623 μg L^{-1} DW, respectively. Within the latter, total phenolic compounds fluctuated from 342.8 to 382 mg GAE L^{-1} FW [153]; total carotenoids varied between 22.4 and 29.1 mg L^{-1} DW, and the ascorbic acid content from 0.22 to 0.33 g kg^{-1} FW [152, 153]. The anthocyanin concentration in pulps and by product, on the other hand, were 3.48 and 3.7 mg 100 g^{-1} DW, respectively, while the flavonoids in pulps and by product were 60.3 and 40 mg 100 g^{-1} DW, respectively [154]. Regarding the above, Zucolotto et al. [155] indicated that C-glycosyl flavonoids are present in several species of *Passiflora* in South America. Furthermore, Da Silva et al. [154] informed that values for β-carotene fluctuated from 57.93 to 1362.07 μg 100 g^{-1} DW for both pulp and by product. It is worth noting, that piceatannol, a compound with an important antioxidant characteristic, is present in peel and seeds of the maracuyá fruit [156]. Moreover, the total antioxidant activity was found to reach values from 409.13 to 805.5 μM TE L^{-1} FW in this fruit [153], while Marcoris et al. [157] indicate values ranging from 1279 to 1460 μM TE L^{-1} FW. Total dietary and soluble fiber is another important characteristic attributed to this species, with values fluctuating from 35.5 to 81.5 g 100 g^{-1} DM. These values are higher in comparison to other tropical fruits such as *Mangifera indica, Ananas comosus*, and *Psidium guajava* [158]. The maracuyá fruit has several special characteristics that are beneficial for human health, described in **Table 2**. Within the latter, the most important properties are the sedative

and anxiolytic activities, which are common to several other species of the *Passiflora* genus [159]. Evaluation of aqueous extracts of pericarp fruit on rats concluded that a sedative effect was obtained with an oral administration of 300 mg kg^{-1} [160]. This effect was corroborated by a dose-dependent decrease of the locomotor-activity. Similar studies using ethanolic extracts of maracuyá leaves in rats exhibited sedative effects at 400 mg kg^{-1} [161]. Figueiredo et al. [162] reported that 130 mg kg^{-1} of bark flour of maracuyá fruits showed a sedative effect in rats. On the other hand, antiproliferative properties on cancer cells evaluated in SW480 and SW620 cells lines showed that cell growth in both lines was inhibited with 50–500 μg mL^{-1} of leaf ethanolic extracts and maracuyá fruit juice [163]. The polysaccharide peel of maracuyá fruits was also evaluated for its antiinflammatory effects and antidiabetic properties. In this context, the reduction of the inflammation was associated with the liberation or synthesis of histamine and serotonin, in response to a polysaccharide fraction of the maracuyá fruit [164]. Moreover, flour peel of maracuyá fruits in diabetic rats showed a decline in glucose content in the blood [165], probably associated with the high fiber level in this fruit tissue, which could prevent absorption of carbohydrates [166, 167]. Furthermore, triglycerides levels significantly decreased with 25 mg kg^{-1} of flour peel, however, no changes in total cholesterol levels were observed [165]. Still, Barbalho et al. [167] concluded that maracuyá fruit juice could improve the lipid profile, including the triglycerides, cholesterol, LDL-cholesterol, and HDL-cholesterol levels.

Figure 8. Fruits of *Passiflora edulis* commonly name maracuyá.

4. Conclusion and perspectives

A wealth of information in the field of phytochemical compounds and their impact on human health has been generated. Nowadays, it is possible to affirm that fruits and vegetables must be a part of daily diet. This is not simply a recommendation, but must be treated as an urgent

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requirement to ameliorate human health, especially in decreasing chronic nontransmissible diseases. We believe that additional efforts of governments and diverse organisms related with human health are necessary in order to highlight the benefits of these food types. Coriander and kiwifruit have remarkable characteristics and are excellent functional foods. We highlight these species for their wide range of benefits in different human diseases and their worldwide distribution. Likewise, further investigation is required to understand the mechanisms associated with several biochemical and physiological processes induced by fruit and vegetable intake in humans. Furthermore, we consider that leeks and artichokes have special potential as functional foods. Although there is a lot of information about the beneficial effects of fruits, we believe it is possible to extend studies to other organs like leaves and stems in artichokes, and roots in leeks, because they can offer additional benefits to human health.

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Chapter 10

Selenium Biofortification and the Problem of its Safety

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Additional information is available at the end of the chapter

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Abstract

Selenium is an essential mineral element to humans and animals because it is an important component of selenoproteins that are important for functioning of the metabolism. Because of poor soil conditions in various regions of the world, the enrichment of edible plants with selenium via the biofortification strategy has been implemented. However, selenium in the context of plant mineral nutrition appears twofold due to its biofortifying character at low concentrations and toxicity at high concentrations. In this sense, understanding of the functional mechanisms in which selenium is involved is important, ranging from its absorption and assimilation in organic compounds to its beneficial or harmful effects, considering its role in food security and human health. Therefore, this chapter addresses the key aspects related to selenium in the soil-plant-man environment and the narrow limit between biofortification and toxicity, as well as the main scientific findings on this mineral element in the biochemical, physiology and plant nutrition contexts.

Keywords: selenate, selenite, sulfur, toxicity, metabolism

1. Introduction

The discovery of selenium (Se) by Swedish chemist Jons Jacob Berzelius about 200 years ago initiated the start of several studies with this chemical element in living organisms [1, 2]. However, it was [3] who identified Se as an essential nutrient for the growth and development of bacteria, mammals and birds. It is currently considered as an essential micronutrient for humans and animals, as well as for certain lower organisms such as algae, fungi and bacteria [4].

Se is a trace element widespread in various geochemical environments [5], and its average content in the earth's crust is estimated at 0.05 mg kg^{-1}. However, concentrations exceeding 0.5 mg kg^{-1} can be found. Selenium levels are slightly higher in mafic rocks; however, they rarely exceed 0.1 mg kg^{-1}. In sedimentary rocks, Se is associated with the clay fraction, and thus, its abundance is higher in clayey sediments compared to sandstone and limestone. Selenium exists in four valence states, of which the state −2 predominates in organic compounds. The forms selenite (Se^{4+}) and selenate (Se^{6+}) do not form stable compounds in geochemical environments and are preferably adsorbed by minerals, particularly clay minerals, and oxides and hydroxides of Fe and Mn [6]. In the soil-plant system, the plant has the important role of recycling and releasing Se forms from the soil to the food chain. In this sense, the Se concentration in agricultural products and forage depends on the Se content in the soil and its availability [7–9]. However, Se availability in the soil is restricted, and its content is relatively low due to weathering and acidity [6, 7, 9, 10].

Because of the chemical similarity between Se and sulfur (S), the metabolism of Se in higher plants is closely related to S. Therefore, its absorption by the roots of plants is governed by the solubility of adsorbed forms and the transformation of organic Se forms (**Figure 1**). Due to the ability of plants to absorb Se, they can be classified according to Se accumulation in their tissues, including the classes of hyperaccumulators (1000–15,000 mg Se kg^{-1} dry weight), accumulators (>100 mg Se kg^{-1} dry weight) and non-accumulators (<100 mg Se kg^{-1} dry weight) [11]. Many species of the genera *Astragulus, Xylorrhyza* and *Stanleya* are typically Se accumulators because they are capable of growing in selenium-rich soil without showing any negative effect of Se toxicity, accumulating Se contents in the range of 20–40 mg Se g^{-1} DM while presenting no toxic effect [12, 13].

Because the range of Se concentrations in the soil varies, for example, from 8000 mg kg^{-1} in soils of the Russian city of Tuva to 0.005 mg kg^{-1} in soils of Finland and China [15], there is close relationship between Se deficiency in soils and the appearance of disease symptoms related to low Se intake in humans and mammals. Various efforts have thus been made to enrich agricultural feed crops with Se via fertilization or genetic breeding in a strategy called biofortification [16, 17]. According to this strategy, the supply of Se to the plant promotes a positive response in human health, preventing the onset of diseases related to low intake of this nutrient. In this sense, several studies report the positive effect of using Se in biofortification of lettuce [18], tomato [19], broccoli [20, 21], cucumber [22] and carrot crops [23].

In this context, biofortification of edible plants appears to be important from a nutritional point of view because selenium participates in constitution of selenoproteins, for example, the active site of antioxidant enzymes from the group of glutathione peroxidases (GSH-Px), which are reactive oxygen species detoxifying enzymes [24, 25]. There are several isoenzymes of the glutathione peroxidase family (with 4 g atom per mole of protein): cellular glutathione peroxidase (GSH-Px 1); glutathione peroxidase of the intestinal epithelium (GSH-Px 2); plasma glutathione peroxidase (GSH-Px 3); hydroperoxide phospholipid glutathione peroxidase (GSH-Px 4); and glutathione peroxidase present in the sperm (GSH-Px 5). Of these, GSH-Px 1 is the most abundant in mammalian selenoproteins [26]. Additionally, Se is of great importance

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to human health because of its presence in chemical constitution of the iodothyronine deiodinase enzyme, which is involved in metabolism of the thyroid hormone [27].

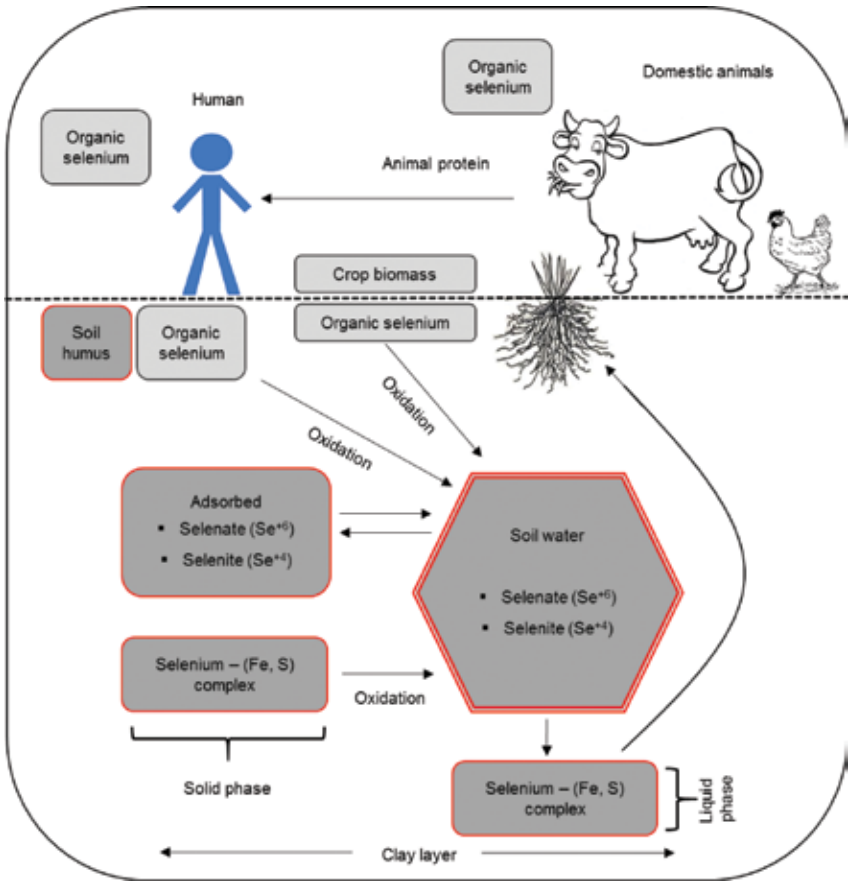

Figure 1. Selenium in the soil-plant-atmosphere system. Plants absorb selenate or selenite from the soil solution. Se concentration in the soil solution depends on the solubility of Se forms present and the biological transformation of organic Se forms. Adapted with permission from [14].

Despite the biofortifying character of Se in plants, it also has a toxic effect when provided to plants in high concentrations. This toxicity results from the substitution of S for Se in cysteine and methionine amino acids, reducing the number of disulfide bonds and this altering the structure and functionality of proteins, causing a negative impact on plant growth. Furthermore, another toxic effect of Se during its assimilation into organic compounds results in depletion of the nonenzymatic antioxidant, glutathione. Therefore, there is an imbalance between detoxification and formation of free radical species, which results in a significant oxidative burst and consequent reduction in plant growth [28].

It should be emphasized that biofortification/toxicity of Se is well reported in literature [18, 21–23, 29]; however, the transition between biofortification and toxicity by Se is narrow and

depends on the concentration and source, as well as the plant genotype. Despite this, literature does not report Se levels in plants based on determination of critical Se concentrations with regard to leaf content and Se concentrations in the culture medium. This shortcoming complicates the adoption of one Se concentration or a narrow range of concentrations that promote plant growth at the expense of biochemical, physiological and nutritional disorders promoted by toxic Se levels.

In this chapter, we sought to address the key aspects inherent to selenium and its functional relationships in the plant environment, as well as the intrinsic importance of food security regarding this nutrient, considering the main current scientific findings in the biochemical, physiological and nutritional fields of plants.

2. Selenium metabolism

In previous studies, it was shown that Se was absorbed by passive diffusion [30, 31]; however, it was recently shown that selenate is absorbed by the sulfur carrier, while selenite is absorbed by the phosphate carrier, and both processes are dependent on energy expenditures [32, 33]. Among the inorganic (selenate and selenite) and organic forms of selenium [selenomethionine (SeMet) and selenocysteine], selenomethionine in canola and wheat plants is the form that present the highest absorption rate and rapid translocation to the shoots [34]. Due to the chemical similarity between S and Se, both present the same route of absorption and assimilation, competing for the same carrier membrane [16]. After absorbed through the sulfur carrier (Sultr) and translocated to the shoot, selenate can be assimilated in chloroplasts and reduced to selenite in a reaction catalyzed by the enzyme ATP sulfurylase (APS) and then into selenide [16].

Non-accumulating plants can concentrate Se because APS has limited catalytic activity. On the other hand, accumulating plants overexpress APS resulting in accumulation and tolerance to high Se concentrations. Selenide may be incorporated into the S-amino acid similar to selenocysteine which can be converted to selenomethionine (SeMet) in three enzymatic steps. Incorrect insertion of the amino acid selenomethionine/selenocysteine in proteins can cause the formation of protein aggregates that promote disruption of important cellular functions [35, 28]. The incorporation of Se in proteins may occur when Se is converted to less toxic forms, because some plant species present nonprotein organic compounds containing Se such as methylselenocysteine (MeSeCys), γ-glutamyl-MeSeCys and/or selenocysteine [36]. Se can be volatilized by plants through the dimetilselenide or dimetildiselenide compounds, synthesized from selenomethionine and methylselenocysteine, respectively [16]. Se accumulating plants have the synthesis of methyl-SeCys catalyzed by the enzyme SeCys methyltransferase (SMT), accumulating methyl-SeCys, a nonprotein amino acid. Furthermore, methyl-SeCys may be converted into dimetildiselenide, a volatile compound. Expression of the enzyme SMT in non Se accumulating plants increases accumulation of Se in the form of methyl-SeCys, and its activity is related to tolerance to Se accumulation [16].

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In the biochemical field, the metabolic pathways of plants are interconnected by means of some compounds. In the case of Se and nitrogen (N), the metabolism of these inorganic elements is interconnected by means of the O-acetylserine compound. Therefore, alterations to the S metabolism induced by Se interfere with that of N with respect to the metabolism of amino acids and proteins [16], considering that the amino acids methionine, phenylalanine, tyrosine and tryptophan are precursors of glucosinolate, while phenylalanine is a precursor of phenolic compounds. Thus, variations in the synthesis of these amino acids influence the synthesis of nutraceutical compounds such as glucosinolate and phenolic compounds [16].

Several studies report the positive impact of Se on the plant metabolism, particularly due to its abiotic stress mitigating effect. In this sense, Se plays an important role in increasing the activity of antioxidant enzymes to contribute to the detoxification of reactive oxygen species, considering that this mineral element participates in the active site of these enzymes. These enzymes, called glutathione peroxidases, appear quite active in plants subjected to various abiotic stresses such as drought stress [37, 38], salinity [39] and heavy metal toxicity [40], conferring stress tolerance to plants. This effect of Se is evident, because when supplied at concentrations of 10 and 50 μM of selenate beneficial effects were observed in wheat plants grown under appropriate and reduced N availability. In this study, Se promoted a better response of the parameters fluorescence and gas exchange, with a positive impact on the growth of wheat plants [41].

On the other hand, Se in toxic concentrations may compromise energy synthesis by redox reactions (*i.e.*, photosynthesis and respiration) due to substitution of S for Se in the cysteine amino acid residue. Cysteine constitutes an important site for binding and stabilization of Fe-S metal centers, heme groups and ions participating in the flow of electrons in the mitochondria and chloroplasts. In this regard, it is speculated that substitution of the cysteine amino acid residue by selenocysteine in proteins rich in Fe-S metal centers disturbs the flow of electrons in the mitochondria and chloroplasts [28]. This fact implies the reduction of energy synthesis, and consequently reduced plant growth.

Moreover, the reduction of selenite to selenate via the S metabolic pathway demands a great glutathione input, a biochemical component involved in important redox reactions in cellular homeostasis [42]. This fact explains the decrease in root growth of plants induced by selenate when it is assimilated into organic compounds, since there is a depletion in the cellular glutathione content [28]. This was observed in brassica plants of *Stanleya albescens* sensitive to Se toxicity, which when exposed to toxic concentrations of this element had their growth compromised due to oxidative stress caused by the increased leaf accumulation of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) in contrast to the more tolerant genotype *Stanleya pinnata*, which has a high glutathione content [43].

The metabolisms of nitrogen (N) and S are interconnected by means of the compound O-acetylserine [16]. Thus, the supply of Se to plants may interfere with the N metabolism. It was indicated that the supply of Se to barley plants reduced the nitrate assimilatory process because of reduced activity of the nitrate and nitrite reductase enzymes in leaf and root tissues. However, intensity of the reduction was greater when Se was supplied in the form of selenite [44].

It was recently demonstrated that toxic concentrations of Se promote reduction of plant growth in *Arabidopsis thaliana* due to incomplete mobilization of starch reserves overnight, reduced expression of genes encoding the synthesis of endotransglucosilase/endohidrolase enzymes and expansins, as well as nutritional disorders [45]. Therefore, despite the benefits of low Se concentrations, it has a large negative impact on the plant metabolism when provided in high concentrations on plant growth by affecting metabolic processes of energy acquisition, cell expansion, and absorption and assimilation of essential nutrients.

3. Selenium biofortification

Selenium is an essential inorganic element for humans and animals, and one of the organic forms of Se, methylselenocysteine, appears to be an effective food source of Se [16]. Se is incorporated into a range of selenoproteins involved in several important metabolic activities such as synthesis of thyroid hormones and antioxidative activity [46, 47]. Selenium is an important inorganic component for the antioxidant metabolism of enzymes, making up part of the active site of enzymes from the group of glutathione peroxidases (GSH-Px) which plays an important role in detoxification of free radicals. The GSH-Px catalyze the reduction of hydroperoxide radicals (H_2O_2 for instance) by the oxidation of glutathione (GSH), a nonenzymatic component of the antioxidative metabolism [48].

It is noted that the accumulation of Se in foods is closely related to the content of this nutrient in the soil. However, consumption of foods poor in Se or low ingestion of foods containing Se is associated with the emergence of numerous diseases such as cancer, type II diabetes, heart disease, pulmonary dysfunction, seizures in children, impaired development and cerebral functions, as well as pregnancy and conception [49–51].

Currently, Se deficiency affects about 1 billion people worldwide due to soils lacking this mineral nutrient in some countries [52]. This edaphic characteristic was registered in countries such as Sweden, Finland, USA and China [16, 53, 54]. Because there is a close relationship between plant mineral nutrition and human health, food fortification with Se, via a strategy known as biofortification, is an effective way to add Se to human food and prevent the emergence of diseases related to deficient Se intake. This strategy proved to be effective due to the fact that Se presents chemical similarity to S, and both have the same carrier membranes and biochemical pathway of assimilation [28, 33].

Because vegetables are considered an important source of bioactive compounds that contain polyunsaturated fatty acids, phytochemicals such as flavonoids and glucosinolates, many of which can inhibit cell proliferation, induce apoptosis and act synergistically when combined in foods [55], some vegetable groups are more suitable for biofortification because they are natural accumulators of Se such as brassicas [16]. This group of plants has significant levels of glucosinolates, a substance of great nutraceutical interest. In this context, recent studies confirm the biofortifying effect of Se in edible plants in the group of brassicas. For example, in a major study [21] showed the positive impact of Se biofortification in broccoli. This study

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found that the broccoli extract showed high levels of phenolic compounds, significant antioxidant effect and anti-proliferative activity of tumor cells on the effect of Se in the synthesis of glucosinolates and phenolic compounds, and anticancer activity, see [16, 56].

Although Se biofortification is a strategy undertaken in edible and domesticated plants, it can occur naturally, as in the Brazilian Amazon, where important food sources of Se are the shrub species of the family Lecythidaceae, *Bertholletia excelsa* Humb. and Bonpl and *Lecythis usitata* Miers, which produce nuts presenting high Se concentrations [57, 58]. For these species, biofortification is a natural phenomenon, because the average levels of Se in their nuts range from 0.03 to 512 μg g^{-1} [59, 60]. Thus, consumption of only one nut per day appears to be sufficient to meet the daily Se requirement of an adult, because according to the Scientific Committee on Foods of the European Commission, the human daily intake requirement of Se is around 55 μg dia^{-1} [27]. However, the production of nuts is seasonal and the season of low production affects the nutrition of people near regions of natural occurrence of these two species [61, 62].

Among domesticated plants of food interest, the biofortification strategy can be carried out by providing Se to the soil, nutrient solution or leaf. However, considering large-scale plantations, the crop cycle and the economic value of the Se sources can make biofortification expensive, with the need for less costly and more practical strategies. One example is pelletizing the seeds of Se accumulator species, like radish, because of its short life cycle. Another strategy is to add Se sources to fertilizers used in agriculture. This strategy was adopted by Whelan and Barrow [63] in cultivation of the grass *Trifolium subterraneum* L in a soil classified as podzol laterite. These authors used a fertilizer containing 1% Se, composed of the sources Na_2SeO_4 and $BaSeO_4$ in a 1:1 ratio, which is characterized by slow release of Se in the soil. This strategy proved to be interesting due to the fact that there is a synchrony between plant growth and Se liberation, a fact which favors Se absorption and culture biofortification based on soil-plant interaction.

4. Toxicity of selenium

From the point of view of plant nutrition, the effects of Se are twofold, since at low concentrations it can promote biofortification but at high concentrations it triggers toxicity in plants [64]. This duality of effects on plant growth is related to the narrow range between biofortification and toxicity, which in literature is not well established based on determination of critical Se levels. It is understood that the critical toxicity level in the soil or nutrient solution is that which implies 10% reduction in plant growth, since the benefit of fertilization reaches its maximum between 90 and 95% of relative growth of the culture [65]. In plants, the effect of Se toxicity is dependent on the Se concentration and source, as well as plant genotype. However, for the same Se concentration, selenite appears to be more toxic than selenate. This fact is justified by the rapid incorporation of selenite into organic compounds still in the root system [28, 66]. In general, symptoms of Se toxicity in plants are characterized by reduced growth, as observed in lettuce [67], spinach [68], cucumber [64] and pea [29]. Reduced plant growth, as a

symptom of Se toxicity, is based on two biochemical mechanisms related to Se absorption and assimilation in organic compounds.

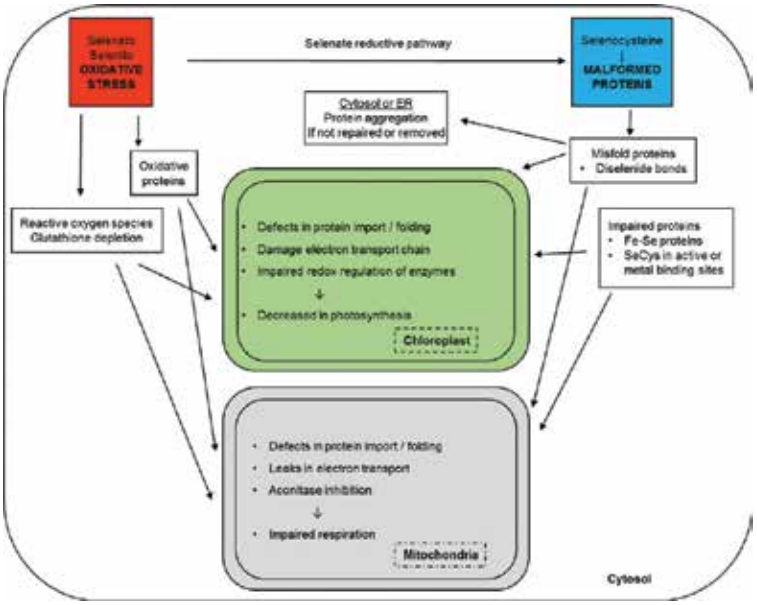

Figure 2. Model describing the two distinct mechanisms of Se toxicity in plants. Inorganic Se contributes to oxidative stress, while its reduction to selenocysteine may inadvertently substitute the amino acid cysteine and create malformed selenoproteins. The possible targets and ramifications of oxidative stress induced by Se and nonspecific selenoproteins are proposed. Adapted with permission from [28]. These symptoms were observed in the study of toxicity to Se sources and concentrations in the lettuce cultivar Vera, conducted under hydroponic conditions in a study of Ref. [71]. These symptoms were most intense in the source selenite at Se concentrations >8 μM (**Figure 3**). In this study (unpublished data), two response patterns were evident for lettuce plants with respect to Se sources and concentrations in the shoot, root and leaf area growth.

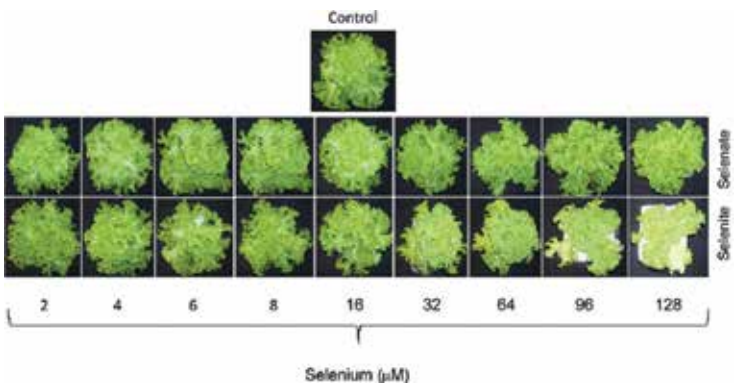

Figure 3. Visual aspect of applying increasing concentrations of selenite and sodium selenate to lettuce plants. Sources [71].

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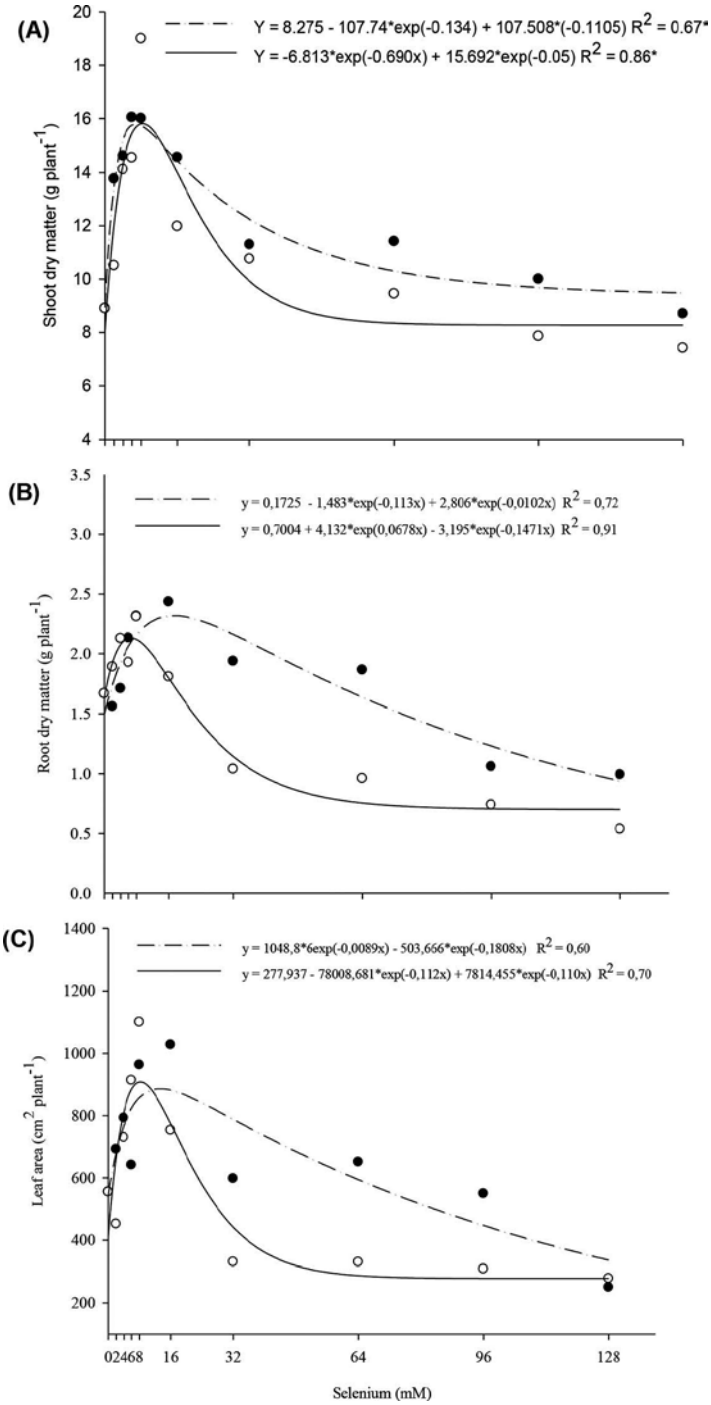

Figure 4. Leaf area (A), shoot (B) and root dry mass (C) of plants and the lettuce cultivar Vera submitted to selenium sources and concentrations in hydroponic cultivation. Selenite (–°–); Selenate (--•--). Source: [71].

The first mechanism is related to substitution of S for Se in the amino acids cysteine and methionine. This exchange of S for Se implies substitution of the amino acids cysteine and methionine for their analogs selenomethionine and selenocysteine during protein synthesis. This substitution is more deleterious in relation to cysteine, because this amino acid residue is of great importance in the structure and function of proteins and formation of disulfide bonds, enzymatic catalysis, metal bonding sites and redox state regulation. It is also suggested that iron as a metal cofactor complexed to selenocysteine can interrupt the flow of electrons in chloroplasts and mitochondria (**Figure 2**), since these cytoplasmic organelles have electron transport systems with supramolecular organization [28]. Therefore, any replacement of S by Se impedes electron flow due to changes in the protein conformational structure, and hence energy synthesis coordinated through the electron transport chain. The second mechanism is related to the participation of glutathione (GSH), a tripeptide active in cellular redox homeostasis regulation [69] and in the selenite reduction stage [28]. The participation of GSH in this step is crucial, since under Se toxicity conditions there may be a functional imbalance of GSH, since there is increased GSH demand for selenite reduction compared to hydroperoxide reduction in reactions catalyzed by glutathione peroxidase (**Figure 2**).

This imbalance in favor of selenite reduction triggers an oxidative burst that results in reduced plant growth [28]. However, plants considered accumulators or hyperaccumulators have biochemical mechanisms that prevent Se incorporation in proteins, by adding the methyl radical to the intermediate compound selenocysteine which is volatilized [16, 28]. From a symptomatology point of view, Se toxicity is characterized by reduced growth and chlorosis of plants [70].

In the lower concentrations of selenite or selenate, there was greater shoot, root and leaf area growth (**Figure 4A–C**). However, concentrations >14 and 16 μM of Se (selenite and selenate) for the shoots, and 9.7 and 30 μM (selenite and selenate) for the root resulted in growth reduction in these organs and more intense chlorosis in plants grown in the presence of selenite (**Figure 4A–C**). These responses were similar to those observed in lettuce [18] and cucumber [64] grown under sources and increasing Se concentrations.

5. Limits between selenium biofortification and toxicity

Plant growth is affected by excess Se, and the sensitivity of plants to toxicity of this mineral element is dependent on its concentration and source. However, in literature, the limits between Se biofortification and toxicity are not evident and based on critical toxicity levels which consider 10% reduction in plant growth [65, 72]. For example, Ríos et al. [18] observed that the concentration of 40 μmol L^{-1} selenate, in contrast to concentrations of selenite, was optimal for lettuce growth and biofortification. However, these authors reported a reduction of plant growth when the selenate concentration was >40 μmol L^{-1}.

It should be noted that the above results reported by the limits between Se biofortification and toxicity are not evident and based on critical toxicity levels which consider 10% reduction in plant growth [65, 72]. For example, Ríos et al. [18] contradict those presented by

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Hawrylak-Nowak [67], which places the limit of lettuce biofortification at concentrations 15 and 20 μmol L^{-1} for the sources of selenite and selenate, respectively, by the fact that no negative impact was observed on growth and the content of photosynthetic pigments at these Se concentrations. To investigate the effect of Se in lettuce, Ríos et al. [73] observed that the provision of selenate was not toxic up to the concentration of 80 μmol L^{-1}. On the other hand, selenite concentrations >5 μmol L^{-1} showed symptoms of toxicity related to reduced growth. These authors attributed growth reduction in plants to the nutritional imbalance of macro- and micronutrients, as well as the oxidative stress caused by toxic levels of selenite used in the study.

In cucumbers, the application of selenate and selenite showed toxic effects with in concentrations >80 and 20 μmol L^{-1}, respectively [64]. In this study, leaf area, leaf and root fresh weight were severely reduced at selenite concentrations >20 μmol L^{-1}. These authors showed that the concentration of chlorophyllian pigments and fluorescence of chlorophyll were more negatively affected by selenite compared to selenate.

It is known that the human health and plant nutrition are closely linked to soil fertility, and diseases associated with Se deficiency are documented in areas where the content of this element in soil is low, as in China, Denmark, Finland, New Zealand and central and eastern Siberia [74, 75]. However, there is no record of human intoxication by Se due to consumption of plant species of food interest (*e.g.*, corn, rice, beans, wheat, barley, etc.), presumably due to the fact that these plants are not Se accumulators. An interesting fact was recorded on a farm in the state of South Dakota, United States, where the owners recorded low hatchability of chicken eggs or chicks born with deformities and soon died. When investigating the fact, the United States Department of Agriculture identified high levels of Se in wheat grains which were used to feed the chickens [76]. Although literature relates the intake of foods rich in Se to the lower risk of emergence of diseases such as colon, stomach, prostate and lung cancer [51], there are studies that relate Se supplementation to increased risk of death due to prostate cancer [77]. However, the population along the Tapajos River in the Brazilian state of Pará presents high levels of Se in their blood in function of the considerable consumption of nuts from *Bethollethia excelsa* and *Lecythis usitata*, which have high Se levels. In these nuts, the Se concentration is quite variable, within the range from 0.03 to 512 mg g^{-1} [59], well above the daily intake recommended by the Scientific Committee on Foods of the European Commission, which recommends a daily intake of approximately 55 μg day^{-1} [27]. Nevertheless, in this population, the consumption of nuts rich in Se appears to protect against the emergence of diseases resulting from advancing age such as cataract and motor dysfunction [78]. The recommended daily Se intake (**Table 1**) varies in function of age, gender, pregnancy and lactation. Among the sexes, there is no difference with regard to ingestion demands, except when women are pregnant or lactating, where intake should be increased by 9 and 27%, respectively [79]. However, the maximum tolerable daily Se limit at which there occurs initial toxicity symptoms (**Table 2**) characterized by hair loss, and the appearance of brittle nails is similarly dependent on sex, age, pregnancy and lactation [79].

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	45 mcg*	45 mcg*		
7–12 months	60 mcg*	60 mcg*		
1–3 years	90 mcg	90 mcg		
4–8 years	150 mcg	150 mcg		
9–13 years	280 mcg	280 mcg		
14–18 years	400 mcg	400 mcg	400 mcg	400 mcg
19* years	400 mcg	400 mcg	400 mcg	400 mcg

Source: [79].
*Adequate Intake (AI).

Table 1. Recommended dietary allowances for selenium.

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	15 mcg*	15 mcg*		
7–12 months	20 mcg*	20 mcg*		
1–3 years	20 mcg	20 mcg		
4–8 years	30 mcg	30 mcg		
9–13 years	40 mcg	40 mcg		
14–18 years	55 mcg	55 mcg	60 mcg	70 mcg
19–50 years	55 mcg	55 mcg	60 mcg	70 mcg
51+ years	55 mcg	55 mcg		

Source: [79].
*Breast milk, formula, and food should be the only sources of selenium for infants.

Table 2. Tolerable upper intake levels for selenium.

6. Conclusions and future perspectives

This chapter addressed the main issues referring to the mineral element selenium, its history of discovery, its presence in the soil-plant system and its main functions in mammals and humans, always based on recent scientific findings that can guide students, teachers and researchers in their studies. Also assessed were the mechanisms of absorption, transportation and assimilation of selenium in organic compounds, as well as its biofortifying and toxic effect in plants. Moreover, its essentiality in mammals has been addressed in order to emphasize its importance in food and human health due to its function in the human metabolism. It was therefore sought to present the importance of this nutrient in the plant-human system, and therefore provide information for future studies seeking to clarify other functional aspects of selenium in plants.

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Chapter 11

Functional Properties and Maillard Reaction Product Formation in Rye-Buckwheat Ginger Cakes Enhanced with Rutin

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Additional information is available at the end of the chapter

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Abstract

A study of functional properties and Maillard reaction progress in rye-buckwheat ginger cakes supplemented with low and high rutin amount was conducted. The cakes were formulated on rye flour substituted by flour from husked buckwheat or flour from roasted buckwheat groats at 30 % level. The dough was either spontaneously fermented for 72 h at 21 °C, or the fermentation step was omitted. The cakes were baked at 180 °C for 18 min. Fortification of rye-buckwheat ginger cakes by low and high level of rutin was associated with the beneficial progress of the Maillard reaction towards the formation of melanoidins, while furosine formation at the early stage of Maillard reaction was decreased. However, loss of the nutritional value due to the formation of high amount of fluorescent (carboxymethyllysine (CML)) and fluorescent-intermediate compounds was observed. It has also been proved that rye-buckwheat ginger cakes fortified with low and high rutin doses are a rich source of biologically active compounds. Therefore, the cakes showed a high ability to inhibit the formation of advanced glycation end products (AGEs) in vitro and revealed high antioxidant potential. These findings may be important factors in complete evaluation of functional properties of ginger cakes. Stronger influence of rutin enrichment was observed in cakes baked from fermented-like dough than without this process.

Keywords: maillard reaction products, antioxidant potential, AGE inhibition, bioactive compounds analysis, buckwheat-based product, ginger cakes

1. Introduction

Ginger cakes are traditional pastries from Central and Eastern Europe. The very important feature of ginger cakes is their ability to remain fresh and savoury for a long time. Currently, ginger cakes are baked on the basis of wheat and rye flour. However, a traditional recipe for ginger cakes was based on the use of rye flour. In Poland, rye (*Secale cereale* L.) is an important grain for bread making and cookies production; therefore, in 2012 18 % of cereal products were made of rye [1]. Rye grain is considered to be a good source of biologically active compounds like antioxidants [2]. Referring to the up-to-date literature, it is highlighted that there is a lack of wider use of buckwheat in pastry.

The buckwheat is a rich source vitamin B1 and B2, lysine, protein with balanced amino acid composition [3], flavonoids [4], phytosterols [5], soluble carbohydrates, D-chiro-inositol and other fagopyritols [6] and thiamin-binding proteins [7]. Buckwheat is also rich in antioxidant compounds such as flavonoids, phenolic acids, tocopherols, reduced glutathione, inositol phosphates and melatonin [8]. Furthermore, buckwheat contains a high amount of rutin (quercetin-3-rutinoside) and has antioxidant, anti-inflammatory and anticarcinogenic properties [9]. According to various chemical compositions, buckwheat-based products were found to display several biological activities, including the increasing number of lactic acid bacteria in rat intestine, treatment of allergic inflammation, reducing the serum glucose level, suppressing cholesterol level, inhibiting protease and scavenging free radicals [10, 11]. These healthy and dietary benefits of buckwheat are main aspects in determining the usage of buckwheat to produce functional products.

The wide spectrum of buckwheat-based bakery and pastry products, e.g. bread, biscuits, crackers, cookies or muffins, was designed by researchers [12]. Mancebo et al. [13] observed that consumers' rating of cookies prepared from buckwheat did not reach high quality score which was mainly related to unpleasant and pungent taste of buckwheat. Therefore, Filipčev et al. [14] noted that 30 % of buckwheat flour is appropriate to create buckwheat-based product with high sensorial acceptability. Chlopicka et al. [15] also showed that 30 % addition of buckwheat flour is highly acceptable, and moreover buckwheat bread has a high antioxidant potential. Moreover, while analysing gluten-free products' sensory profiles, Loredana et al. [16] suggested that the optimum buckwheat flour addition is different for cake, cookies and muffins. The optimum amount in cake was established on the level of 30 % and 10 % for cookies, while for muffins 20 %. Not only the optimisation of the recipes but also technological process parameters for buckwheat-based product preparation have acquired an increasing interest. Lee [17] achieved high overall acceptance for steam bread (wheat with 3 % of buckwheat flour). However, the addition of buckwheat flour was not as high as in the previous studies yet, during the steaming process, not as high amount of an undesirable Maillard reaction products may be formed. Moreover, it is said that dough fermentation step can lead to nutritive and antinutritive compound formation, but some studies also suggested that fermentation process negatively influenced sensory properties of Turkish bread yufka supplemented with 10 % of buckwheat flour [18, 19].

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The baking process is inherent in Maillard reaction product formation. The Maillard reaction is a reaction which might have nutritional and toxicological effects on processed food. The Maillard reaction is initiated by the reaction between the carbonyl group of a reduced sugar and a free amino group of proteins, and then subsequent and parallel reactions go. The progress of the reaction can be considered in the context of early, advanced and final Maillard reaction product formation such as furosine (ε-N-2-furoylmethyl-L-lysine) [20], fluorescent intermediary compounds (FIC) formed at the advanced stage [21], carboxymethyllysine (CML) [22] and melanoidins [23]. The latter are responsible for product's colour formation, nevertheless possessing the ability to scavenge free radicals. The degradation of proteins is usually expressed as FAST index [24], based on the measurement of the fluorescence of tryptophan and formation of intermediatory compounds. Moreover, in thermally treated food *via* Maillard reaction, dietary advanced glycation end products (dAGEs) can be formed [25]. dAGEs are an important contributor to the total pool of AGEs formed in the living organism and can induce oxidant stress and inflammation resulting in increasing risk of diabetes and cardiovascular diseases [26]. The AGE accumulation in the body can be regulated by low dAGE diet or by consuming food with natural AGE inhibitors such as plant extracts and plant products, which are good source of antioxidant polyphenols [27, 28]. Thereby, the high antiglycation potential of wheat-buckwheat bread extract has been recently reported [29].

Gathering all the above-mentioned information led to enriching the traditional ginger cakes recipe with 30 % of light buckwheat flour or flour obtained from roasted buckwheat groats. The new recipe for buckwheat ginger cakes has been created, which fits well with contemporary trends in the bakery. Furthermore, characterisation of the changes occurring during fermentation and baking processing and effect of rutin supplementation should be valuable for the understanding of quality and safety of buckwheat ginger cakes. To achieve the aim, the evaluation of the total phenolics; rutin; products of early, advanced and final Maillard reaction stages; and antioxidative capacity of rye-buckwheat ginger cakes enriched with rutin was addressed in this study.

2. Research methods

2.1. The rye-buckwheat ginger cake preparation

The ginger cakes were prepared using a mix of rye flour (70 %) and light buckwheat flour/flour from roasted buckwheat groats (30 %). Then the rye-buckwheat ginger cake recipe was modified by the addition of low (50 mg/of rutin/100 g of flour mix) and high rutin (100 mg/of rutin/100 g of flour mix) dosage. The rutin dosage added to buckwheat ginger cakes was adjusted to rutin content in one tablet of OTC drugs. The control cake was prepared from rye flour. The ingredient list used for rye-buckwheat ginger cake preparation is included in **Table 1**. All the ingredients were well mixed, and then half of dough was set aside, and the other half was cut into regular discs and baked at 180 °C for 18 min. The first half of dough was spontaneously fermented for 72 h at 21 °C in fermented chamber. Then, the fermented dough was prepared as the previous one. The cakes were freeze-dried and powdered after

baking and cooling. The powdered samples were stored at −20 °C until analysis of functional properties and Maillard reaction product formation.

Ingredients	RGC	BERGC-1	BERGC -1L	BERGC -1H	BERGC -2	BERGC -2L	BERGC -2H
Rye flour [g]	100	70	70	70	70	70	70
Light buckwheat flour [g]	–	30	30	30	–	–	–
Flour from roasted buckwheat groats [g]	–	–	–	–	30	30	30
Buckwheat honey [g]	50	50	50	50	50	50	50
Sugar [g]	20	20	20	20	20	20	20
Baking soda [g]	3	3	3	3	3	3	3
Butter [g]	25	25	25	25	25	25	25
Spice mix for ginger cakes [g]	2	2	2	2	2	2	2
Rutin [mg]	0	0	50	100	0	50	100

Table 1. The list of ingredients used for rye-buckwheat ginger cake formulation.

Sample description: RGC (control), rye ginger cake; BERGC-1, buckwheat-enhanced rye ginger cake formulated on (1) light buckwheat flour; BERGC-1L, buckwheat-enhanced rye ginger cake with low rutin dose; BERGC-1H, buckwheat-enhanced rye ginger cake with high rutin dose; BERGC-2, buckwheat-enhanced rye ginger cake formulated on (2) flour from roasted buckwheat groats; BERGC-2L, buckwheat-enhanced rye ginger cake with low rutin dose; and BERGC-2H, buckwheat-enhanced rye ginger cake with high rutin dose.

2.2. The determination of total phenolic, rutin contents and antioxidant capacity in ginger cakes

The initial step included preparation of ginger cake extracts. Therefore, 100 mg of powdered samples was extracted with 1 ml of 80 % (v/v) methanol solution. Then, the mixture was treated by ultrasounds (30 s) and vortexed (30 s) three times. After centrifugation (6860 rpm at controlled temperature 4 °C, 5 min) the supernatant was collected into 5-ml flask. That step was repeated five times to achieve the final extract concentration 20 mg/ml.

The total phenolic content (TPC) was measured using Folin-Ciocalteu reagent according to Przygodzka et al. [30], whereas the rutin content was determined with HPLC with UV detector (330 nm), reported by Zielińska [31]. The antioxidant properties were determined by measurement of scavenging ability against ABTS radical cation, DPPH radical and superoxide anion radical ($O_2^{\bullet -}$) [30]. The measurements were carried out using spectrophotometer UV-160 1PC (Shimadzu, Japan) and Photochem® apparatus (Analytical Jena, Germany). The Trolox was used as a standard.

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2.3. The Maillard reaction product determination: furosine, fluorescent intermediary compounds (FIC), carboxymethyllysine (CML) and melanoidins

In these buckwheat ginger cakes, markers of early (furosine), advanced (carboxymethyllysine (CML), total fluorescent intermediate compounds) and final (melanoidins) Maillard reaction compounds were identified and quantified.

The furosine content was determined as described by Delgado-Andrade et al. [32], using HPLC (Shimadzu, Japan) with PDA detector setup at 280 nm. To determine the furosine quantity, the external standard of furosine at concentration range 0.2–9 μg/ml was applied. In the next step, the fluorescent intermediary compounds (FIC) was measured. The total sum of intermediatory compounds in buckwheat ginger cake extracts was determined according to procedure described by Delgado-Andrade et al. [21]. The analysis was followed by enzymatic hydrolysis step using pronase E to break the bindings between intermediatory compounds and proteins. The fluorescent readings were registered at extinction wavelength 347 nm and emission, 415 nm, using a luminescent spectrofluorometer (LS-50B, PerkinElmer, USA). The results are expressed as fluorescence intensity (FI) per milligram of dry matter.

The degradation of proteins (nutritional value) was expressed as FAST index according to Damjanovic Desic and Birlouez-Aragon's procedure [24]. The FAST index was calculated as a ratio of the fluorescence of intermediatory compounds, measured at extinction wavelength 347 nm and emission, 415 nm, using a luminescent spectrofluorometer (LS-50B, PerkinElmer, USA), to fluorescence of tryptophan (extinction ,290 nm, and emission, 340 nm) and described as a percentage.

The carboxymethyllysine (CML), one of the intermediatory compounds, was quantified and determined by HPLC method. The CML extraction was followed by a detailed description of Peng et al. [9]. The OPA reagent solution, which is a mixture of 10 mg of *o*-phthaldialdehyde (OPA) in 2 ml of methanol and the CML determination, was evaluated by HPLC (Dionex, USA) with fluorescent detector (SFLD-3400RS, Dionex, USA). The detector settings were established as the excitation wavelength 455 nm and emission, 340 nm, whereas the oven temperature was adjusted at 35 °C and flow rate 0.2 ml/min. The CML was separated on Luna® 3 μm C18 column (Phenomenex, USA) and eluted in isocratic gradient by water with 0.05 % of *o*-phosphoric acid and acetonitrile with 0.05 % of *o*-phosphoric acid. For quantitative analysis, calibration curve of CML standard was prepared in the range from 2.5 to 20 μM. The results were expressed in μg per gram of dry matter.

The formation of final Maillard reaction products was estimated as reported previously by Zieliński et al. [33]. The absorbance of buckwheat ginger cake methanolic extracts was measured at 410 nm using UV-Vis spectrophotometer (Shimadzu, Japan). Final results were expressed as the absorbance units (AU).

2.4. The evaluation of buckwheat ginger cake inhibitory activity against advanced glycation end-product formation

The inhibitory effect on formation of advanced glycation end products (AGEs) in ginger cakes was studied according to the procedure described by Szawara-Nowak et al. [29] in two in vitro

model systems: bovine serum albumin-glucose (BSA-glu) and bovine serum albumin-methylglyoxal (BSA-MGO). The fluorescence intensity was measured at the excitation wavelength 330 nm and emission 410 nm using a luminescent spectrofluorometer (LS-50B, PerkinElmer, USA). The results are expressed in percentage inhibition of AGE formation. Aminoguanidine solution (1 mmol/l) was used as a positive control in this experiment.

3. Results and discussion

3.1. The results of total phenolic and rutin contents and antioxidant capacity in buckwheat ginger cakes

Table 2 shows total phenolic content (TPC) in rye-buckwheat ginger cakes prepared with and without dough fermentation-like step, respectively. The substitution of rye flour by buckwheat flours at level of 30 % w/w on total flour basis resulted in higher TPC values in rye-buckwheat ginger cake BERGC-1 and BERGC-2 obtained without dough fermentation-like step than RGC, 52 and 85 %. In contrast, this effect was not seen in BERGC-1 and BERGC-2 cakes after dough fermentation-like preparation. Furthermore, the addition of low and high rutin doses increased the TPC. The highest TPC was noted in BERGC-2H cake after dough fermentation-like process, whereas the result was almost two times higher than in BERGC-2. Higher TPC values were observed in ginger cakes after 72 h at constant 21 °C fermentation-like process. It can be related to the positive influence of fermentation process of dough on effective formation of antioxidants [34]. Moreover, all types of rye-buckwheat ginger cakes reached higher TPC values than did ginger nut biscuits [14]. It means that this innovative buckwheat-based product is a good source of phenolic compounds.

It is generally known that rutin is the main bioactive compound in buckwheat-based products [9], and for this reason, the rutin content in rye-buckwheat ginger cakes was analysed. The results of rutin content are presented in **Figure 1**. The rutin identification in RGC, obtained both with or without dough fermentation-like process, was related to the buckwheat honey usage in the recipe. Except for buckwheat honey, another crucial source of this flavonoid was light buckwheat flour and flour from roasted buckwheat groats. Therefore, rutin content increased almost twice in BERGC-1 and BERGC-2 cakes due to the 30 % substitution of rye flour by buckwheat flours. The highest rutin content was noted in BERGC-1H cake after dough fermentation-like process; this result was almost 25 times higher than in BERGC-1. In the case of cakes with omitted fermentation-like step, it was noted that BERGC-1 and BERGC-2 with low and high rutin doses have 7 times higher rutin content in BERGC-1L and 15 times in BERGC-1H. The analogous tendency was observed in BERGC-2L and in BERGC-2H, 12 times and 15 times, respectively. Moreover, a similar trend was noted in BERG-1 and BERG-2 obtained after dough fermentation-like process. Generally, the fermentation-like process caused some changes in dough, resulting in higher rutin content in cakes after this process. Compared to Filipčev et al. [14], the rutin content in rye-buckwheat ginger cakes (BERGC-1 and BERGC-2 with dough fermentation-like step) was higher than it was noted in ginger nut biscuits (wheat-buckwheat flour, 70:30).

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Type of ginger cakes	TPC	DPPH	ABTS	PCL
Dough *without* fermentation-like preparation				
RGC	2.52 ± 0.10eG	5.15 ± 0.65fI	17.96 ± 0.87cE	3.45 ± 0.07fG
BERGC-1	3.85 ± 0.27dF	7.53 ± 1.14e	28.12 ± 2.08aA	7.95 ± 0.71bcC
BERGC-1L	3.79 ± 0.16dF	9.16 ± 0.10dE	23.94 ± 1.12bC	7.64 ± 0.07cdC
BERGC-1H	4.97 ± 0.16cD	9.37 ± 0.24dE	27.18 ± 0.74aA	10.36 ± 0.08aA
BERGC-2	4.66 ± 0.32cD	8.74 ± 0.77eF	23.48 ± 0.70bB	6.93 ± 0.12dD
BERGC-2L	5.52 ± 0.36bC	12.15 ± 0.36bC	8.64 ± 0.28dG	5.49 ± 0.56eE
BERGC-2H	7.20 ± 0.46aB	17.74 ± 0.29aA	24.49 ± 0.53bB	8.65 ± 0.79bB
Dough *with* fermentation-like preparation				
RGC	4.80 ± 0.14dD	6.87 ± 0.05fH	17.41 ± 0.87cE	4.84 ± 0.06fF
BERGC-1	4.91 ± 0.23cdD	7.20 ± 0.21efG	20.19 ± 1.16abCD	5.93 ± 0.80cE
BERGC-1L	5.71 ± 0.49bC	7.78 ± 0.28dG	21.25 ± 0.37abC	5.96 ± 0.53cE
BERGC-1H	5.80 ± 0.42bC	8.18 ± 0.08cF	21.62 ± 0.90aC	7.32 ± 0.42bC
BERGC-2	4.26 ± 0.14eE	9.40 ± 0.35cE	19.72 ± 0.36cD	4.91 ± 0.79cdF
BERGC-2L	4.63 ± 0.36deD	11.02 ± 0.45bD	14.23 ± 0.65bF	5.97 ± 0.62cE
BERGC-2H	8.43 ± 0.37aA	14.55 ± 0.68aB	21.03 ± 1.41abC	8.97 ± 1.17aB

Table 2. The total phenolic content (TPC) and antioxidant capacity of rye-buckwheat ginger cakes determined by DPPH, ABTS and PCL methods.

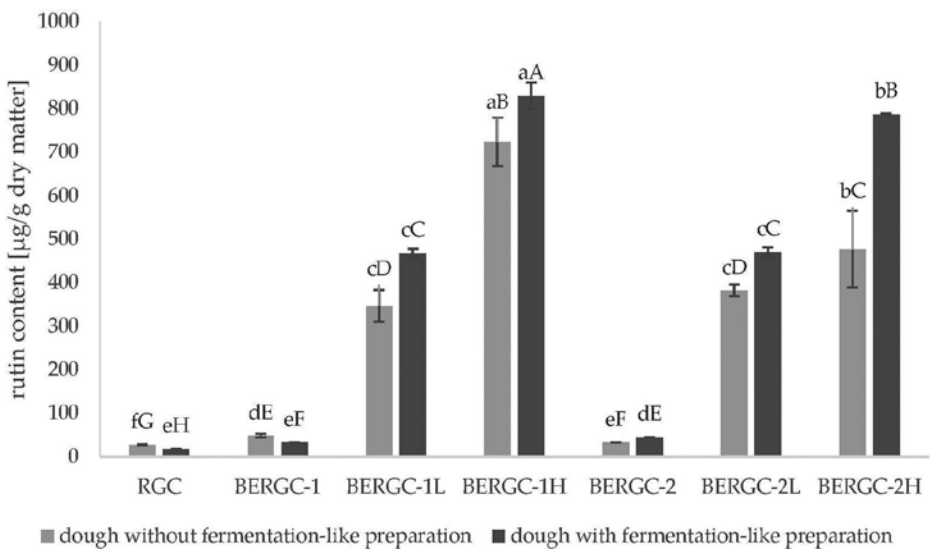

Figure 1. Rutin content in rye-buckwheat ginger cakes from dough obtained with or without fermentation-like preparation. Sample description under **Table 1**.

The 80 % methanol-water extracts of rye-buckwheat ginger cakes were examined for their free radical scavenging activity against $DPPH^{\bullet}$, $ABTS^{+\bullet}$ and $O_2^{\bullet-}$ radicals. The results are collected in **Table 2**. In DPPH method, the highest scavenging ability was noted in BERGC-2H without dough fermentation-like process. In general, higher antioxidant capacity was noted for ginger cakes made of flour from roasted buckwheat groats (BERGC-2) and in cakes from dough without fermentation-like treatment. These results are in accordance with Sedej et al. [35], in which authors maintained that buckwheat groat possesses the strongest DPPH scavenging ability. The 16 % increase of antioxidant activity values was noted in BERGC-2 compared to BERGC-1, 89 % in BERGC-2H to BERGC-1H. A similar increasing trend of antioxidant capacity vs. rutin addition was found for rye-buckwheat ginger cakes with dough after fermentation-like step. The antioxidant scavenging ability of rye-buckwheat ginger cakes against $DPPH^{\bullet}$ was higher than in ginger cakes evaluated from rye flours [33]. For the ABTS method (**Table 2**), the highest scavenging ability was noted in BERGC-1H without dough fermentation-like process. In this case, higher antioxidant capacity as 11 % was noted for ginger cakes made of light buckwheat flour (BERGC-1) and, as previously in DPPH method, in cakes from dough without fermentation-like treatment. The increase of almost 56 % in antioxidant activity values was noted in cakes after buckwheat flour incorporation to achieve BERGC-1 and 31% to BERGC-2 without fermentation-like usage. Then the influence of low and high rutin application was not observed. For cakes after dough fermentation-like process, the 16 % increase of antioxidant activity values was noted in cakes after buckwheat flour incorporation to achieve BERGC-1 and 13%—BERGC-2. The results were similar for BERGC-1H and BERGC-2H. At least, in PCL method the highest scavenging ability was noted, as well as for ABTS method, in BERGC-1H without dough fermentation-like process. Therefore, higher antioxidant capacity was noted for ginger cakes made of light buckwheat flour (BERGC-1) and, as previously in DPPH and ABTS method, in cakes from dough without fermentation-like treatment. The antioxidant activity values have 2.3 times increase in cakes after buckwheat flour incorporation to achieve BERGC-1 and two times in BERGC-2 (dough without fermentation-like step). Then the influence of low and high rutin application was not observed. For cakes after dough fermentation-like process, no spectacular increase of antioxidant activity values was noted in cakes after buckwheat flour incorporation BERGC-1 and BERGC-2. Furthermore, a significant increase of ability to scavenge superoxide anion radicals was observed after addition of high rutin dose, 23 % in BERGC-1H compared to BERGC-1 and 83 % in BERGC-2H in comparison to BERGC-2.

Sample description under **Table 1**. TPC is expressed in mg of rutin eq. per gram of dry matter. Antioxidant capacity is expressed in μmol of Trolox eq. per gram of dry matter. Values are means and standard deviations (n = 3).

3.2. The results of Maillard reaction products formed during buckwheat ginger cake baking

In **Table 3**, data of furosine, fluorescent intermediary compounds (FIC), carboxymethyllysine (CML) and melanoidin contents formed *via* Maillard reaction are summarised.

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Type of ginger cakes	Furosine (mg/g DM)	Total FIC (FI/mg DM)	CML (μg/g DM)	Melanoidins (AU)
Dough *without* fermentation-like preparation				
RGC	0.94 ± 0.08aA	132.8 ± 0.08eI	12.70 ± 1.20dE	0.40 ± 0.01eI
BERGC-1	0.54 ± 0.01bC	166.6 ± 3.05dG	15.60 ± 1.00cD	0.75 ± 0.01bB
BERGC-1L	0.58 ± 0.01bC	208.2 ± 5.08aC	15.90 ± 2.00bcD	0.76 ± 0.00bB
BERGC-1H	0.53 ± 0.07bC	187.6 ± 3.33cE	11.90 ± 0.90dE	0.91 ± 0.01aA
BERGC-2	0.52 ± 0.02bC	200.8 ± 11.53abCD	17.90 ± 1.20abC	0.42 ± 0.01eI
BERGC-2L	0.29 ± 0.02cD	202.2 ± 4.51abD	19.10 ± 0.60aC	0.50 ± 0.01dE
BERGC-2H	0.27 ± 0.01cD	198.2 ± 0.19bD	17.90 ± 1.20aC	0.62 ± 0.01cC
Dough *with* fermentation-like preparation				
RGC	0.49 ± 0.10cC	125.7 ± 5.62eJ	12.69 ± 1.15dE	0.40 ± 0.01fI
BERGC-1	0.80 ± 0.06bB	151.3 ± 2.09dH	27.63 ± 1.95aA	0.45 ± 0.00dG
BERGC-1L	0.14 ± 0.03dE	250.9 ± 1.29aA	18.67 ± 1.66cC	0.35 ± 0.00gJ
BERGC-1H	0.17 ± 0.01dE	230.7 ± 2.49bB	17.98 ± 0.88cC	0.49 ± 0.00bE
BERGC-2	0.76 ± 0.08bB	172.0 ± 0.01cF	24.36 ± 1.31bB	0.43 ± 0.00eH
BERGC-2L	0.98 ± 0.04aA	174.2 ± 6.62cF	23.84 ± 0.80bB	0.48 ± 0.00cF
BERGC-2H	0.47 ± 0.04cC	175.6 ± 6.25cF	22.96 ± 0.90bB	0.55 ± 0.00aD

Table 3. Data of furosine, fluorescent intermediary compounds (FIC), carboxymethyllysine (CML) and melanoidin contents formed via Maillard reaction.

At the early stage of Maillard reaction, furosine was analysed due to its influence on nutritional protein damage in thermally treated food products [36]. According to obtained results, furosine was formed in all types of ginger cakes. In cakes from non-fermented-like dough, light buckwheat flour or flour from roasted buckwheat groats, limited formation of furosine was observed from 0.94 in RGC to 0.54 mg/g of dry matter in BERGC-1 and to 0.52 mg/g of dry matter in BERGC-2. Furthermore, the highest reduction of furosine, being about twofold, was noted in BERGC-2L and BERGC-2H. In cakes with dough after fermentation-like preparation, 63 and 55 % higher formation of furosine was noted in BERGC-1 and BERGC-2 than in RGC. These findings are in accordance with other studies where high protein content of light buckwheat flours and from roasted buckwheat groats was confirmed [38]. Then, the protective effect of rutin application was observed. In contrast, in BERGC-2L the polyphenolic protective effect was not observed. The most effective furosine decrease 4.7-fold and 1.6-fold was noted for BERGC-1H and BERGC-2H, respectively. Moreover, the lowest furosine content was determined in BERGC-1H with fermentation-like step during dough preparation. The furosine content formed in rye-buckwheat ginger cakes reported in this study was at the same level as previously described in enteral formula [37] and rye ginger cakes [33].

In the next step, the total fluorescence of intermediatory compounds (total FIC) formed at the advanced stage of Maillard reaction was studied. The formation of FIC is related to nutritional

loss due to the formation of new molecules from lysine-free amino residues and reducing sugars [21]. These results are presented in **Table 3**. The total FIC were determined in all types of cakes within the range of 132.8–208.2 FI/mg in rye-buckwheat ginger cakes from non-fermented-like dough and 125.7–250.9 FI/mg in cakes from fermented-like dough. The addition of buckwheat flours to ginger cake recipe from dough after fermentation-like processing influenced the increasing total amount of FIC formation around 1.25 times and 1.51 times in BERGC-1 and BERGC-2, respectively. The lower formation of total FIC was noted after incorporation of high rutin dose compared to ginger cakes with low rutin dose. The most significant decrease 1.1-fold in BERGC-1H was noted. Similar findings were noted in ginger cakes prepared from dough after fermentation-like process. The buckwheat flour incorporation increased FIC formation 1.2-fold and 1.36-fold in BERGC-1 and BERGC-2, respectively. Then the application of low and high rutin content increased total FIC value up to 65 % in BERGC-1L. These results may suggest that FI compounds are more likely to be formed after the addition of phenolic compounds such as rutin. The FIC values remain at the same level after rutin application to cakes made of flour from roasted buckwheat groats (BERGC-2L and BERGC-2H).

In **Table 3**, the results of carboxymethyllysine (CML) content in rye-buckwheat ginger cakes enriched with rutin, made in two dough preparation procedures, are collected. The CML is known as a nonfluorescent intermediatory compound and characteristic marker of advanced Maillard reaction stage [38]. The CML was identified in all ginger cake samples. The addition of buckwheat flours to ginger cakes made of dough without fermentation-like step influenced increasing CML content around 1.22 times and 1.41 times in BERGC-1 and BERGC-2, respectively. Then, enrichment with rutin proceeded to achieve 24 % lower amount of CML in BERGC-1H than in BERGC-1, whereas no change was observed for BERGC-2H. In cakes evaluated from dough after fermentation-like step, the buckwheat flour addition increased around twofold CML content in BERGC-1 and BERGC-2. Then, addition of rutin decreased CML amount to 1.5 times in BERGC-1H. However, in cakes made of flour from roasted buckwheat groats, no effect was observed. In general, the lowest CML content was found in GC-1H (without dough fermentation-like preparation) 11.9 μg/g of dry matter. According to high values of CML content, it may be observed that fermentation-like preparation of dough negatively influenced intensified formation of CML. The restricted parameters of fermentation process, e.g. using specific bacterial strain and temperature, are required to control CML formation in further studies. Moreover, CML formation was linked to furosine content ($r = 0.63$), suggesting that loss of nutritional quality of elaborated rye-buckwheat ginger cakes was caused by Maillard reaction progressing at the advanced stage.

Sample description under **Table 1**. Furosine is expressed as mg/g of dry matter. Total FIC is expressed in fluorescence intensity (FI) per mg of dry matter. Melanoidin content is expressed as arbitrary units (AU). Values are means and standard deviations ($n = 3$).

In **Figure 2**, the results of FAST index are displayed. In ginger cakes prepared from fermented-like dough, the FAST values ranged from 461 to 1309 % and in cakes without previous fermentation-like preparation from 271 to 1477 %. The obtained results were at least twice lower than those described for ginger cakes made from rye flour [33]. The addition of buck-

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wheat flours to ginger cakes made of dough without fermentation-like process showed FAST index value on the same level for BERGC-1 as in RGC, while for BERGC-2 FAST value was above twice higher. Then, enrichment with rutin proceeds to achieve 2.8 higher FAST in BERGC-1H than in BERGC-1, whereas no change was observed for BERGC-2H. In cakes evaluated from dough after fermentation-like step, the buckwheat flour addition decreased around 2.6-fold and 1.4-fold FAST in BERGC-1 and BERGC-2, respectively. Then, addition of rutin increased FAST values up to 5.4 times in BERGC-1H. Moreover, in cakes made of flour from roasted buckwheat groats, no significant effect of rutin supplementation was observed. An increase of FAST values was observed in using light buckwheat flour in ginger cake recipe. In contrast, in ginger cakes baked from flour from roasted buckwheat groats, no significant effect was observed. However, their FAST values were significantly higher than in raw buckwheat groats [33].

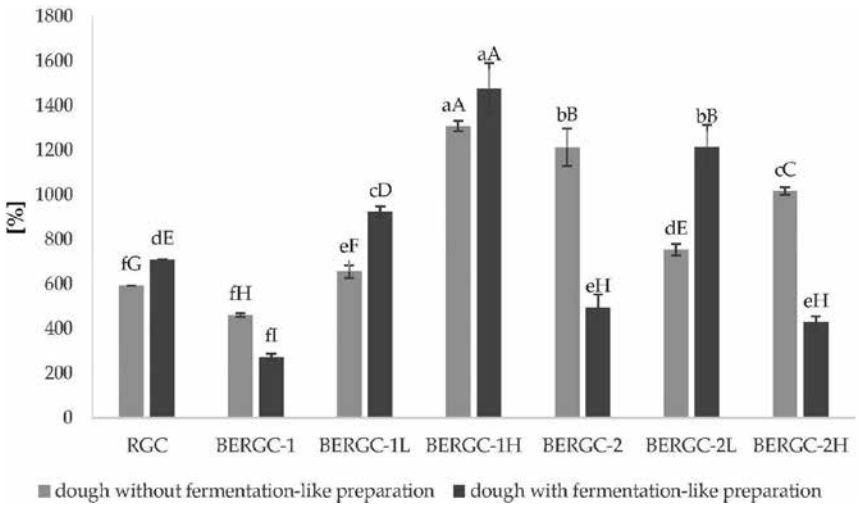

Figure 2. FAST index evaluation in rye-buckwheat ginger cakes obtained from dough with or without fermentation-like process.

The results of brown pigment formations as melanoidin, as the markers of the last stage of Maillard reaction, are shown in **Table 3**. As it may be observed, these brown polymers were formed in RGC as well as in new elaborated rye-buckwheat ginger cakes enriched with rutin. The addition of buckwheat flours to ginger cakes made of dough without fermentation-like process influenced on increasing melanoidin content by 88 % and 5 % in BERGC-1 and BERGC-2, respectively. Additional increase up to 21 % (BERGC-1) and 48 % (BERGC-2) was observed after rutin substitution. Therefore, the most advanced melanoidin formation process was observed in BERGC-1H. In cakes evaluated from dough after fermentation-like step, the results were similar. The obtained values noted in our study were slightly higher than those previously found in ginger cakes from rye flour, but they were twice as those in ginger cakes formulated on rye and wheat flours mix [39]. Moreover, melanoidin formation was found to be positively correlated with antioxidant capacity measured by ABTS test (r = 0.61) and PCL

assay (r = 0.84) and DPPH (r = 0.94) as well as TPC and rutin content in cakes without fermentation-like process (r = 0.97 and 0.64). The slightly lower correlation coefficients for ginger cakes prepared from fermented-like dough were noted. Our findings are in accordance with previous studies, where positive correlation between melanoidin formation and antioxidant activity was proved [40].

3.3. The inhibitory activity of buckwheat ginger cakes against advanced glycation end-product (AGE) formation

The inhibitory ability of rye-buckwheat ginger cake extracts, prepared from dough without or with fermentation-like step, against AGE formation was evaluated using in vitro BSA-MGO and BSA-glu model systems. The obtained results are presented in **Figures 3** and **4**.

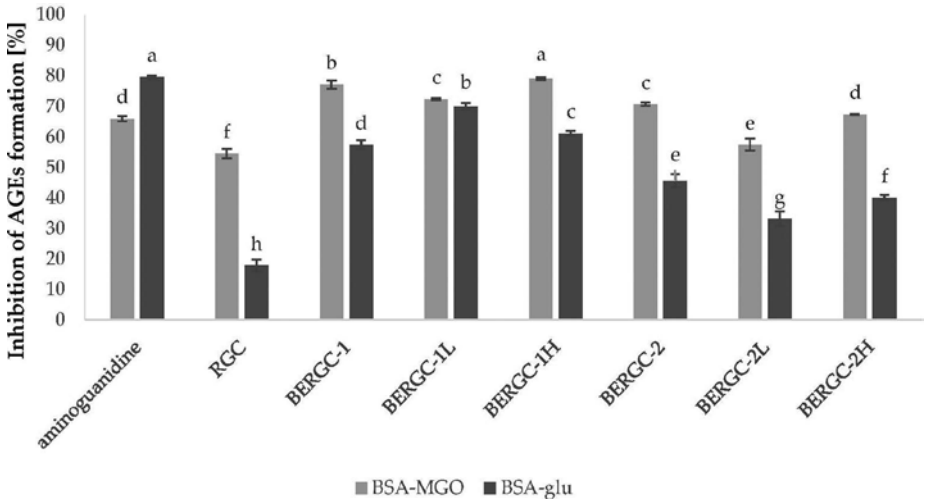

Figure 3. Inhibition of AGE formation in rye-buckwheat ginger cakes from dough without fermentation-like preparation. Sample description under **Table 1**.

Firstly, it was found that in BSA-MGO model in cakes, prepared without fermentation-like preparation, the AGE inhibitory activity increased after usage of buckwheat flours almost 42 and 30 % in BERGC-1 and BERGC-2, respectively (**Figure 3**). Their inhibitory activity values were higher than for aminoguanidine, a medicine used during medical treatment against diseases related to AGE accumulation in human tissues [29]. The enrichment of rye-buckwheat ginger cakes with rutin increased inhibitory activity and the highest value was noted in cakes with high dose of rutin (BERGC-1H). The application of light buckwheat flour in ginger cake formula (BERGC-1) offered also higher values of AGE inhibitory activity than in flour from roasted buckwheat groat incorporation (BERGC-2). In BSA-glu model, also the usage of buckwheat flours almost 23 and 56 % increased inhibitory activity of BERGC-1 and BERGC-2, respectively. However, in this model system, inhibitory effect of aminoguanidine was higher reaching 80 %. Also, the addition of low and high rutin dosages did not increase the inhibitory

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ability. Then, in BSA-MGO model system but in cakes baked from fermented-like dough, 30 % of light buckwheat flour addition increased the AGE inhibitory ability from 59 to 65 %, while no change was obtained after flour from roasted buckwheat groats addition. Therefore, in BSA-MGO model system, the highest values of inhibitory activity of ginger cakes were noted for BERGC-2H. The same results were obtained in BSA-glu model system, whereas the highest inhibitory activity was achieved in BERGC-2H (48 %). According to these results, it has been confirmed that the antiglycation activity was strongly correlated with polyphenolic compound content and the scavenging ability measured for rye-buckwheat ginger cakes obtained from fermented-like dough. It may be summarised that the effect of rutin enrichment was clearly seen in cakes obtained with fermented-like dough, even if the inhibitory activity was slightly lower than those cakes produced from non-fermented dough. Our findings are confirmed in previous studies of antiglycation and antioxidative activities of buckwheat breads [29]. These breads substituted by light buckwheat flour inhibited at 40 % the AGE formation and breads baked from flour from roasted buckwheat groats at 60 % measured in BSA-glu model system, in BSA-MGO at 20 % and 40 %, respectively [29]. Moreover, the high AGE inhibitory potential of coriander, which is an ingredient of spice mix, may contribute to the total antiglycation ability of rye-buckwheat ginger cakes [41].

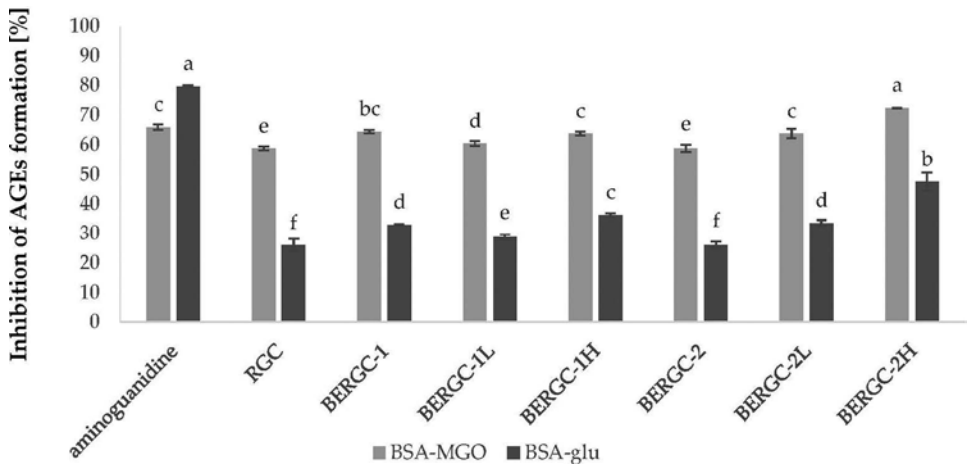

Figure 4. Inhibition of AGE formation in rye-buckwheat ginger cakes from dough obtained with fermentation-like preparation. Sample description under **Table 1**.

4. Conclusions

The new functional product as rye-buckwheat ginger cakes enriched with rutin has been elaborated, and data of total phenolics; rutin contents; antioxidative capacity measured by ABTS, DPPH and PCL methods; and characterisation of Maillard reaction markers have been provided. Moreover, the inhibitory activity against AGE formation using in vitro model

systems BSA-MGO and BSA-glu has been studied. The enrichment of rye-buckwheat ginger cakes with rutin improved their increased total phenolic content and antioxidant properties. The protective effect on furosine formation has been observed, whereas melanoidin formation has been stimulated. In contrast, the loss of nutritional quality of rye-buckwheat ginger cakes enriched with rutin has been noted due to the formation of CML and FI compounds at the advanced stage of MR. Moreover, high antiglycation potential of rye-buckwheat ginger cakes enriched with rutin has been confirmed. The relationship between antiglycation ability and rutin content and antioxidant capacity has been found. The addition of buckwheat flours as well as rutin supplementation in ginger cakes has influenced the increase of AGE inhibitory potential.

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Chapter 12

Role of Meal Replacements on Weight Management, Health and Nutrition

Andrew Shao

Additional information is available at the end of the chapter

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Abstract

Meal replacements are a safe and effective tool for weight loss and weight management and beyond. Recent research and concepts suggest that the category can provide additional metabolic and nutritional benefits not previously recognized. Recent studies indicate that use of protein-enriched meal replacements helps maintain lean body mass during weight loss, providing additional metabolic benefits in the form of improved insulin sensitivity and reduced inflammation. Depending on the formulation, meal replacements can have a low glycemic index and have a high nutrient density relative to energy density, the latter being an important aspect highlighted in government dietary guidance. While well defined in some markets, there is a need to establish clear regulatory standards in other key markets to ensure a level playing field and proper recognition of the category.

Keywords: meal replacement, weight loss, body composition, glycemic index, nutrient adequacy, regulation

1. Introduction

Globally, obesity rates remain high, and although incidence has plateaued in some countries (e.g., US men), rates of related comorbidities continue to escalate, such as, type II diabetes. Nutrition survey data suggest that populations are becoming overfed, yet undernourished, due to the poor nutrient density of the diet, contributing simultaneously to elevated rates of chronic disease and nutrient inadequacy. Meal replacements (MR)—a prepackaged, calorie-controlled product in a bar or powder mix that can be made into a shake or beverage—have long been validated as safe and effective tools for weight loss (and weight maintenance). More recent studies have indicated that high-protein MR are also effective at maintaining lean body

mass and reducing visceral body fat during weight loss. This review focuses on MR that do not require medical supervision (those classified as medical foods).

Depending on the formulation, MR also possess the advantage of having a low glycemic index (GI) value; low-GI diets have been linked to improved weight maintenance and reduction in risk of diabetes and ocular disease. Many nutrition researchers and authoritative bodies around the world have highlighted the need to improve the nutrient density of diets as a means to reduce obesity while maintaining optimal nutrition status. MR also tend to be nutrient dense, meaning that they possess a high ratio of essential nutrients relative to calories.

Some markets have established clear regulatory standards and definitions for the composition and marketing claims for MR (e.g., Codex, Canada, EU, Brazil, Korea, Indonesia). However, several large markets (e.g., US, Mexico, China, Russia, India) still lack these important standards, in turn limiting research opportunities and recognition by governments, healthcare professionals and consumers of the value the category provides.

The aim of this chapter is to review the extensive body of literature validating the safety and effectiveness of MR as weight loss and weight maintenance tools; explore the benefits of MR beyond weight loss, including maintenance of lean body mass and low glycemic index; discuss the concept of nutrient density, its importance in nutrition and how MR fit into a nutrient-dense diet; and discuss the need for regulatory standards to be established in those countries that currently lack a definition for MR.

2. Meal replacements for weight loss and weight maintenance

According to the most recent global analysis, obesity rates continue to rise at an alarming level overall, reaching 50% of the population in some countries (**Figure 1**), with the prevalence in women rising faster than that for men. Globally, the prevalence of obesity now exceeds that of underweight (NCD Risk Factor Collaboration 2016). Although obesity rates in some developed countries appear to have leveled off (e.g., US men) [1], comorbidities, such as type II diabetes, continue to rise. The World Health Organization (WHO) estimates the prevalence of diabetes has doubled worldwide since 1980 and resulted in 3.7 million deaths in 2012, with combined direct and indirect costs estimated in the $billions annually [2]. With overweight and obesity recognized as the strongest risk factors for type II diabetes, the WHO recommends obesity prevention, through healthy diet and physical activity, as a key approach.

Few tools have been validated as safe and effective in the treatment or prevention of obesity and overweight. Bariatric surgery is effective at treating those who are morbidly obese, yet it is associated with substantial risks and postsurgery complications, including nutrient deficiency. While advances in science and technology have eventually provided several efficacious pharmaceutical drugs for obesity treatment, the effects are modest and associated with a myriad of side effects [3], and many FDA-approved prescription weight loss drugs have been subsequently withdrawn from the market due to safety concerns [4]. In contrast, nearly 150 studies demonstrate that use of MR (in various forms) safely reduces energy intake and results in sustainable weight loss (**Table 1**). A systematic review published concluded that

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MR safely and effectively produce sustainable weight loss [5]. The systematic review included six randomized, controlled MR intervention studies of at least 3 months duration, involving adults with a body mass index (BMI) ≥ 25 kg/m^2.

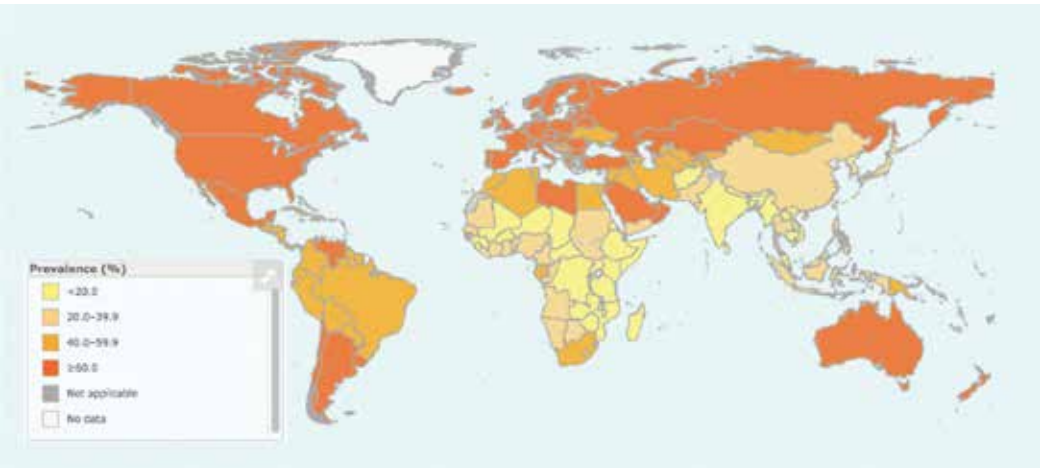

Figure 1. World Health Organization Global Health Observatory (GHO) data. Global overweight and obesity prevalence. *Source*: World Health Organization http://www.who.int/gho/ncd/risk_factors/overweight/en/.

Approach	Category	Effectiveness for obesity treatment—long term (>1 year)	Side and adverse effects
Pharmacological	Prescription drug	5% total body weight (Khera 2016)	Significant and serious, with some drugs having received FDA approval, then subsequently withdrawn from the market
Bariatric surgery	Medical device	30% of total body weight in the morbidly obese (Chow 2016)	High risks associated with surgery and postsurgery complications, including nutrient inadequacy or deficiency
Meal replacements	Conventional food and medical food	7–8% total body weight (Heymsfield 2003)	Only nonserious (nuisance) effects reported

Table 1. Relative comparison between pharmacological, surgical and meal replacement approaches to obesity treatment and prevention.

More recent studies have demonstrated MR effectiveness at maintaining weight loss up to several years. Intervention studies involving MR use with a year or more of follow-up have shown a range of sustained weight loss from 2% up to 11% of baseline body weight (**Figure 2**) [6–21].

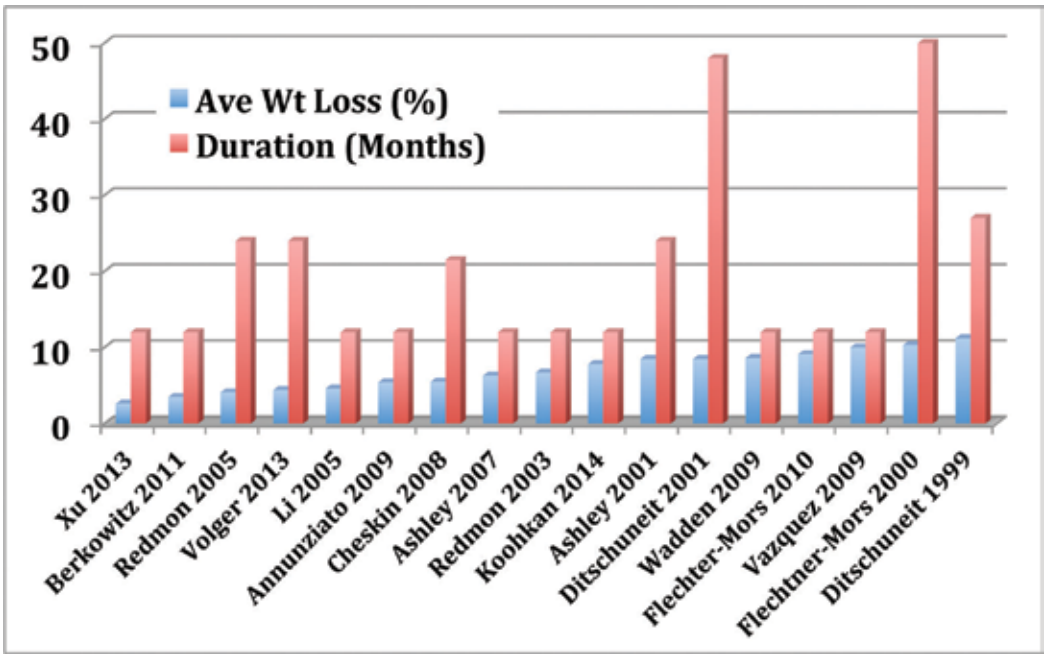

Figure 2. Weight loss and maintenance from randomized controlled trials ≥1 year in duration involving meal replacement.

Portion size is a key factor in determining energy intake and may be closely linked to obesity. Research indicates that portion size is directly correlated with energy intake, suggesting that controlling portion size is an effective approach to reduce energy intake and combat obesity [22]. Among the few portion control tools researched to date, liquid MR are considered among the most effective and consistent, particularly if combined with other efforts to encourage consumption of high-nutrient-dense, low-energy-dense foods [22]. Furthermore, MR promote adherence to a restricted calorie diet due to simple preparation and convenience compared to preparing and cooking low-calorie foods at home. MR generally contain a tight range of total calories, macro- and micronutrients (**Figure 3**), and are a nutrient-dense tool, especially useful for supporting adherence to a calorie-restricted diet through portion control.

Satiety and appetite are known to impact total energy intake, as well as food choices and eating behavior. Both are regulated by a combination of mechanical and endocrine effects ranging from the gut to the brain. With respect to diet, protein has been identified as an important contributor to satiety, defined as the absence of hunger between meals. Dietary protein can induce satiety through several mechanisms including thermic effects and induction of gut hormones such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) and ghrelin [23]. Intervention studies show that increased protein intake, using protein-enriched MR, is effective at increasing satiety, reducing hunger sensations, decreasing energy intake and facilitating weight loss in obese subjects [24, 25].

Many authoritative bodies around the world have sanctioned the use of MR for weight loss and control. As far back as the mid-1980s, Codex Alimentarius recognized the use of MRs for

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weight control [26]. In 2010, the European Food Safety Authority (EFSA) concluded that MR are effective for both weight loss and weight maintenance [27]. Most recently, the Academy of Nutrition & Dietetics (AND) rated strongly the use of MR as part of a comprehensive weight management program [28].

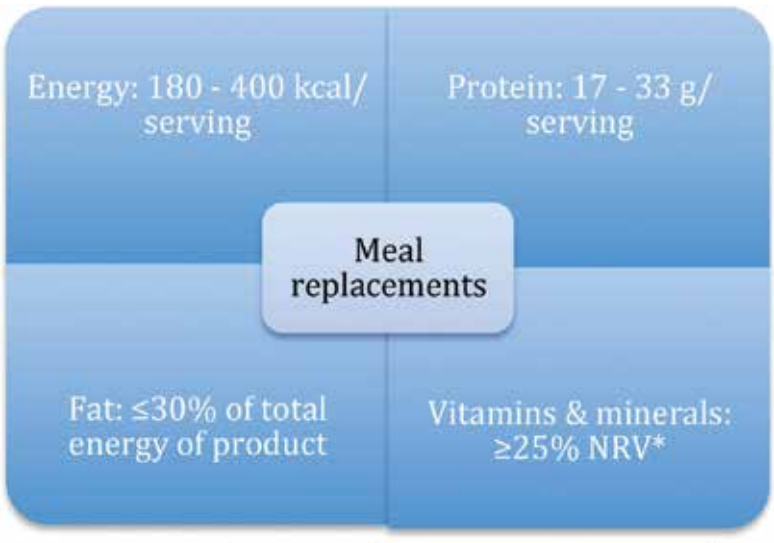

Figure 3. General macro- and micronutrient composition of meal replacement products.

3. Metabolic benefits of meal replacement

Weight loss in obese subjects during an intervention is comprised of water, fat and lean (muscle) mass. The amount and extent of fat and muscle loss depend on the specific weight loss intervention. As lean mass determines the basal metabolic rate (BMR), the goal for any weight loss program is to lose fat mass, while preserving muscle mass. This helps to maintain a higher BMR, which in turn helps to maintain energy expenditure, which can often decline with weight loss. Use of protein-enriched MR products has been shown to effectively maintain lean body mass during weight loss [24, 29], particularly when combined with resistance exercise [30].

Glycemic index (GI) represents a measure of the ability or rapidity of a given food to raise an individual's postprandial blood glucose level. GI is determined for a given food in reference to a standard food, usually white bread, and reflects the blood glucose-raising ability of digestible carbohydrates in a given food [31]. Examples of relative GI values of different foods can be found in **Table 2**.

A growing body of evidence suggests that the GI and glycemic load (GL, a measure of how much a given food will raise an individual's blood glucose level following consumption) of the diet play an important role in human metabolic functions and health. High GI foods and a

high GL stimulate a rapid rise in insulin levels, which on a chronic basis can result in insulin resistance [32, 33]. The GL of a food is calculated by multiplying its GI by the amount of carbohydrate it contains per serving, and then dividing by 100. GL is a function of the amount of carbohydrate intake and the GI of the food. In contrast, GI is an inherent property of a food, independent of the amount of carbohydrate ingested. The GI value of a diet can impact insulin sensitivity and glucose metabolism [34]. Blood sugar levels have also been implicated in appetite control, suggesting that. Furthermore, MR promote adherence to a restricted calorie diet due to simple preparation and convenience compared to preparing and cooking low-calorie foods at home. The GI of a diet may impact overall food and energy intake [35]. Accordingly, low-GI diets have been shown to be an effective approach for managing diabetes [36, 37] and obesity [38, 39]. The combination of a high-protein, low-GI diet in obese subjects is effective at inducing weight loss and maintenance of lean body mass [25, 36, 40]. Although it varies by formulation, MR tend to be high in protein and have a low GI (<55), making them ideal for incorporation into an overall low-GI diet plan.

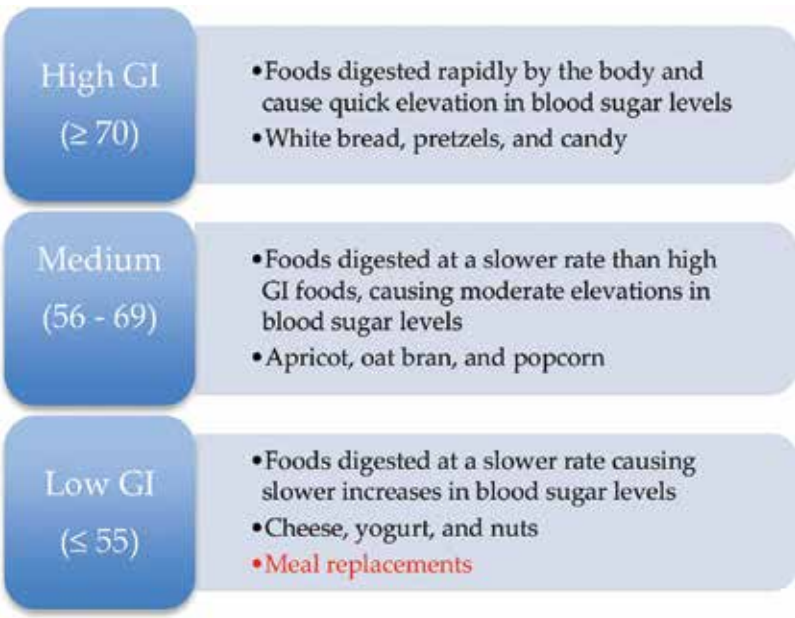

Table 2. Glycemic index (GI) values of select foods.

As with insulin sensitivity, the degree of intrabdominal and visceral fat is tightly linked to metabolic syndrome. Surrounding the body's critical organs, such as the heart and liver, visceral fat stimulates systemic inflammation and is known as an increasingly serious risk factor for chronic diseases, including cardiovascular disease and diabetes [41]. In simple terms, "sarcopenic obesity" can be defined as low skeletal muscle mass and strength combined with excess body fat, much of which is visceral fat [42, 43]. The concept has also been described as "thin outside, fat inside" or "TOFI" [44]. Related to obesity, individuals can have the same body mass index (BMI), but vastly different inflammatory states and risk levels due to

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differences in distribution and degree of visceral fat [45]. As there is as yet no medical cure, resistance and strength exercise, combined with a high-protein diet, is recommended as one of the only effective means of addressing sarcopenic obesity and complications of excess visceral fat [30, 46]. When used in conjunction with reduced total calorie intake and resistance exercise, MR can also be effective at reducing visceral fat [19, 30, 47].

With respect to safety, use of MR for weight control and other metabolic benefits is among the safest approaches studied. Many individual intervention studies [48–50] as well as systematic reviews [51] have confirmed that MR safely facilitate weight loss and maintenance.

4. Meal replacements and nutritional adequace

According to the *2015 Dietary Guidelines for Americans*, individuals should consume more nutrient-dense foods to better balance meeting nutritional needs while avoiding excess calories or energy [52]. A position paper from the Academy of Nutrition & Dietetics concluded that there is a positive association between dietary energy density and increased adiposity [53]. Nutrient density is a term referring to the amount of essential nutrients in a food relative to the amount of energy (calories) that food delivers. High-nutrient-dense foods provide a high level of nutrients with relatively low caloric value, and low-nutrient-dense foods provide a high level of calories with relatively low nutrient content [54, 55]. Examples of nutrient-dense foods include fruits, vegetables, whole grains, lean meats and dairy.

In the United States, more than half of the population fails to achieve the recommended intakes for key nutrients, including vitamins A, C, D and E, fiber, magnesium and potassium [56], all of which have been deemed "nutrients of concern" or "shortfall nutrients" by the 2015 Dietary Guidelines Advisory Committee [57]. Incorporation of more nutrient-dense foods into the diet is an effective approach to achieve proper nutrient adequacy without adding excess calories.

Overweight and obese individuals are at even higher risk than the general population of experiencing nutrient deficiency, particularly vitamin D [58]. This is believed to be due, in part, to overconsumption of a high-energy-dense and low-nutrient-dense diet [59], a phenomenon described as "overfed but undernourished" [60]. Furthermore, weight loss regimens, particularly those involving rapid weight loss, can lead to compromised nutritional status [61].

With a modest amount of calories, added essential vitamins, minerals and fiber, MR are considered to be a nutrient-dense food. Indeed, a variety of studies demonstrates that use of MR during a weight control regimen helps to ensure adequate intake of essential nutrients [12, 62–64].

5. Meal replacement definitions and standards

In some markets around the world, regulations exist to define MR, both in function and in composition. The definition, specific authorized claims for weight loss or management and composition standards (for both macro- and micronutrients) vary by country (**Table 3**). The *Codex Alimentarius* composition standards for MR were established back in 1991 and have

served as the basis for the definition in a number of other markets [65]. Establishing regulations and composition standards has served as the basis for sanctioning weight loss benefit claims for MR and has facilitated harmonization in multi-country regions (e.g., EU). Together with the plethora of data supporting the safety and efficacy of MR, these standards have also led to increased use, research and acceptance of MR by the healthcare professional community [66].

	Codex	Australia	Brazil	Canada	Chile	EU	Indonesia	Korea	US & China
Energy	200–400 kcal	≥200 kcal	≥200–400 kcal	≥225 kcal	≥200–400 kcal	200–250 kcal	≥200 kcal	≥200–400 kcal	None
Protein	25–50% of total energy; ≤125 g/day	≥12 g	25–50% energy of product and <125 g	20–40% energy of product	25–50% energy of product and <125 g	25–50% energy of product	≥12 g	≥10% NRV	None
Fat	≤30% of total energy	None	≤30% energy of product	≤35% energy of product	≤30% energy of product	≤30% energy of product	≤13 g	None	None
Fat from linoleic acid	≥3% of total energy of linoleic acid (glycderide form)	None	≥3% energy of product	≥3% energy of product	≥3% energy of product	≥1 g	None	None	None
Linoleic acid & linolenic acid ratio	None	None	None	4:1–10:1	None	None	None	None	None
Vitamin	33–25% of specified amount in Codex 181-1991 (depend on # of servings/ day)	Specific minimum indicated	Specific minimum indicated	Specific minimum indicated	Specific minimum indicated	≥30% NRV	≥25% RDA	≥25% NRV	None
Mineral	33–25% of specified amount in Codex 181-1991 (depend on # of servings/ day)	Specific minimum indicated	Specific minimum indicated	Specific minimum indicated	Specific minimum indicated	≥30% NRV w/ specific limit on Na & K No min limit: F, Cr, Cl, Mo	≥25% RDA	≥25% NRV	None
Essential amino acids	None	None	None	None	None	Yes—profile WHO 1985	None	None	None

Table 3. Comparison of standards and regulations for meal replacements in various markets around the world.

However, in other markets with high obesity prevalence, including the United States, Mexico, China and Russia, no such standards have been established. The reasons for the lack of MR

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regulations and standards in these countries vary, but are tied closely to the existing food and/or dietary supplement policy and regulatory framework. For example, in the United States, composition or identity standards are not expressly required in order for products to bear health benefit claims. For MR, as with conventional foods and dietary supplements, the ability to bear a weight loss claim is predicated on the availability, quality and quantity of scientific substantiation, not a formal definition for MR or composition standards [67]. In contrast, in the case of Mexico, MR are regulated under the category of food supplements. By regulation, food supplements are not permitted to bear claims of any kind [68], thus eliminating the ability to communicate a weight loss benefit for the category and reducing the need to establish a definition. Finally, in China, MR are regulated under the health or functional food category [69]. Products in this category are required to go through animal and/or human testing (depending on the desired claim) as part of a premarket registration process. This testing requirement to validate the health food product prior to market has precluded the need for a specific MR definition or standard.

Establishing full recognition of the health benefits of MR in these markets may ultimately require a formal definition and composition standards. Indeed, the absence of a formal regulation for MR has allowed the category to be inappropriately targeted with antiobesity policies aimed at, for example, curbing the public's consumption of sugars. In Mexico, MR are subject to the same tax aimed at reducing intake of sodas and other sugar-sweetened beverages as part of a broader public health initiative [70]. In the United States, similar policy has been proposed at both the Federal [71, 72] and state levels [73] and has passed at the local level [72, 74]. In some cases, MR have been exempted (Berkley, CA), and in others, this exemption has not been expressly granted (Philadelphia). Imposing such policy on MR seems incongruent with the state of the evidence, which clearly demonstrates that MR are part of the obesity solution, not the problem.

The absence of a formal definition for MR may negatively impact the consumer, as products claiming to be a MR may not meet basic compositional expectations. Consumers conceivably stand to benefit from a standard or regulation by receiving properly formulated and consistent products. The absence of a formal definition has also prevented the category from being included in potentially beneficial public policy aimed at obesity and disease prevention. Without a clear standard of identity and recognition of its health benefits, MR cannot be included in government-sponsored programs such as Flexible Savings Accounts or Health Savings Accounts.

6. Summary and conclusions

Rates of obesity and comorbidities continue to rise worldwide. MR are among the safest most effective tools available demonstrating significant and long-term weight loss. MR use provides benefits well beyond weight loss, including body composition and metabolic benefits from its low glycemic index. As a nutrient-dense food, MR are also effective at achieving and maintaining nutrient adequacy without delivering excess calories. Although well defined in some markets, MR still lack a formal definition and regulation in several key markets around the world. The absence of this formal recognition and composition standards has left the category vulnerable to onerous public policy while being excluded from potentially beneficial

policy. Efforts to establish formal regulations in these key markets should be considered in order for the category to provide its full impact on obesity and public health.

Conflict of interest statement

Dr. Shao is a full-time employee of Herbalife International of America, Inc, a global nutrition company that manufactures and markets nutritional products (functional foods and dietary supplements), including meal replacement products.

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Chapter 13

Antioxidant Properties of Dark Wheat Bread with Exogenous Addition of Buckwheat Flour

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Additional information is available at the end of the chapter

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Abstract

In this study, the antioxidative/reducing activity of buckwheat-enhanced dark wheat breads (BEDWBs), based on the substitution of dark wheat flour (DWF) with buckwheat flour (BF) or flour from roasted buckwheat groats (BFR) at levels of 10, 20, 30 and 50% (w/w), was investigated. The antioxidative activity was measured against the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) and the superoxide anion radical ($O_2^{-\bullet}$) by photochemiluminescence (PCL), reducing power by Fe(III) reduction and directly by cyclic voltammetry (CV) technique. The Fe(II) chelating capacity was also provided. The substitution of dark wheat bread with white and roasted buckwheat flour up to 50% (w/w) resulted in higher scavenging capacity against free radicals. The chelating and reducing power were above threefold higher as compared to a reference dark wheat bread. The improved antioxidant properties of buckwheat-enhanced dark wheat breads were due to the incorporation of buckwheat flour polyphenols. The high correlation noted between the total phenolic content and antioxidant capacity suggested that these assays may be used to characterize the cereal products enriched by buckwheat flours. Overall, buckwheat-enhanced dark wheat bread could be applied as food with more efficient antioxidant properties.

Keywords: buckwheat flours, dark wheat flour, breads, antioxidant/reducing capacity, chelating activity, reducing power

1. Introduction

Development of products which positively affect the consumer health is an important aspect followed by the food industry. This assignment may be realized when process of the industry is concentrated on the natural antioxidants. Antioxidants present in food can protect against lipid and protein autoxidation. It is very important to quantify the antioxidant properties of different types of food. In addition, a special attention is devoted to the processing methods to maintain the beneficial antioxidant properties of food [1].

Presently, wheat flour is widely used in bread making; however, other types of flour are also used. Rye and spelt types of flour are preferred due to the content of micro- and macronutrients and fibre [2, 3]. Recently, the potential usage of buckwheat flour as a functional component in food has been demonstrated [4]. Buckwheat (*Fagopyrum esculentum* Moench), commonly, cultivated in Russia and China, is added to the other cereal grains because of likenesses in usage [5]. Buckwheat is a rich source of nutrients (lysine, vitamins B, carbohydrates) [6] and antioxidants such as vitamin E, glutathione, phytic acid [7], phenolic acids and flavonoids—mainly rutin, with anti-inflammatory, anti-carcinogenic and anti-glycation properties [4, 8, 9]. Buckwheat polyphenols can function as antioxidants in one or more possible ways: as reducing agents, as compounds that scavenge free radicals, as chelating agents of metals that catalyse oxidation reactions and thus limiting their ability to initiate free radical chain reactions or by inhibiting oxidative enzymes such lipoxygenases [10–13]. However, processing conditions may considerably affect biological activity of polyphenols. There are many studies regarding negative effects of thermal processing on the phenolic compounds including flavonoids. The type of heat transfer and processing conditions are the major factors responsible for the observed decrease in the flavonoid content in food [7, 14–16]. Having all these evidences, buckwheat mill products seem to be an attractive ingredient in the bakery industry [17–20]. The recent evidences have shown that the intake of bread with addition of buckwheat flour in the recipe resulted in a positive increase of antioxidant potential in humans [17]. This finding, due to the quality properties of buckwheat bread as described by Lin et al. [21], can make it favourable for developing a healthy diet. Recently, a number of food products containing buckwheat has been investigated such as buckwheat-enhanced ginger nutty cakes [18], buckwheat enriched wheat bread [17, 20, 21] and buckwheat cakes [18, 19, 22]. Therefore, due to the nutritional value and beneficial effects on human health, buckwheat and partially buckwheat-based products form a pool of potential functional food [4, 10].

For the overall characterization of a new food product, more often the antioxidative capacity is used. There is a variety of analytical methods to assess the antioxidant capacity of food. However, there is no single standard method that would be used to determine the antioxidant capacity of the complex matrix and give consistent, unquestionable results in confrontation with other analytical methods. Therefore, it is advisable to use more than one method. A part of analytical methods is based on scavenging of non-natural free radicals, other deal with lipid peroxidation chemical markers. These methods need a little preparation, small amounts of reagents and are quick [13].

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Many antioxidant capacity approaches have been suggested to assess antioxidant properties of food products and to clarify their relationships with antioxidants. Among them, ABTS, DPPH, reducing power assay and metal chelating activity are used for the assessment of antioxidant capacity of food [1, 23–25]. The ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) is a spectrophotometric method based on inhibition of green colour in the presence of an antioxidant. DPPH (2,2-diphenyl-1-picrylhydrazyl) is based on the assumption that antioxidants are the hydrogen donors. This spectrophotometric method uses the DPPH radical, which changes from purple to yellow in the attendance of antioxidant compounds. However, ABTS is soluble in water and in alcoholic solutions, but DPPH is soluble only in organic solvents. The photochemiluminescence (PCL) method is based on the scavenging activity against the superoxide anion radical. The chelation of Fe(II) ions may cause significant antioxidative effects by delaying metal-catalysed oxidation [26, 27]. The reducing power assay involves the formation of coloured complexes, in the presence of antioxidants, with potassium ferricyanide, trichloroacetic acid and ferric chloride. The increase of absorbance of the reaction mixture is related to the reducing power of the samples. Currently, a mixture of the methods should be used to evaluate the antioxidant capacity of food *in vitro* to cover all aspects of antioxidant effectiveness [1, 12, 25]. Therefore, the aim of this study was to characterize the dark wheat bread with exogenous buckwheat addition as a source of antioxidative activity for humans.

2. Materials and methods

2.1. Chemicals and reagents

n-Hexane and methanol (HPLC-grade) were provided by Merck (Darmstadt, Germany). 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid (AA) and sodium dodecyl sulfate (SDS) were from Sigma Chemical Co. (St. Louis, MO, USA). PCL ACW (antioxidant capacity of water-soluble substances) and PCL ACL (antioxidant capacity of lipid-soluble substances) kits were from Analytik Jena AG (Jena, Germany). Monobasic potassium phosphate, dibasic potassium phosphate, ethylenediaminetetraacetic acid (EDTA), ferric chloride and ferrous chloride were purchased from POCh (Gliwice, Poland). Water was purified with a Mili-Q-system (Millipore, Bedford, USA).

2.2. Preparation of buckwheat-enhanced dark wheat breads

Dark wheat flour (DWF) and buckwheat (variety Kora) flour were purchased from a healthy food store in Olsztyn, Poland. The flour from roasted buckwheat groats (BFR) was procured from a local company in Poland. The dry matter in BF, BFR and DWF was 87.6, 89.7 and 87.0%, whereas protein content was 10.6, 14.3 and 8.1%, respectively. BF or BFR was used to replace DWF at levels of 10, 20, 30 and 50% (w/w). Buckwheat-enhanced dark wheat breads (BEDWBs) and reference dark wheat bread (DWB) were baked in a laboratory bakery. **Table 1** shows the

buckwheat-enhanced dark wheat breads formulation and baking conditions. Three pieces of each type of bread was baked. Samples were freeze-dried, milled and sieved through of 0.6 mm, and then were stored at –20°C before using for analysis.

Ingredient and conditions	Addition of buckwheat flours (%)				
	0	10	20	30	50
Dark wheat flour (g)	350	315	280	245	175
Buckwheat flour (g)	–	35	70	105	175
Roasted buckwheat flour (g)	–	35	70	105	175
Water (mL)	228	228	228	228	228
Salt (g)	3.5	3.5	3.5	3.5	3.5
Yeast (g)	10.5	10.5	10.5	10.5	10.5
Fermentation					
Temperature (°C)/time (min)	37/90	37/90	37/90	37/90	37/90
Pieces of dough (g)	250	250	250	250	250
Proofing (75% rh)					
Temperature (°C)/time (min)	37/25	37/25	37/25	37/25	37/25
Baking					
Temperature (°C)/time (min)	250/30	250/30	250/30	250/30	250/30

Table 1. Buckwheat and reference dark wheat breads formulation and baking conditions.

2.3. Preparation of bread crude extracts for measurement of antioxidant capacity by ABTS and DPPH assays, and reducing capacity by cyclic voltammetry (CV)

Bread samples (0.25 g) were extracted in triplicate at 25°C with 5 mL of 67% aqueous methanol using Thermomixer comfort (Eppendorf, Germany) by shaking at 1400 rpm for 60 min. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R centrifuge, Eppendorf, Germany). After that, the 67% methanol extracts were directly used to determine the antioxidant capacity.

2.4. Preparation of hydrophilic and lipophilic bread extracts for measurement of antioxidant capacity by photochemiluminescence assay

Hydrophilic extracts: About 0.1 g of bread samples were extracted in triplicate for 3 min with 1 mL of deionized water using Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany) and the fresh supernatants were used to determination of antioxidant activity formed by water-soluble antioxidants (ACW). Lipophilic extracts: About 0.1 g of bread samples were extracted in triplicate for 3 min with an n-hexane and methanol (1:4 v/v) using Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany) and the fresh supernatants were used to determine the antioxidant capacity of lipid-soluble antioxidants (ACL).

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2.5. Antioxidant capacity measured by ABTS, DPPH and PCL assays

2.5.1. ABTS assay

For the determination of the antioxidant activity was using the method described by Re et al. [28]. The $ABTS^{\bullet+}$ stock solution was diluted with 67% methanol to the absorbance of 0.70 ± 0.02 at 734 nm. Appropriate, solvent blank was used in each assay. The Trolox standard curve was determined in the range of 0.1–2.5 mM. The measurements were performed by a spectrophotometer UV-160 1PC with CPS-controller (Shimadzu, Japan). The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter (DM).

2.5.2. DPPH assay

$DPPH^{\bullet}$ scavenging activity was determined as described previously in details [29]. The Trolox standard solutions were prepared in 67% methanol in the range of 0.1–2.5 mM. The measurements were performed by a spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter.

2.5.3. Antioxidant capacity measured by photochemiluminescence (PCL) assay

The PCL assay was carried out using the method according to Popov and Lewin [30]. This method consists in determining the superoxide anion radicals generated by luminol (under UV light) in the presence of antioxidants. The antioxidant capacity of buckwheat bread extract was determined using the analytical kits, which are designed to determine the antioxidant activity of the hydrophilic (ACW) and lipophilic (ACL) compounds, as reported previously by Zielińska et al. [29]. Measurements were performed with a Photochem® apparatus (Analytik Jena, Leipzig, Germany). PCL values are showed as a sum of ACW and ACL. The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter.

2.6. Metal chelating activity of buckwheat-enhanced dark wheat breads

Bread samples (0.1 g) were extracted in triplicate with 1 mL of 0.2 M phosphate buffered saline (PBS, pH 7.4), which contains 1% (m/v) SDS for 30 s using VC 750 type sonicator (SONICS, USA) followed by vigorously shaking for 30 s using Genie-2 type vortex (Scientific Industries, USA). That stage was repeated three times. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany). The supernatants were directly used to determine Fe(II) chelating power of breads. Chelating power was measured using the method of Wang et al. [31]. To the reaction tube was added 0.25 mL of 1 mM $FeSO_4$, 0.25 mL of fresh bread extract, 1 mL of PBS (pH 7.4) with 1% (m/v) SDS, 1 mL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), 0.4 mL of 10% NH_2OH HCl and 2.1 mL of ethanol. The mixture was shaken and left at room temperature for 20 min. The absorbance at 522 nm was determined and used to evaluate Fe^{+2} chelating activity using ethylenediaminetetraacetate (EDTA) as a standard. The standard curve was constructed within the range of 0.125–2.0 mM of EDTA. The Fe(II) chelating capacity of samples was measured in triplicate using a temperature-controlled spectropho-

tometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). Results were expressed as μmol EDTA equivalents/g DM.

2.7. Measurement of reducing power of buckwheat-enhanced dark wheat breads

Bread samples (0.25 g) were extracted in triplicate at 25°C with 5 mL of 67% aqueous methanol using Thermomixer comfort (Eppendorf, Germany) by shaking at 1400 rpm for 60 min. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R centrifuge, Eppendorf, Germany). After that, extracts were dried at 40°C using a rotary evaporator. Then samples were dissolved in 5 mL of phosphate buffer (0.2 M, pH 6.6) and were used immediately for the measurement of reducing power of bread extracts. The reducing power was determined by Oyaizu [32] with minor modification according to Liyana-Pathirana and Shahidi [33]. The assay mixture contained 1 mL of sample, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, incubated at 50°C for 20 min. Then, 2.5 mL of 10% TCA was added to the mixture and centrifuged for 5 min (2000 × g, 4°C). Exactly 2.5 mL of the extract of sample was mixed with 2.5 mL water and 0.5 mL of 0.1% $FeCL_3$ and was measured at 700 nm using a spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). A standard curve was prepared using ascorbic acid within the range of 0.015–0.5 mM and the reducing power was expressed as μmol ascorbic acid equivalents/g DM.

2.8. Measurement of reducing capacity of buckwheat-enhanced dark wheat breads by cyclic voltammetry

The cyclic voltammetry experiments were performed in 67% methanol bread extracts mixed with 0.1 M sodium acetate–acetic buffer (pH 4.5) at ratio 1:1 (v/v) according to Zielińska et al. [29]. The sodium acetate–acetic buffer acted as a supporting electrolyte for cyclic voltammetry measurements. A micro-electrochemical cell (with the total volume of 200 μL), made all of Teflon, was used during the course of this experiment. Three electrodes: a glassy carbon (GC) working electrode (BAS MF-2012, 3 mM diameter), an Ag/AgCl (3.5 M KCl) reference and a Pt (0.5 mM diameter coiled Pt wire) counter electrode constituted the cell. Working electrode was hand-polished with 0.05 μm alumina-water paste (BAS CF-1050), using BAS (MF-1040) polishing cloth and then rinsed with ultra-pure water and methanol. The cyclic voltammetry experiment was performed in the range of 100–1100 mV at a potential sweep-rate of 100 mV s^{-1} at room temperature using a potentiostat/galvanostat G 750 (Gamry Ins., USA). The total charge below the anodic wave curve of the voltammogram was calculated. The cyclic voltammograms of Trolox solutions over the concentration range of 0.05–2.5 mM was determined. The reducing capacity of buckwheat-rich wheat breads was expressed in terms of μmol Trolox/g DM.

2.9. Statistical analysis

Results of the chemical analyses are illustrated as mean values and the standard deviation of three independent measurements. The obtained results were analysed with one-way ANOVA. Fisher Least Significant Difference (LSD) test at a significance level of $p < 0.05$ was performed

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for post-hoc comparison. The Statistica ver. 5.0 software was used (General Convention and Statistica, StatSoft, USA, 1995).

3. Results

Free radical scavenging activity of food extracts should be determined by using different techniques to evaluate the antioxidant capacity of food *in vitro* to cover all aspects of antioxidant effectiveness. Recently, we provided evidences for the main differences in bioactive compounds content as well as in antioxidant properties of two types of buckwheat flours, e.g. BF and BFR when compared to DWF [34]. The estimated values of antioxidant capacity of flours based on the relative abilities of 67% methanol crude extracts to scavenge the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals in comparison to Trolox showed the following order: BF > BFR > DWF. Moreover, chelating and reducing power of two types of buckwheat flour showed a comparable level, being higher than determined for DWF. A well-illustrated difference in the reducing capacity of buckwheat flours and dark wheat flour is presented on **Figure 1** when cyclic voltammetry technique was applied.

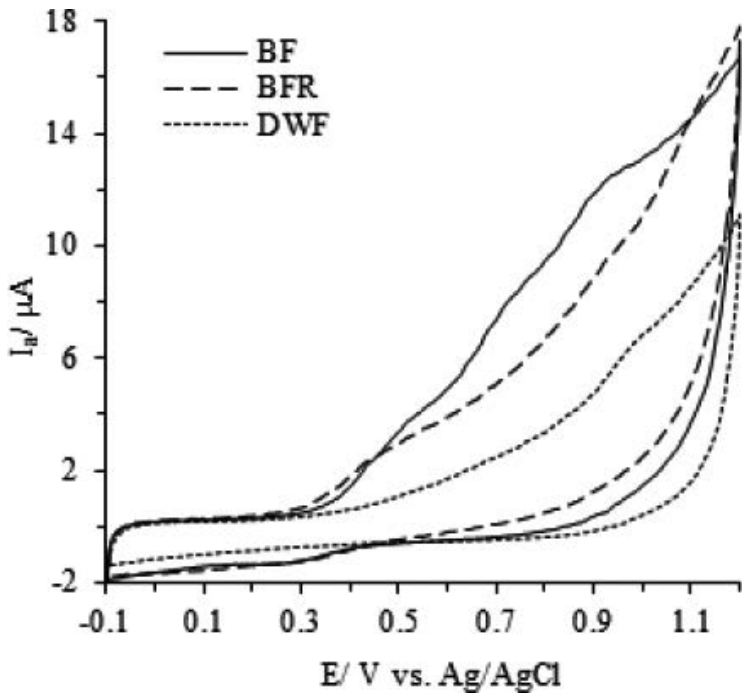

Figure 1. Cyclic voltammograms of buckwheat flour (BF), flour from roasted buckwheat groats (BFR) and dark wheat flour (DWF). Measurements were performed with 67% methanol extracts (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v); scan rate 100 mV s^{-1}. The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated flour extracts.

3.1. Antioxidant capacity of buckwheat-enhanced dark wheat breads as measured against free radicals

The antioxidant activity of buckwheat-enhanced dark wheat breads determined by ABTS, DPPH and PCL assays is shown in **Table 2**. The PCL values show the sum of antioxidant capacity of the hydrophilic (ACW) and lipophilic (ACL) fractions of bread (**Figure 2**). The rank of scavenging effect of reference DWB extract was 5.24 ± 0.24 μmol Trolox/g DM (DPPH assay) > 4.31 ± 0.07 μmol Trolox/g DM (ABTS assay) > 1.48 ± 0.01 μmol Trolox/g DM (PCL assay). The addition of BF or BFR in the range of 10, 20, 30 and 50% in the bread formula caused a significant ($p < 0.05$) increase in antioxidant capacity as compared to the reference DWB. The highest scavenging activity was found in BEDWBs with addition of 50% of BF (for ABTS assay 15.02 ± 0.90 μmol Trolox/g DM, for DPPH assay 8.36 ± 0.12 μmol Trolox/g DM and 3.18 ± 0.07 μmol Trolox/g DM for PCL assay). A similar rank of values was noted in BEDWBs after substitution of DWF by BFR at 50% level (13.99 ± 0.05 μmol Trolox/g DM, 9.20 ± 0.17 μmol Trolox/g DM and 4.43 ± 0.06 μmol Trolox/g DM, respectively). The increased substitution level of DWF by BF or BFR resulted in higher ACL values as compared to ACW (**Figure 2**).

Type of bread	% of buckwheat flours	Antioxidant capacity (μmol Trolox/g d.m.)		
		ABTS	DPPH	PCL
Dark wheat bread (DWB)	0	4.31 ± 0.07a	5.24 ± 0.24a	1.48 ± 0.01a
Buckwheat-enhanced dark wheat breads	10	8.07 ± 0.15b	5.82 ± 0.07b	1.55 ± 0.01b
(BEDWBs) with BF	20	10.06 ± 0.24c	7.48 ± 0.15c	1.72 ± 0.03b
	30	10.82 ± 0.78c	8.24 ± 0.04d	2.54 ± 0.16d
	50	15.02 ± 0.90e	8.36 ± 0.12d	3.18 ± 0.07e
Buckwheat-enhanced dark wheat breads	10	7.13 ± 0.22b	5.99 ± 0.08b	1.92 ± 0.04b
(BEDWBs) with BFR	20	8.83 ± 0.05c	7.68 ± 0.12c	2.07 ± 0.11c
	30	10.03 ± 0.23c	9.00 ± 0.17d	2.94 ± 0.04d
	50	13.99 ± 0.05d	9.20 ± 0.17d	4.43 ± 0.06e

Values are means of three determinations ± standard deviation. Values within column followed by the same letter are not significantly different at 95% confidence level. PCL values show the sum of ACW and ACL values.

Table 2. Antioxidant capacity of bread samples determined against ABTS, DPPH and PCL assays.

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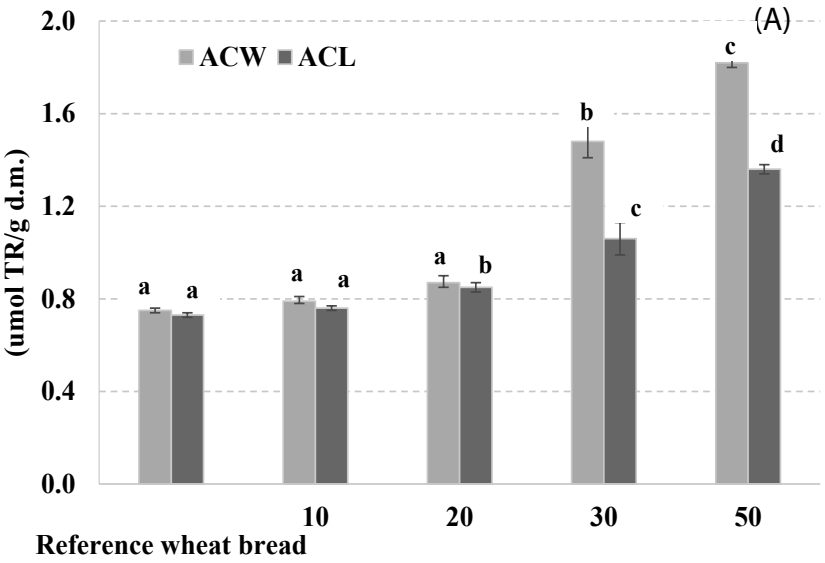

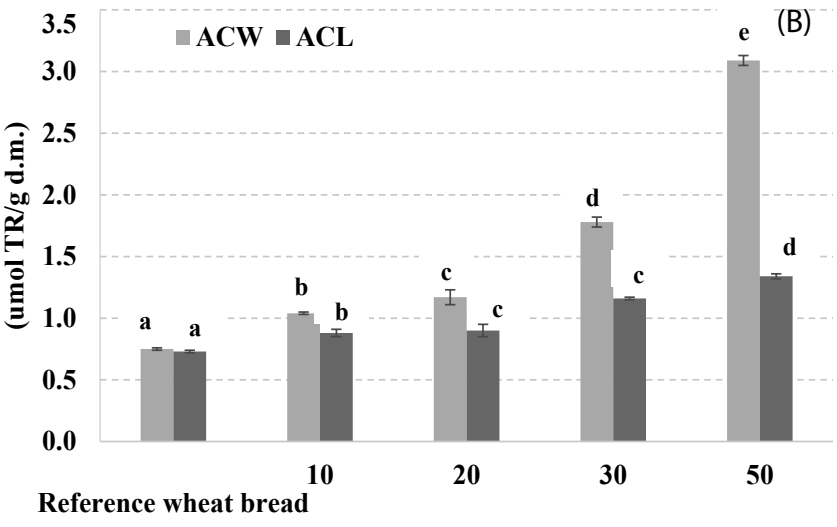

Figure 2. Antioxidant capacity of buckwheat-enriched dark wheat breads formed by hydrophilic (ACW) and lipophilic (ACL) antioxidants. (A) DWF was substituted by BF. (B) DWF was substituted by BFR.

3.2. Reducing power and capacity of buckwheat-enhanced dark wheat breads

Table 3 illustrates the reducing power of BEDWBs as determined by the potassium ferricyanide method. The reducing power of BEDWBs was higher ($p < 0.05$) than noted for DWB. It was found that substitution of DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on the total flour basis caused an increase of the reducing power of BEDWBs. The highest level of DWF substitution (50%) by BF of BFR resulted in 2.5-fold increase of the reducing power of breads as compared to the reference DWB.

Type of bread	Substitution level (%)	Fe(II) chelating capacity	Reducing capacity by CV method	Reducing power by Fe (III) reduction
Dark wheat bread (DWB)	0	9.89 ± 0.01a	1.86 ± 0.11ab	1.76 ± 0.00a
Buckwheat-enhanced dark	10	10.81 ± 0.12b	1.79 ± 0.12a	2.56 ± 0.03b
wheat breads (BEDWBs) with BF	20	10.86 ± 0.09b	3.24 ± 0.21c	3.34 ± 0.09c
	30	13.14 ± 0.32c	3.35 ± 0.22c	3.59 ± 0.05c
	50	13.71 ± 0.05d	4.05 ± 0.27d	4.40 ± 0.26d
Buckwheat-enhanced dark	10	12.32 ± 0.15bc	2.43 ± 0.16b	3.37 ± 0.29c
wheat breads (BEDWBs) with BFR	20	13.48 ± 0.09c	2.40 ± 0.16b	3.93 ± 0.01c
	30	13.78 ± 0.34d	3.57 ± 0.24c	4.62 ± 0.01d
	50	13.97 ± 0.20d	4.92 ± 0.31d	5.34 ± 0.06e

Values are means of three determinations ± standard deviation. Values within column followed by the same letter are not significantly different at 95% confidence level.

Table 3. Reducing power (μmol ascorbic acid equivalents/g DM), reducing capacity (μmol Trolox/g DM) and Fe(II) chelating capacity (μmol EDTA equivalents/g DM) of buckwheat-enhanced dark wheat breads.

A special focus was put on the cyclic voltammetry (CV) experiments as a novel technique. The cyclic voltammograms of 67% MeOH extracts from breads were recorded as it is shown on **Figure 3**. The reducing capacity of BEDWBs was higher ($p < 0.05$) than noted for DWB. The reducing capacity of BEDWBs provided by CV assay was comparable to their reducing power determined by the potassium ferricyanide method (**Table 3**), and antioxidant capacity provided by photochemiluminescence (**Table 2**). In contrast, reducing capacity of BEDWBs was about threefold lower than antioxidant capacity determined against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals.

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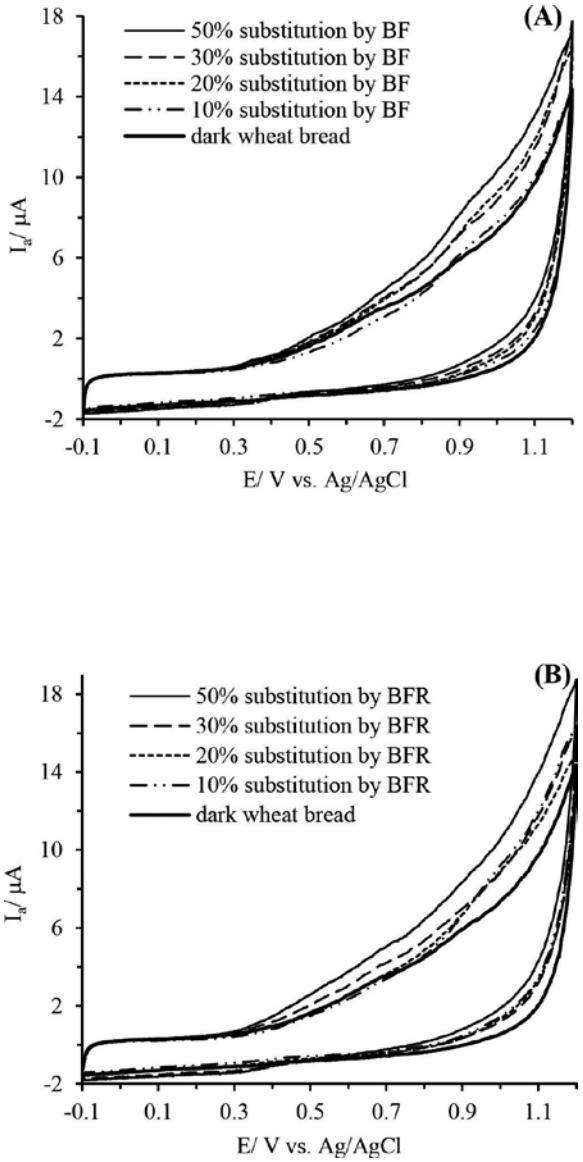

Figure 3. Cyclic voltammograms of buckwheat-enriched dark wheat breads. (A) DWF was substituted by BF. (B) DWF was substituted by BFR. Measurements were performed with 67% methanol extracts (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v); scan rate 100 mV s^{-1}. The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated bread extracts.

3.3. Fe(II) chelating capacity (ChC) of buckwheat-enhanced dark wheat breads

The results of Fe(II) chelating capacity of BEDWBs are summarized in **Table 3**. It was found that DWB as well as all types of BEDWBs contained compounds with Fe(II) chelating capacity. Both buckwheat types of flour were a good source of these compounds since substitution of

DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on the total flour basis resulted in increased chelating capacity of breads. The highest Fe(II) chelating capacity was noted for BEDWBs with 50% substitution of DWF by BF or by BFR. This level of DWF substitution resulted in 40% increase in the chelating capacity of bread as compared to the reference DWB (9.89 ± 0.01 μmol EDTA/g d.m.).

4. Discussion

The obtained results show that substitution of DWF by two types of buckwheat flour, especially by BF, enhanced the antioxidant properties of BEDWBs. This clear beneficial effect may be due to the enrichment of DWB in bioactive compounds, including rutin with well-recognized antioxidant properties. These results are consistent with the results obtained by Zielińska et al. [14] and Zieliński et al. [35]. Similarly, Lin et al. [21] showed that supplementation of wholegrain buckwheat flour in wheat bread resulted in increase of the antioxidant properties more than the application of light buckwheat flour. Whereas Yoo et al. [15] and Błaszczak et al. [16] found that rutin content in buckwheat groats is greatly reduced by thermal processing (by approximately 60%). This finding may explain the lower antioxidant capacity of buckwheat-enhanced dark wheat bread based on flour from roasted groats as compared to bread formulated with buckwheat flour as it was shown in this study. Many researchers also argue that phenolic compounds as well as compounds formed in Maillard reaction (e.g. HMF, furfural and acrylamide) play a significant role in scavenging of free radicals [36, 37]. However, the formation of Maillard reaction compounds can disguise actual reduction of phenolic contents and antioxidant capacity as well as loss of antioxidant activity in bread samples throughout the heat treatment [38].

Furthermore, the present study showed that BEDWBs were more effective scavengers of radical cation ($ABTS^{\bullet+}$) than $DPPH^{\bullet}$ radicals and superoxide anion radical ($O_2^{-\bullet}$). These differences were statistically significant ($p < 0.05$) (**Table 2**). An inverse relationship was found in the reference DWB. This trend in the rank of the radical scavenging activity was demonstrated by Floegel et al. [39] and Xu et al. [40]. Similarly, Sakač et al. [38] observed clear differences in antioxidant capacity between the light buckwheat enriched bread and wholegrain buckwheat enriched bread.

One of the significant mechanisms to defend against oxidative damage and lipid peroxidation is to chelate metal ions. In this study, we observed significant differences in metal chelating activity and reducing power between the reference DWB and BEDWBs (**Table 3**). Especially, supplementation of BFR has contributed to an increased metal chelating activity of BEDWBs. Whereas, Sakač et al. [38] found significant differences in antioxidant capacity measured by metal chelating activity and reducing power between the light and wholegrain buckwheat enriched breads. Enhancement of the antioxidant activity of bread after application of buckwheat flour from milled roasted groats can be related to the modification and/or degradation of phenolic compounds and formation of Maillard reaction products such as melanoidins, which may also act as antioxidants [36, 37]. It is possible that the enhancement of bread

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with BF or BFR contributed to metal chelating activity due to the rutin content, since rutin is well-known as a potent metal chelator. Symonowicz and Kolanek [27] reported that the interactions of phenolic compounds with metal ions caused the formation of chelates. Metal chelation may be important to limit the formation of free radicals, thus reduce oxidative stress. Several studies have confirmed that flavonoids possess antioxidant properties due to their ability to chelate metal ions [26, 27]. Filipčev et al. [18] observed clear differences in metal chelating activity between the buckwheat and rye cookies. They also noticed that buckwheat enriched cookies (in amount 30, 40 and 50%) show a higher antioxidative properties than cookies enriched in rye. In this study a special focus was put on the cyclic voltammetry methodology, which allowed rapid screening of the electrochemical profile of buckwheat-enhanced dark wheat bread samples. This reducing capacity of BEDWBs was based on the electrochemical behaviour and chemical properties of the electroactive compounds being in bread [41]. In this study was found that substitution of DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on total flour basis caused almost a linear increase of the reducing capacity of BEWBs (**Table 3**). It should be mentioned that practical limitation of CV methodology was that the working electrode had to be frequently cleaned to remove residues of sample from its surface and to maintain its sensitivity. However, the advantage of CV was related not to do requiring the use of reactive chemicals.

5. Conclusions

This paper shows the beneficial role of the addition of buckwheat in bakery products. It highlights aspects of buckwheat as a food ingredient and possible use as flour in bakery products. The obtained results indicate that the improved antioxidant properties of buckwheat-enhanced dark wheat bread might be enhanced due to the incorporation of phenolic compounds, mainly rutin and quercetin, which had been shown to possess antioxidant activity. Overall, buckwheat breads could be developed as a source of antioxidant activity for humans.

6. Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Chapter 14

Rice Bran as a Functional Food: An Overview of the Conversion of Rice Bran into a Superfood/Functional Food

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Additional information is available at the end of the chapter

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Abstract

Rice bran is a byproduct of the rice milling process; it constitutes 10% of rice, with a potential global production of 48 million tons per year. The major portion of this is used as animal feed or discarded as waste material. However, rice bran is attracting attention from researchers because it is widely available, cheap and rich in nutrients such as protein, fat, carbohydrates, bioactive compounds and dietary fiber. Many food-processing techniques that have improved rice bran resources have been pioneered, such as enzyme treatment and fermentation. We have been investigating the functional role of rice bran since 2003. Our experiments revealed that rice bran and its active compounds, such as γ-oryzanol, tocopherol, tocotrienol, adenosine and ferulic acid, play a role as a functional food. In this review, we summarize how rice bran is a super food and functional food to illustrate the global interest in rice bran and its functional aspects and medicinal qualities. We also describe the techniques to prepare functional bran and the composition and health benefits of functional bran, which may encourage entrepreneurs to produce rice bran-based food on a large scale and meet the global demand for super foods and functional foods.

Keywords: functional bran, bioactive compounds, adenosine, ferulic acid, γ-oryzanol

1. Introduction

Throughout the history of human civilization, food has been developed to provide nutrition and sustain health. In this regard, the development of "functional foods" is gaining momentum,

because consumers in developing and developed countries wish to maintain better health [1]. The concept of a "functional food" originated in the 1980s in Japan, from where it migrated to Europe and the United States [2]. In general, a functional food is a modified food that improves health and well-being beyond the effects of the nutrients it contains. Generally, foods can be made functional foods by increasing the concentration of, adding, or improving the bioavailability of particular components. Food is considered functional when it can be established that it enhances body function or reduces the risk of diseases [3].

Functional foods have recently emerged as convenient, inexpensive and promising candidates to prevent chronic health problems. Rice bran, a byproduct of the rice milling process, is derived from the outer layer of the rice grain; it contains a number of nutrients and biologically active compounds. Rice bran is often processed using stabilization, fractionation, enzymatic treatment, or fermentation. This treated rice bran is called functional bran. The identification of the bioactive components contained in rice bran has reinforced its status as a functional food.

Experiments have revealed that enzyme-treated or ethanol-extracted rice bran (6% of the diet for 8 weeks) improves blood pressure (BP), the lipid profile and glucose metabolism. Furthermore, adenosine supplementation (10 mg/kg body weight), an active component of functional bran, improved metabolic syndrome in stroke-prone spontaneously hypertensive rats (SHRSPs) [4]. In addition, ferulic acid (FA) supplementation (9.5 mg/kg body weight), another active compound in functional bran (0.19 mg/100 g of rice bran), improves BP and the lipid profile [5]. Thus, the aim of this review was to analyze the evidence of rice bran as a functional food as well as the global interest in rice bran.

2. Compositional distinctiveness of rice bran

The composition of rice bran differs with the variety of rice, geographical conditions and processing methods. Rice bran, the outer layer of the rice grain, accounts for 8–10% of the total weight of the grain; however, it contains most of the nutrients: carbohydrates (34–62%), lipids (15–20%), protein (11–15%), crude fiber (7–11%) and ash (7–10%). In particular, rice lipids and bioactive components are concentrated in rice bran [6, 7]. Fatty acids such as palmitate (21–26%), linoleate (31–33%) and oleate (37–42%) are predominant in rice bran. In addition, due to its high content of polyunsaturated fatty acids, rice bran is considered a healthy food [7, 8]. Significant quantities of bioactive compounds such as γ-oryzanol, tocotrienol, tocopherol and α-sitosterol as well as dietary fibers such as α-glucan, pectin and gum have been found in rice bran [9, 10]. Specifically, γ-oryzanol, the main antioxidant present in rice bran, has a 10-times higher antioxidant activity than tocopherol, while tocotrienol has 40–60 times greater antioxidant activity than tocopherol. However, the proportions of these phytochemicals vary with the type of rice cultivar [11]. In addition, rice bran contains 4-hydroxy-3-methoxycinnamic acid (FA), which has photoprotective and antioxidative effects [12–14].

The health information website SelfNutritionData (http://nutritiondata.self.com) reports that one cup of crude rice bran provides 88 calories and that 28 g of rice bran contains 5.8 g of fat, 1.2 g of which is saturated and 4.2 g of healthy unsaturated fatty acids. According to Walter

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Willett, a nutritionist from Harvard University (https://www.hsph.harvard.edu/walter-willett), heart health is directly related to the ratio of unsaturated to saturated fatty acids in the diet. In addition, one cup of rice bran delivers 13.9 g of carbohydrate and 3.7 g of protein, which is 7% of our daily requirement and a single serving of rice bran delivers more than half of our daily nutritional requirements of thiamine, niacin and pyridoxine.

3. Rice bran as functional bran

3.1. Fermentation

Owing to its disease-preventing properties, rice bran is popular in the food industry. Interestingly, the antidiabetic and antidyslipidemic activities of rice bran have been reported in different animal model experiments [15–17]. Furthermore, the active components in processed rice bran promote health; indeed, the processing itself adds value to the rice bran [18, 19]. Such treated rice bran may protect against metabolic syndrome by attenuating hypertension, dyslipidemia and insulin resistance; it is a candidate functional food because it prevents oxidative stress in rat and mouse models [20–23]. To create a more applicable and functional bran, several fermentation processes have been used to enhance its nutritional value. Rice bran fermented using *Saccharomyces cerevisiae* has anti-stress and anti-fatigue effects. Furthermore, the polysaccharide extracts of rice bran fermented using *Lentinus edodes* showed an anti-cancer effect and they prevented defective immune responses; the water extracts of the same fermented rice bran had an anti-photoaging effect [24, 25]. Moreover, brown rice fermented using *Aspergillus oryzae* has a suppressive effect on dextran sulfate sodium-induced ulcerative colitis and it inhibits inflammation-mediated cell infiltration [26, 27]. Rice bran extract fermented using *Lactobacillus plantarum* improves functional recovery and reduces cognitive impairment after ischemic brain injury in a rat model [28, 29]. Fermentation using different microbes can increase the levels of bioactive compounds as well as the availability of functional food. For instance, fermentation using *Rhizopus oryzae* increases the protein content of rice bran (43%); it also increases the levels of phenolic compounds, which have high antioxidant activity, by breaking down lignin in the substrate cell wall [30].

3.2. Compositional improvement as functional bran

Rice bran is processed to inactivate lipases and other nutritional inhibitors such as field fungi, bacteria and insects, to reduce their toxicity without damage to the protein quality of rice bran. The rice bran must be stabilized using suitable techniques while bran layers are removed from the endosperm during milling. Specifically, to achieve proper stabilization, each individual bran particle must have the same moisture content, depending on the time and temperature. Furthermore, to inactivate the enzymes in the rice bran that are responsible for rancidity, different stabilization methods are used. Among these, microwave energy offers an alternative energy source for stabilization [30–32]. Next, stabilization fractionation is performed. This is an important step in industrial processing; it involves the conversion of rice bran into various parts that contain more desirable than undesirable components. Subsequently, the different

fractions are centrifuged to separate the insoluble fiber fraction—called rice bran fiber—from the aqueous dispersible fraction—called rice bran soluble. The mixture of both insoluble and soluble extracts is called rice bran balance. Using different technologies, the bran is fully stabilized and the oil is removed. The resultant food-grade, defatted rice bran is temporarily stored in food grade silos until it can be used in edible applications. Bleaching of the edible oil typically leaves minor flavor and odor compounds that must be removed by steam distillation before the oil is used. Steam distillation is the final step in the processing of edible oil, whereby any off-flavor and residual free fatty acids left in the oil are removed.

We produced two types of rice bran fraction: Driselase® fraction (DF) and ethanol fraction (EF). To process the rice bran, 500 g bran was agitated in 1.0 L of 70% ethanol for 2 h; this yielded two fractions: the solid and filtered fractions. The DF was derived from the solid fraction. Driselase® is a commercial plant cell wall-degrading enzyme mixture containing cellulase, xylanase and laminarinase; however, it is esterase free. The solid fraction of rice bran was dried at room temperature and then suspended in 10 mM acetate buffer (500 mL) containing Driselase (0.2 mg/L) from *Basidiomycetes* spp. The bran was treated in this manner overnight at 37°C; the suspension was then filtered and finally lyophilized. As a result, Driselase-treated rice bran had increased quantities of bioactive components that improve glucose and lipid metabolism in the SHRSPs—a genetic animal model of metabolic syndrome [33, 34].

4. Extraction, isolation and identification of the active components in rice bran

Different conventional methods are used to extract the bioactive compounds from plant materials. Of these, solvent, supercritical fluid, microwave-assisted and ultrasonic-assisted extraction are notable. Microwave-assisted extraction and ultrasound-assisted methods are used to detect antioxidant and anticancer bioactive components in the plant extracts [35, 36]. Imsanguan et al. described conventional solvent extraction, with different modifications at different temperatures (32–60°C), using 100 ml hexane at a rotating speed of 200 rpm for 24 h to extract γ-oryzanol from rice bran [37]. However, this conventional technique does not fully remove toxic solvent residues from the final product; for this reason, Herrero et al. used the prominent technique of supercritical fluid extraction, which offers better extraction and purification of bioactive compounds [38]. Zigoneanu et al. described antioxidant extraction from rice bran oil using microwave-assisted extraction, which uses electromagnetic radiation in the range of 0.3–300 GHz [39].

We developed the DF method to identify active components in the rice bran. As already described, the DF was derived from the solid fraction and chromatographed onto a silica gel column. One fraction derived from the methanol eluate was further fractionated using an octadecylsilane (ODS) column. The active fraction was obtained from the methanol/water (20–70%) eluate and separated by high-performance liquid chromatography (HPLC) using an ODS column. The BP-lowering activity of each fraction was examined using a single oral administration to male, 14-week old SHRSPs; we found that gavage of a certain fraction at 40 mg/kg

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body weight decreased BP significantly 1, 2, 4 and 6 h after administration. The chemical structure of this fraction was determined using fast atom-bombardmentmass spectrometry, as well as NMR analyses; we then identified adenosine as the active compound [4].

γ-Oryzanol was initially acknowledged as a single component when it was extracted from rice bran oil. Subsequently, 10 fractions were isolated using reverse-phase HPLC and their structures were determined using gas chromatography-mass spectrometry. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate were identified as the major components of γ-oryzanol [40–42].

Phenolic compounds were identified in the rice bran using sequential fractionation and subfractionation using Sephadex LH-20 chromatography with 40% acetone. The total phenolic content was highest in the subfraction portion and the major phenolic acid was identified as FA (178.3 μg/mg) using HPLC and liquid chromatography-electrospray ionization-tandem mass spectrometry analyses [43, 44]. The chemical structure of the active components of rice bran is illustrated in **Figure 1**.

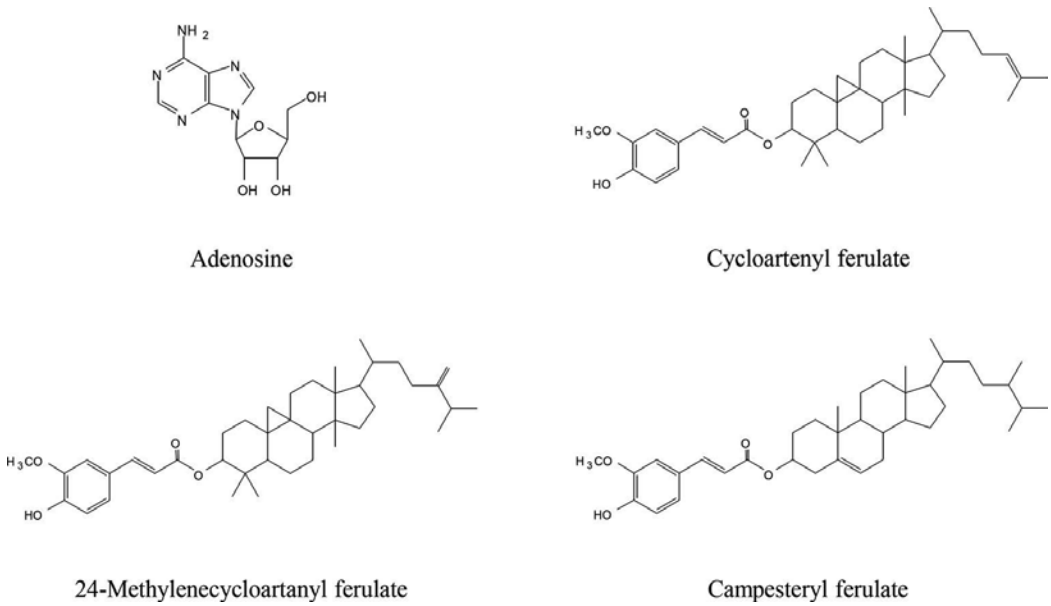

Figure 1. Chemical structure of bioactive components of rice bran.

5. Role of bioactive components

5.1. Hypertensive aspects

The following factors increase the risk of diet-related disorders such as obesity, cancer and cardiovascular disease (CVD): consumption of fewer plant-based foods, changing dietary

patterns, increased consumption of Westernized food and socioeconomic conditions. High BP, one manifestation of CVD, continues to be a major cause of morbidity and death and one public health strategy is dietary management of high BP. Studies have shown that a 5-mm Hg decrease in BP is related to a 16% decrease in CVD [45]. The risk factors for CVD are higher plasma cholesterol levels, lower high-density lipoprotein (HDL) levels and higher low-density lipoprotein (LDL) levels. Several bioactive compounds from rice bran that have been identified and used may reduce the risk of CVD. For instance, angiotensin-converting enzyme (ACE) inhibitors reduce BP via the renin-angiotensin system and FA has plasma ACE-inhibitory activity. In this regard, Ardiansyah et al. showed that food supplementation using FA reduces high BP by inhibiting plasma ACE activity [5]. Later, the novel compound adenosine, isolated from the DF fraction, was also found to have BP-lowering activity. Specifically, single-dose or long-term orally administered adenosine may reduce BP in the spontaneous hypertension of the SHRSP model [4]. Administered adenosine increases plasma nitric oxide levels, which in turn increase vasodilation. Adenosine also causes potent vasodilation by activating adenosine receptors (A2) on vascular smooth muscle; moreover, it stimulates K+ATP channels, resulting in the hyperpolarization of smooth muscle [4].

The risk of CVD is elevated in conditions of oxidative stress. Urinary 8-hydroxydeoxyguanosine (8-OHdG) serves as a sensitive biomarker of oxidative stress resulting in genetic damage. FA significantly reduces urinary 8-OHdG levels; thus, it can reduce CVD risk factors [4, 5].

Diets containing cholesterol-lowering phytochemicals and antioxidants can prevent the progression of atherosclerotic lesions. Experiments have demonstrated that γ-oryzanol possesses potent anti-atherogenic and antioxidant activity. Furthermore, in a rat model of two-kidney, one-clip renovascular hypertension, Boonla et al. described the vasorelaxant and antihypertensive effects of peptides derived from rice bran protein hydrolysates [46]. **Table 1** describes the anti-hypertensive roles of various bioactive components of rice bran.

5.2. Metabolic disorder aspects

Metabolic disorders consist of metabolism-related diseases, including hyperglycemia, hypercholesterolemia, hypertriglyceridemia and insulin resistance; they accompany type 2 diabetes mellitus, obesity and CVD. Rice bran and its various active components, prevents or ameliorates metabolic disorders. Specifically, a rice bran enzymatic extract-supplemented diet can prevent the adipose and macrophage changes associated with diet-induced obesity in mice [54]. In addition, the antihyperlipidemic effects (lower cholesterol and triglyceride levels) of α-tocopherol have been investigated in F344 rats fed a Western diet [55]. Pigmented rice, which contains anthocyanins and proanthocyanidins concentrated in the bran layer, stimulates glucose uptake by 3T3-L1 adipocytes—a key function in glucose homeostasis. Specifically, basal glucose uptake is increased two to three fold, while mRNA levels of both GLUT1 and GLUT4 are upregulated [56]. γ-Oryzanol and FA ester with phytosterols—both of which are abundant in rice bran—prevent high-fat and high-fructose diet (HFFD)-induced metabolic syndrome [57]. In addition, only γ-oryzanol treatment is more effective than FA in significantly decreasing the liver index and hepatic triglyceride content. Decreased serum C-reactive protein and IL-6 levels and increased serum adiponectin concentration confirmed that FA and γ-

oryzanol can be used as dietary supplements to alleviate the deleterious effects of HFFD [57]. Adenosine, in particular, effectively mitigates metabolic syndrome in SHRSP [50]. Specifically, single-dose and long-term oral administration of adenosine improves hyperlipidemia and hyperinsulinemia; it also regulates body weight gain and food intake. Studies have shown that enhanced plasma adiponectin levels alleviate hyperinsulinemia and that dietary adenosine can elevate plasma adiponectin and increase insulin sensitivity. Adenosine administration for 3 weeks downregulates mRNA levels of glucose-6-phosphatase, a gene encoding the rate-controlling enzyme of hepatic gluconeogenesis. Adenosine also plays an important role in regulating hepatic mRNA expression of genes involved in β-oxidation, fatty acid synthesis and AMP-activated protein kinase [4, 50]. In conclusion, various active components of rice bran ameliorate metabolic-related diseases.

Rice bran component	Species	Dose	Effect	Ref
Adenosine	Rat	10 mg/L drinking water	↓ Blood pressure Blood ↓ TC, LDL-C, TG, FFA, glucose, insulin, leptin, ↑ HDL-C, adiponectin Liver ↓ TC, TG	[4]
Ferulic acid	Rat	9.5 mg/kg BW	↓ Blood pressure Blood ↓ TC, TG, ACE activity	[5]
Peptides-derived from rice bran protein	Rat	50–100 mg/kg BW	↓ Blood pressure Blood ↓ ACE activity, ↑ NO Thoracic aorta ↑ eNOS, ↓ $p47^{phox}$ NADPH oxidase subunit	[46]
γ-Oryzanol	Rat	0.5–2% diet	Blood ↓ TC, LDL-C, HDL-C, VLDL-C Liver ↓ cholesterol esters, TG	[47]
γ-Oryzanol	Rat	10 mg/kg BW/day	Blood ↓ TC, PL, TG, FFA, free cholesterol	[48]
γ-Oryzanol	Rat	1% diet	Blood ↓ TC, LDL-C, VLDL-C, TG, PL, ↑ HDL-C	[49]
Adenosine 5′-monophosphate	Rat	87.5 mg/kg diet	↓ Blood pressure Blood ↓ TG, glucose, insulin, ↑ HDL-C, adiponectin Liver ↓ TC, TG	[50]
γ-Oryzanol	Human	300 mg/day	Blood ↓ TC, LDL-C, TG, lipid peroxides, ↑ HDL-C	[51]
Tocotrienol	Human	200 mg/day	Blood ↓ TC, LDL-C, Apo B, platelet aggregation	[52]
Rice bran oil	Human	50 g/day	Blood ↓ TC, LDL-C, TG	[53]

ACE, angiotensin-converting enzyme; ApoB, apolipoprotein B; BW, body weight; FFA, non-esterified fatty acid; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; PL, phospholipid; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol; eNOS, endothelial nitric oxide synthase.

Table 1. Summary of bioactive components and their prospective effects on blood pressure and metabolic parameters in different species.

5.3. Anti-cancer aspects

Dietary factors have a significant effect on the risk of cancer. Only 5–10% of all cancer is heredity; all other incidents are directly or indirectly correlated with lifestyle and dietary habits. If dietary supplements are used appropriately, they may reduce the incidence of cancers

in humans by as much as 30% [58, 59]. Phytic acid extracted from rice bran has anticancer activity against hepatocellular carcinoma (HepG2) cells, wherein apoptotic activity was evaluated by expression analysis of apoptosis-regulatory genes (i.e., p53, Bcl-2, Bax, Caspase-3 and -9) [60]. Similarly, δ-tocotrienol (δ-T3) is reportedly useful as an anticancer agent against human colorectal adenocarcinoma (DLD-1) cells under both normoxic and hypoxic conditions. *In vivo*, oral administration of rice bran tocotrienol (mainly γ-T3; 10 mg/mouse/day) significantly inhibited tumor growth in nude mice [61]. Tumor cells produce reactive oxygen species, which damage cellular integrity. Cycloartenyl ferulate, a major component of γ-oryzanol, successfully inhibits proliferation in the colorectal adenocarcinoma SW480 cell line because of its antioxidant activity [62].

5.4. General health-promoting aspects

Rice bran itself has health benefits, while rice bran oil and isolated active components have immune stimulatory effects. Rice bran that is rich in phytosterols, γ-oryzanol and compounds with antioxidant properties may modulate the immune system. In addition, rice bran has several generalized health-promoting characteristics. For example, rice bran supplementation enhances gut health by encouraging the growth and colonization of *Lactobacillus rhamnosus* and it provides effective protection against human rotavirus diarrhea in pigs by modulating gut permeability [63].

Long-term supplementation has a positive impact on survival, cognition and brain mitochondrial function, which may delay Alzheimer's disease [64]. Rice bran supplements can also be used as ergogenic supplements by body builders and athletes [65] and they may mitigate menopausal symptoms such as hot flashes, as well as bone loss in older women who suffer from osteoporosis [66]. Rice bran can be regarded as a source of plant-derived active compounds and as an alternative to expensive vitamin sources from animals. For instance, different colored rice bran has micronutrients, including a rich reserve of β-carotene, which can be converted to vitamin A [67]. **Table 2** describes the role of various bioactive components of rice bran for general health.

Rice bran component	Health aspects	Dose	Effect	Ref
Rice bran	HRV induced diarrhea prevention in gnotobiotic pigs	10%	↑ Intestinal IFN-γ and total IgA levels	[63]
γ-Oryzanol	Brain aging in NMRI mice	4 g/kg diet for 6 months	↑ Mitochondrial proteins	[64]
γ-Oryzanol	Human health growth	500 mg/day	↑ Muscular strength (bench press and squat) and vertical jump power	[65]
γ-Oryzanol	Postmenoposal osteoporosis in rats	0.3% crystalized oryzanol	↑ Estrogen, Bone mineral	[66]

HRV, human rota virus; IFN-γ, interferon gamma; IgA, immunoglobulin A.

Table 2. Summary of bioactive components and their prospective effect on generalized health aspects.

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6. Conclusion

Previously, rice bran was only used in animal feed or discarded as waste. However, now it is treated as a potential source for the preparation of nutraceuticals. In this review, the therapeutic role of rice bran itself and of its bioactive and novel components has been described briefly from different clinical points of view. We noted that rice bran has various health benefits in terms of disease prevention and that it can be used to treat humans and experimental animals with no side effects. Owing to its significant nutritive and therapeutic value, rice bran may enhance well-being and health, as well as reduce the risk of disease, providing health benefits and improving quality of life. Thus, rice bran can be considered a super food and/or functional food. However, the true potential of functional bran could be developed using new biotechnological methods.

In conclusion, there is a strong demand for the enrichment of functional bran components in different diet-based approaches that mitigate lifestyle-related disorders. Entrepreneurs should be encouraged to consider rice bran as a major source of bioactive components for the developments of super foods.

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Section 4

Pet Foods with Functional Properties

Chapter 15

Benefits of Super Food and Functional Food for Companion Animals

Mary Ememe and Chukwuma Ememe

Additional information is available at the end of the chapter

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Abstract

This chapter reviews super foods and functional foods for companion animals such as dogs, cats, and horses. Super foods are considered to be beneficial for health and well-being, whereas functional foods are fortified or enhanced foods that may provide a health benefit beyond the traditional nutrient they contain. Super foods for dogs and cats include blueberries, sweet potatoes, broccoli, cocoa, tomatoes, spinach, banana, strawberry, apples, carrots, coconut oil, quinoa, kale, and raw honey. Examples of functional foods for dogs and cats include omega-3–enriched egg, fatty fish, soybean oil, nuts, yogurt, and oatmeal. These food products help pets fight disease, maintain healthy skin and shiny coat, improve healthy digestion, maintain joints and strong bones, boost immune system, promote longevity, boost energy, and maintain good health in general. Many nutrients including essential fatty acids, zinc, vitamin A, vitamin E, and B-complex vitamins are now incorporated in pet foods for normal functioning of the skin and coat condition. Super foods for horses, such as pollen bee, Echinacea, and spirulina, are natural foods that have high-quality vitamins, minerals, cofactors, and enzymes. They support optimal digestive health and boost the immune system in horses. This chapter highlights the benefits derived by consuming super foods and functional foods and some specific claims supported by scientific research of these foods in companion animals.

Keywords: super food, functional food, cats, dogs, horses, nutrients

1. Introduction

Super food is a nonmedical, marketing term that refers to natural foods supposed to be useful for health because they are rich in a particular antioxidant or any other nutrient [1]. They are edibles that deliver the maximum amount of nutrients with minimum calories [2]. They help pets fight disease, maintain healthy skin and shiny coat, improve healthy digestion,

maintain joints and strong bones, fight tartar and bad breath, whiten teeth, detoxify the body system, boost immune system, promote longevity, boost energy, and maintain good health in general [3]. Unlike super foods, functional foods are natural or processed foods [1]. They contain considerable levels of biologically active components that impart health benefits beyond the basic essential nutrients [4, 5]. They also provide clinically proven and documented health benefits for the prevention, management, or treatment of chronic diseases [6]. The philosophy of food as medicine was supported by Hippocrates in approximately 400 B.C. [7]. This thinking is the foundation of functional foods used to clarify the idea that food can be a powerful deterrent and cure of many diseases and ailments. Hippocrates believed that the things eaten can help the body fend off disease and food should be the first line of disease defense, used as a disease preventative mechanism. Previous studies have provided great examples of how specific ingredients in some pets' foods can act as medicines [8]. In the past, many of the perceptions about healthy eating have focused on avoiding certain components in foods, such as fat and sugar. Nutritional research has shifted from the prevention of nutritional deficiencies, such as vitamin C and scurvy or niacin and pellagra, to the prevention of chronic diseases [4]. Most pets do not receive an adequate amount of raw, essential nutrients in their diet especially in highly processed commercial pet food. Most of the natural pet foods go through a great deal of cooking, rendering, and sterilizing thereby reducing the essential nutrients [3].

Examples of super food and functional foods are oats, garlic, green tea, red grape juice, red wine, tomatoes, soy products, flaxseed, broccoli, cocoa, blueberries, carrots, sweet potatoes, pumpkin, onions, kale, cherries, and apples [9]. Oats contain beta-glucan, a soluble fiber, which aids to reduce the risk of cardiovascular disease by lowering blood cholesterol. Garlic is rich in allicin and lowers cholesterol levels and blood pressure [10]. It also stimulates immune function and slows the growth of cancer cells. Green tea contains polyphenols and may help prevent cancer [9]. Red grape juice and red wine contain resveratrol, which aids in prevention of heart disease and cancer [11]. Tomatoes contain lycopene, a carotenoid that helps to reduce risk for cancer of the colon, prostate, bladder, and pancreas [9, 12]. Soy products (tofu, tempeh, soy milk, miso, etc.) contain genistein and isoflavones [13]. These compounds contain blood vessel formation that supplies cancer cells and hinder the body's synthesis of estrogen, thereby reducing the risk for breast, ovarian, and prostate cancer [14]. Flaxseed that contains lignans, powerful antioxidants, stops cells from becoming cancerous. It also contains alpha-linolenic acid, a type of plant omega-3 fatty acids that may lessen the risk of heart disease [9]. Broccoli has indoles that protect cells against carcinogens and aids the liver inactivate estrogen-like compounds that may sustain formation of breast cancer [2]. Cocoa has similarly been claimed to cut the risk of heart disease by lowering blood pressure and increasing the elasticity of blood vessels. This is thought to be due to cocoa's high content of compounds called flavonoids [15, 16]. Blueberries, carrots, sweet potatoes, and pumpkin provide healthy vitamins including A, B, and C that are good for a healthy coat and immune system [2]. Onions, kale, cherries, and apples contain quercetin that also helps to fight cancer and heart disease [14, 17]. Other fruits that have super food status include açaí berries and pomegranates. The fruit pulp of açaí berries has been shown to have potent antioxidant properties [18, 19]. Researchers have recognized these components and are trying to determine exactly what benefits they may present [20].

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2. Super foods and functional food for dogs

Super foods and functional foods have positive effect on dogs' health and protect them against a range of diseases [2]. **Figure 1** shows a dog consuming a super food.

Figure 1. A dog consuming a super food [22].

Kale: It is a supercharged leafy vegetable that contains an abundant amount of vitamins, including A, E, C, K, iron, and calcium [21]. It is a good source of antioxidants and helps the liver detoxify the body. It also has anti-inflammatory properties. Kale should be avoided in pets with certain types of bladder stones or kidney disease [2].

Carrots: They are filled with carotenoids, fiber, vitamin C, K, magnesium, manganese, most of the B vitamins, phosphorus, and potassium [22]. They are packed with beta-carotene that aids in the prevention of heart disease and healthy eyesight. Carrots may be used as shavings to "bulk up" main meals in order to help fat dogs lose weight [21].

Pumpkin: It is low in calories and high in soluble fiber, pumpkin helps maintain a healthy digestive tract. It is low in sodium and exceptionally high in carotenoids, potassium, and vitamin C, and has some calcium and B vitamins [2].

Sweet potatoes: They are super high in heart-healthy vitamin A and C and also manganese and iron that are good for a healthy coat and immune system. Their high level of fiber also aids in healthy digestion [21]. Sweet potatoes are among the top vegetables on the nutrition scale and have 150% more antioxidants than blueberries. This tuberous root vegetable acting

as an antioxidant tackles free radicals in the body and helps in healing and disease prevention [2]. Potato fiber, a prebiotic, has been shown to promote the production of many essential molecules and shifted the microbiome in ways that may be vital to gut health [23]. A study in dogs after potato fiber was added in their diet showed that *Faecalibacterium prausnitzii*, a probiotic proliferated butyrate, short-chained fatty acids (SCFAs) increased and there was an overall decrease in fecal pH [23]. Each of these has been involved with lower incidence of infectious bowel disease.

Fish: Fish is an excellent protein source with many essential vitamins and minerals [24]. Oily fishes such as herring, salmon, sardines, mackerel, and anchovies are rich in omega-3 fatty acids [2]. Omega-3s are good for the coat and brain as well as limit inflammatory processes that cause arthritic pain and other chronic canine conditions. Moreau et al. [25] showed that after feeding diets higher in omega-3 fatty acids, there was an improvement in locomotory disability and performance in a dog showing osteoarthritis. Dogs showing atopic dermatitis (AD), a chronic and relapsing common eczematous skin disease, and fed a standardized mixture of fish, potato, and natural compounds (aloe vera, arctium lappa, malva sylvestris, and ribes nigrum) demonstrated a reduction in the overall intensity of each symptom within 20 days [26].

Oilseeds: Many prepared pet foods include omega-6 fatty acids found in oilseeds such as sunflower, canola, and safflower. Omega-6 fatty acids are recognized as important nutrients for reproduction, tissue repair, skin health, and coat condition [27].

Seaweed/Nori: Dried edible seaweed is a Japanese staple, often associated with sushi. It has protein, galactans (a soluble fiber), vitamins C, E and all the Bs, and minerals such as zinc and copper. B-group vitamins and zinc are a key nutrient for healthy skin and are widely incorporated into prepared pet foods to support healthy skin and coats [21].

Seaweed contains some lesser-known sterols and chlorophyll, which have been investigated for their effects on regulating metabolism [2]. Nori may have beneficial effects on fat metabolism, immune function, and antitumor response.

Sugar beet pulp: It contains fiber that is important for digestion and helps to prevent a range of serious health conditions and constipation. Dietary fiber provides bulk and helps to regulate stool volume and consistency [27]. Soluble dietary fiber gives such health benefits including careful stimulation of growth and activity of the bacteria that live in the colon. They also act as natural protective mechanism against invasion by bacteria.

Chia: The seeds of this traditional grain from Mesoamerica have numerous benefits as flax [2]. The nutritional benefits of chia include fiber, omega fatty acids, calcium, antioxidants, and protein.

Coconut oil: The "good fats" in coconut demonstrate the properties of antioxidants and boost vitamin E, promoting tissue health and shiny coats in dogs [21]. Coconut oil is over 90% saturated fat and has antimicrobial, antibacterial, and antifungal properties [28].

Raw honey: Honey is made up of simple sugars—mostly glucose and fructose that add to a range of health concerns, from obesity to diabetes [29]. Raw honey is preferred in dogs

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because it has super food health benefits [30]. This is because it has not been heat treated (pasteurized) or processed and has retained its original nutritional qualities [31]. It is typically thick and milky in appearance. Pasteurization destroys honey's beneficial properties, leaving behind a sugary, high-glycemic sweetener [29]. Feeding raw honey to dogs has such benefits including alkaline-forming, high in antioxidants, containing natural enzymes and nutrients, powerful antibacterial and antimicrobial properties, helps heal ulcers, helps manage diarrhea, and aids indigestion [32]. Raw honey is also helpful in treating topical wounds, including sunburns and mild burns. This is due to the chemical reaction that occurs between glucose in the honey and an enzyme added by honeybees called glucose oxidase. When the honey comes in contact with the skin, glucose oxidase breaks down the glucose into hydrogen peroxide, which is antibacterial. Pasteurized honey, however, is not beneficial for wound treatment [29]. Also consumption of locally grown raw honey helps to prevent seasonal pollen allergies because locally grown raw honey has local pollen spores taken up by the bees, hence consumption leads to gradual production of immunity to the pollen [33, 34]. Manuka honey is the most useful of all honeys. In clinical trials, Manuka honey has helped to destroy bacteria such as methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *S.aureus*, vancomycin-resistant enterococci, and *Helicobacter pylori* [31]. Raw honey should be fed in moderate amount to dogs, a teaspoon per day for small dogs and a tablespoon per day for large dogs. Care should be taken especially in diabetic or overweight dogs. Puppies should not eat raw honey, as it can potentially be contaminated with a botulism-related toxin which their immature immune systems are unable to defend [29].

Blueberries: They are filled with antioxidants known to boost cognitive function in dogs [21]. Blueberries are one of the most popular and well-known super foods, and have been studied frequently by scientists curious about their health properties [35]. The high concentrations of antioxidant and anthocyanins inhibit the growth or kill cancerous human colon cells [36]. Blueberries are also rich in other antioxidants that may prevent and reverse age-related memory decline in rats [37].

Quinoa: It is packed with protein; studies have reported that quinoa is also associated with preventing heart disease and cancer. Because it is nutritionally denser than most processed carbohydrates, it makes an excellent rice and grain alternative [21].

Natural peanut butter: A tasty source of protein used to build and repair muscle tissue; peanut butter is filled with "good fats" that support a healthy cardiovascular system. Caution should be taken while feeding pups with peanut butter as it is high in calorie [21].

2.1. Super foods and functional food for cats

Dandelion: Dandelion leaves and roots may relieve feline allergies and aid with healthy digestion. Roots of pesticide-free dandelions are mostly useful in liver detoxification. Cats sometimes chew the plants hence these help to get a little roughage in their diet [38].

Cranberries: The nutrients in this super berry are known for preventing recurrent urinary tract infections and promoting overall urinary and kidney health [38]. Cranberry is packed with antioxidants and anticancer agents.

Yogurt: Plain (not vanilla or sweetened) whole-milk yogurt is good for cats. Yogurt is rich in protein and calcium, and it is a great source of probiotics [39]. Lactose is already broken down with the culturing of the yogurt, and milk proteins are either removed or reduced, hence it is simple and delicious for cats to digest. It is recommended that cat is gradually introduced to yogurt consumption to avoid allergic reactions [38].

Lean meats: This is a top cat super food. Lean meats such as cooked chicken, beef, or pork with no noticeable fat boost protein in cat's diet. Lean meat is an excellent source of iron and B vitamins, which are involved in kitty's energy metabolism [38]. Fat trimmings should be avoided as too much fat at one sitting puts cat at risk of pancreatitis.

Fish: Fish is a great source of lean protein and omega-3 fatty acids [24]. Oily fishes, such as salmon, sardines, and anchovies, have benefits of omega-3s without doses of mercury [40]. The omega-3 fatty acids in salmon and other oily fish may prevent heart problems in people with a high-cardiovascular risk, as well as ease joint pain experienced by patients with rheumatoid arthritis [24]. These healthy fats have been shown to help cats' coats stay shiny and lustrous to fighting cancer. Most pet foods are heavy on omega-6s, which are proinflammatory. Therefore, to balance the ratio in the body and reduce the danger of inflammation, cats should be fed a diet rich in the omega-3s docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [39].

Eggs: Eggs are a super source of protein and very easy to digest. They also contain essential amino acids responsible for keeping kitty lean and muscular. Cooked eggs are preferable as there are problems with serving cat raw eggs [39]. There is the possibility of food poisoning and protein in raw egg whites, called avidin, interferes with the absorption of the vitamin B biotin, which cat needs for healthy skin and shiny coat [40].

A good rule of thumb is that these super foods and functional foods for cats should not make up more than 15% of their diet [38]. **Figure 2** indicates a cat being fed on super food.

Figure 2. A cat consuming a super food [38].

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2.2. Super foods and functional foods for dogs and cats

Goat's milk: It is a new super food for dogs and cats. It contains very small or no casein at all unlike cow milk. Casein, along with other different proteins, makes cow's milk much more difficult to digest, increasing the likelihood of allergic reactions [41]. Goat milk does not cause much allergic reaction. Previous studies have shown that the fat present in goat milk is very easily absorbed, and the higher concentration of short- and medium-chain fatty acids significantly enhances digestibility. Goat milk also has high amounts of enzymes, vitamins, minerals, trace elements, vital electrolytes, and the naturally occurring prebiotics (fructooligosaccharide) as a possible aid in the treatment of diabetes [41].

Avocado: It may be one of the best sources of essential nutrients for dogs or cats as one powerful super food [42]. Persin is a substance found in the avocado pit, skin, bark, leaves and is harmful to some animals such as birds, rabbits, horses, and goats. However, there have been no reported cases of avocado poisoning in dogs and cats [42].

2.3. Super foods and functional food for horses

Feeding super foods promote a naturally healthy horse. Feeding high-quality super foods is healthier than feeding highly processed foods. Most horse feeds include probiotics and prebiotics, which aid in enhancing the general digestibility of horse's diet, reduce susceptibility to pathogenic bacteria, and improve immune system [43]. Super foods for horses, such as pollen bee, Echinacea, and spirulina, have high nutritional value in the most bioavailable form for horses [44]. This means the horse's body will fully utilize this type of nutrition and receive multiple benefits due to easy absorption of the nutrients and their synergistic properties [45].

Spirulina: Spirulina (**Figure 3**) is a type of blue-green algae (BGA), which is rich in protein, vitamins, minerals, and carotenoids [46]. There are numerous reports in the veterinary literature of BGA down-regulating inflammation in colitis, liver disease, joint disease, and neuropathic pain in horses [47]. It is useful for a variety of equine conditions; it is used in horses for lung allergy issues. Spirulina is probably best known for its ability to boost the immune system. It is high in protein (55–70%), amino acids, vitamin B-12, and vitamin E. It contains essential fatty acids including gamma-linoleic acid (GLA) for heart and joint health. It contains high concentrations of all minerals including trace minerals [45]. It specifically improves the production of IgG antibodies, while down-regulating allergies associated with IgA antibody responses [48]. Spirulina supplementation can improve such equine conditions including Heaves/chronic obstructive pulmonary disease (COPD)/asthma, seasonal respiratory allergies, hives and other skin allergies, sweet itch (summer eczema), and poor immune function. It is easily digestible and highly bioavailable [46].

Psyllium: Studies have shown that feeding a daily dose of psyllium can effectively limit the severity of hindgut ulcers and may aid in the prevention of their occurrence [49]. It creates a barrier and limits the intestinal absorption of acids thus decreasing the incidence of ulcer-

ation. Multiple studies have evaluated the benefits of psyllium's daily use in horses who struggle with right dorsal colitis. Intestinal bacteria ferment psyllium to produce short-chain fatty acids that can aid in the healing of mucosal tissues [50]. Feeding psyllium to horses can actually lower blood glucose and insulin levels [51]. This may aid to ameliorate conditions such as equine metabolic syndrome, insulin resistance, Cushings, and many other common metabolic issues. When psyllium is fed in its proper form, psyllium has the ability to support optimal digestive function. By coating the intestinal lining, it allows for the effective movement of feed and debris through the digestive tract while also promoting maximum absorption of nutrients and water [52].

Figure 3. Spirulina powder [48].

Bee pollen: It contains high concentrations of living enzymes. It is good for older horses or those that have inadequate grazing. Bee pollen contains a full range of super whole food vitamins, minerals, coenzymes, antioxidants, and amino acids. It has helped older horses maintain their muscle tone as they age [45]. Pollen contains high concentrations of living enzymes. However, high heat destroys enzymes; therefore, it is essential that the form is raw whole food. Bee is collected from flower pollen and is a natural food for horses, and can bring them into a healthy springtime bloom anytime of the year [53]. There are numerous reports of the benefits of supplemented bee pollen in horses including improved oxygen utilization, lower heart rates, and firmer muscle tone [54]. A recent pilot study in horses demonstrated bee pollen supplementation on physical fitness parameters, immunological status, and nutritional variables in Arabian horses in training. It was observed that supplementation with a commercial 55% bee pollen for 42 days did not change physical fitness or immunological variables in the horses but it significantly increased feed consumption and nutrient retention in the same horses [54].

Echinacea: A popular herb (purple coneflower, a common ornamental garden plant) stimulates the immune system and helps fend off opportunistic infections such as the common cold. The equine industry typically uses Echinacea as an immune booster to compliment a healthy immune system [55]. Studies by Briggs [56] showed that Echinacea is an immunostimulant and a hematinic agent in horses. Echinacea is known for its antiviral, antibacterial, antioxidant, and anti-inflammatory properties [57]. This is an exceptional herb given to

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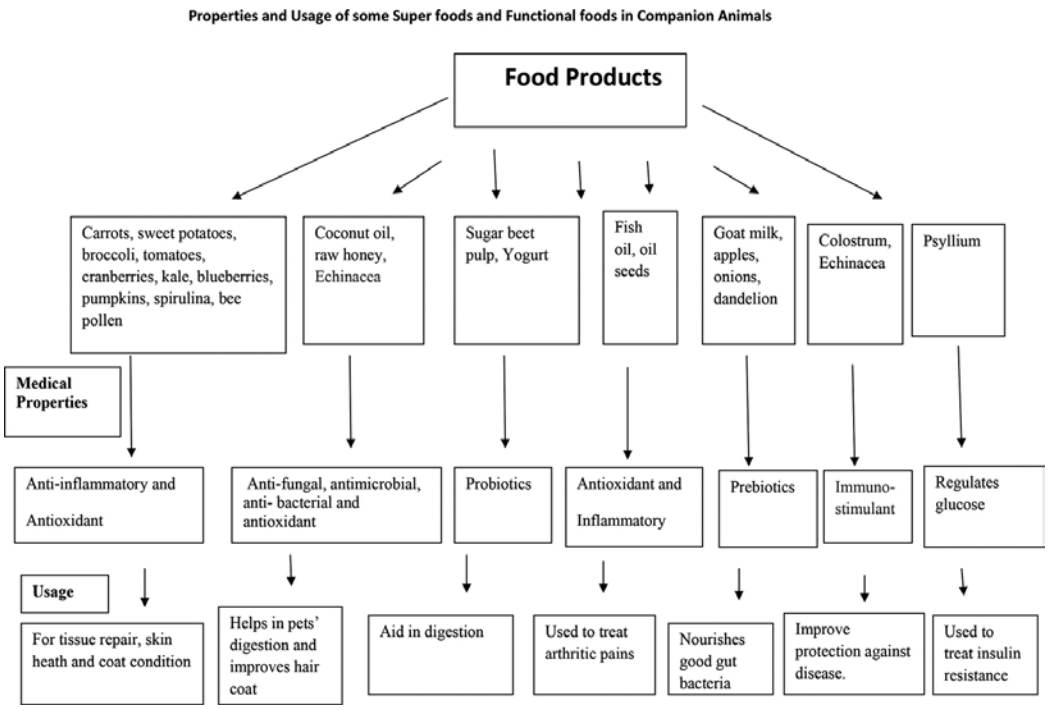
horses usually during seasonal changes when there is likelihood for illness [45]. A study was carried out on eight horses using an Echinacea preparation called Echi-Fend, manufactured by Quinte Botanicals, a division of Bioniche Life Sciences. They were fed 30 ml of Echi-Fend twice a day for 6 weeks and an inactive placebo for another 6 weeks. Blood samples were taken every week and complete hematology and biochemistry screens along with phagocytic function test were carried out. The study showed some effects: while on Echi-Fend, the horses showed a significant increase in the size and the number of circulating red blood cells, as well as an increase in the level of hemoglobin and lymphocytes in the blood. In addition, there was a significant decrease in the levels of circulating neutrophils in the blood. This study clearly demonstrated that Echinacea acts both as an immuno-stimulant and a hematinic agent in horses [56].

Colostrum: It is a complex fluid, rich in nutrients and immune-regulating compounds [58]. Immunoglobulins found in colostrum are very large proteins, far too large to be absorbed directly into the bloodstream at an advanced age. In adult animals, most of these proteins are degraded by digestive enzymes, but some portion of these large molecules is transported across the intestinal lining intact, where it binds to an antigen [59]. Hence, supplementing colostrum to adults horses impacts on their health. Colostrum also protects overall well-being by providing a wide range of factors including lactoferrin, oligosaccharides, peptides, leukocytes, and growth factors [60]. Colostrum from cows is richer in immunoglobulins than from other animal species, thereby offering improved protection against viral and bacterial infections. It is also very low in lactose, making it appropriate for adult horses (who are naturally lactose intolerant) [61]. Other researchers have identified improvements in disorders such as obesity, insulin resistance, leaky gut syndrome, and ulcers [62, 63]. Colostrum supplementation has helped to improve performance in horses by its apparent ability to stimulate neutrophil's oxidative response following prolonged exercise [64].

Fructo-oligosaccharides (FOS): A type of prebiotic, specialized sugars that are super food for bugs to encourage the development of healthy intestinal flora [65]. They give resident microflora (good bacteria) a competitive advantage over pathogenic bacteria and strengthen the stability of the essential flora. The colon and the good bacteria have an essential role in the extraction and assimilation of nutrients to face the daily and sporting requirements but most notably to guarantee the health of the equine athlete [66].

3. Conclusion

Feeding super foods and functional foods to companion animals provides multiple health benefits. Such foods help maintain good immune response, body hair coat, digestive health, locomotion, and general well-being. More investigations need to be carried out to identify other super foods and functional foods for companion animals and determine precisely what benefits they may boast.



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Chapter 16

Functional Pet Foods

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Gianandrea Guidetti and Sergio Canello

Additional information is available at the end of the chapter

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Abstract

Functional foods provide health benefits if they are consumed on regular basis. Some nutraceutical pet diets have been demonstrated to exert health benefits *in vitro* and *in vivo* while also exhibiting palatability to the animals. The aim of this chapter is to provide an overall update of commercially available pet diets with proven efficacy against pathologies with an inflammatory background. Research on pet food is still scarce and biased. The ultimate success of functional pet foods will depend on delivering bioactive components in a predictable and assured manner to effectively reduce the risk of disease and/or support the body. Our investigations outlined the improved health status of sick *dogs* by means of a commercially available nutraceutical pet diet approach. Therefore, additional investigations into the consumption of functional foods in domestic animal nutrition should be done in order to study dietary interventions for disease prevention and treatment.

Keywords: functional foods, nutraceutical pet diets, proven efficacy

1. Introduction

The interest in the efficacy and safety of pet food has been growing worldwide with vegetables, whole grains, fortified active principles, fruits, probiotics, prebiotics, and herbal extracts as the most effective substances available. In addition, the use of antibiotics in the agriculture and intensive farming has also become a relevant concern with consequent potential health risks derived by their entry/accumulation in human food and animal food supply chains.

2. Antibiotic residues in pet food and adverse food reactions

In the last 35 years, a surprising increase of skin and gastrointestinal (GI) diseases in both *cats* and *dogs* has been observed [1]. For instance, *in vivo* studies have widely demonstrated that the most commonly responsible ingredients for the onset of cutaneous and gastrointestinal adverse food reactions are beef, dairy products, wheat, and to some degree, lamb, soy, and fish [2–5]. However, only in a few cases were such adverse food reactions clearly ascribed to the presence of food additives such as dyes, preservatives, or even antibiotics [6–8].

One of the main symptoms of skin-related diseases is severe itching, which may lead to self-inflicted injuries caused by obsessive scratching, while frequent gastrointestinal symptoms are vomiting and diarrhea, which continue to persist after therapy. These phenomena suggest paying particular attention to the history of several examined cases in order to determine their primary and real cause.

We recently identified a specific compound, oxytetracycline (OTC), as the possible underlying cause of most inflammatory pathologies both *in vitro* [9, 10] and *in vivo* [11, 12]. OTC belongs to the class of tetracyclines, which are the most widely and legally used antibiotics in intensive farming, for example, poultry [10], livestock [13], and aquaculture [14], due to their low cost and efficacy [15]. Unfortunately, OTC also has a high affinity for calcium-rich tissues such as bone and teeth [16] and can remain fixed for extended periods in treated animals even respecting withdrawal time [10]. Moreover, pet food production relies on meat (mainly poultry) by-products, which are mechanically separated [17, 18]. This kind of separation, and the common use of important percentages of bone meal mixed with meat meal, generates a bone-based meal-bearing OTC residues that is present in commercially available diets (canned, semi-moist, and especially dry) in a percentage of 20–30% and can accumulate within pet's body.

Odore et al. and Di Cerbo et al. recently demonstrated the significant *in vitro* toxicity of milled bone from chickens treated with OTC, either alone or diluted 1:4, toward peripheral blood mononuclear cell (PBMC) culture ($^{**}p < 0.01$ and $^{***}p < 0.001$, respectively) [9, 10]. Conversely, bone derived by chickens untreated with OTC did not show any cytotoxic effect (**Figure 1**).

Furthermore, Di Cerbo et al. have recently shown the *in vitro* ability of the OTC to induce a significant interferon (IFN)-γ release from human T lymphocytes and non-T cells [9].

Besides the ability to induce mortality of both the T lymphocytes and non-T cells after a 48-h co-incubation, OTC was also able to induce the release of pro-inflammatory cytokines in the first 10–12 h of challenging. More in detail, T lymphocytes increased their IFN-γ production once exposed to OTC or to the culture media conditioned with the bone derived by OTC-treated chickens in order to resemble the same conditions of intensive farming [15].

Both the innate immunity (non-T cells, mainly represented by natural killer (NK) lymphocytes) and the acquired immunity (T lymphocytes, $CD8^+$, and $CD4^+$) [19, 20] resulted to be influenced by the OTC toxicity (**Figure 2**).

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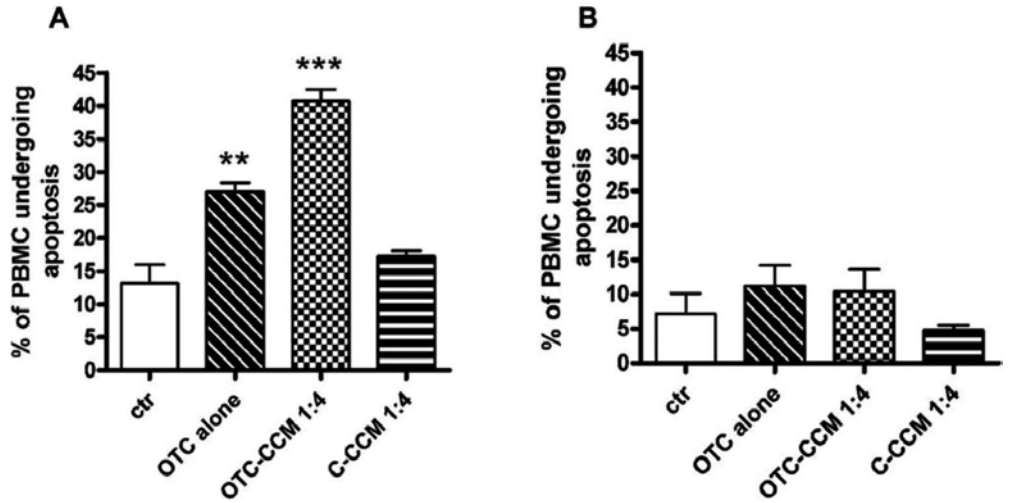

Figure 1. Percentage of PBMC undergoing apoptosis. On the *x*-axis, different cell incubations and conditioned cell culture medium dilutions, after 12h (B) and 24h (A) of incubation, are shown. OTC-CCM indicates the conditioned cell culture medium challenged with a ground bone of chickens treated with OTC; the C-CCM indicates the growth medium challenged with a ground bone of chickens treated without OTC, while OTC alone indicates a growth medium with the addition of 1 μg/ml of OTC. The "ctr" indicates the incubation in the growth medium with Annexin V staining, which has been used as a control of the apoptosis that occurs in the cells when in a culture without any other incubation is maintained; $^{**}p < 0.01$, and $^{***}p < 0.001$ (with the permission of John Wiley and Sons) [18].

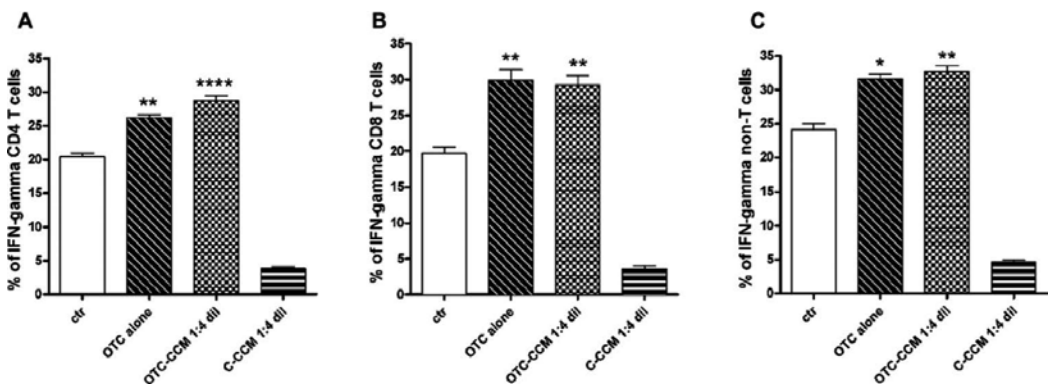

Figure 2. Percentage of IFN-γ production in CD4+ and CD8+ T lymphocytes and in non-T cells. The bar column graphs represent the mean values of the percentage of IFN-γ-producing cells. On the *x*-axis, different cell incubations and conditioned cell culture medium dilutions are shown. OTC-CCM indicates the conditioned cell culture medium challenged with a ground bone of chickens treated with OTC; the C-CCM indicates the growth medium challenged with a ground bone of chickens treated without OTC, while OTC alone indicates a growth medium with the addition of 1 μg/ml of OTC. The condition indicates as "ctr" refers to basal IFN-γ production. All the cell cultures (ctr, OTC alone, OTC-CCM, and C-CCM) were maintained in a growth medium added with PMA and ionomycin to induce cytokine production. Panels A, B, and C show IFN-γ production in CD4+ T lymphocytes, CD8+ T lymphocytes, and in non-T cells, respectively; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.001$ (with the permission of John Wiley and Sons) [18].

In this context, it is known that IFN-γ represents the main cytokine involved in the immune response [21], as well as a crucial element in the onset of impaired tissue homeostasis conditions, typically related to autoimmunity or chronic inflammation [22–30].

These observations clearly reinforce the potential toxicity associated with chronic consumption of poultry bones and derivatives by pets and pave the way for a new concept of food sensitization due to contaminant presence as main enhancers of inflammatory processes, which typically characterize skin and gastrointestinal diseases. Although the Food and Drug Administration [31] and World Health Organization [32] have recently established maximum residue limits in foods, antibiotic residues in foods may still be present [33] thus explaining the persistence of dermatological manifestations in many pets. Moreover, international laws do not impose an antibiotic concentration evaluation in bones and fat, which are considered inedible, thus making pet food dangerous for pet's health [34].

A wide number of scientific reports suggest the possible toxicity and harmfulness of OTC toward human and pet health as a consequence of the consumption of meat derived from intensive farming [15, 35–40].

All of these data may explain why chicken proteins, widely considered hypoallergenic and highly effective from a dietary point of view, play an important role in the etiology of several inflammatory pathologies. It is worth noting that the similarities between these phenomena and food allergies, atopy, and Flea allergy dermatitis have been observed. In spite of the limited evidence that canine food allergy is suggested to resemble a type I hypersensitivity reaction to allergens ingested by food, it cannot be excluded that non-IgE-mediated food allergies may also occur. Although literature reports have been evidenced that the prevalence of food allergies in *dogs* and *cats* is still unknown, the impressive number of cases is not justified merely on the basis of increased allergy spreading in civilized societies. Furthermore, it has been observed that 25% of the *cats* with both chronic GI and skin problems do not clinically express food allergies, while the remaining 75% had only gastrointestinal problems. On the other hand, there are no data regarding food allergies related to gastrointestinal problems in *dogs*. In addition, food allergies can be often confused with pyoderma, pruritic exudative dermatitis or "hot spots."

Based on our recent studies, we investigated the sera of 24 *dogs* with food-adverse reactions, that is, itching, diarrhea, otitis, dermatitis, conjunctivitis, overnight fasting, vomiting, flatulence, interdigital pyoderma, and anal sacs repletion for the presence of any haptens which might be responsible for such conditions by means of an enzyme-linked immunosorbent assay (ELISA) (FS0059, IDLABS™ Inc. Biotechnology, PO Box 1145, Station CSC, London ON N6A 5K2, Canada) according to the manufacturer's instructions [12].

Results indicated the presence of OTC and doxycycline in all animal sera. Although only eight out of 24 *dogs* (33%) showed antibiotic concentrations above the ELISA detection limit (7.5 ng/ml or ppb), all the remaining *dogs* presented serum levels of both antibiotics. OTC serum levels ranged from 2.61 to 56.04 ng/ml (6.30 ± 2.12; mean ± standard error of the mean), whereas doxycycline serum levels ranged from 1.28 to 22.84 ng/ml (5.20 ± 0.89; mean ± standard error of the mean). Our preliminary clinical investigation further confirmed the haptenic toxic-

sensitizing mechanism due to prolonged subliminal oral intake of OTC-enriched bone-meal-based feeds derived from animals grown under a chronic tetracycline administration regime.

3. Herbal extracts: possible pets' health allies

What differentiates common pet food from a functional pet food is the presence of a protein source free of any contaminants, for example, antibiotics and hormones (as happens in intensive farming) as well as the addition of antioxidants, minerals, trace elements, herbal extracts, and medical plants in order to, respectively, stabilize, preserve, and improve the whole nutritional profile of the food.

Many scientific studies clearly demonstrate the efficacy of functional herbal extracts or medical plants for disease prevention or treatment, to improve overall health status or even to delay aging [41].

Based on these observations, we recently studied the anti-inflammatory and antitoxic activity of a well-standardized mixed pool of herbal extracts, as part of a commercially available pet food diet, following an OTC challenge [42]. More in detail, the extracts within the pool were *Ascophyllum nodosum* (66.3%), *Cucumis melo* (1.5%), *Carica papaya* (3.1%), *Aloe vera* (3.1%), *Haematococcus pluvialis* (1.1%), *Curcuma longa* (2.3%), *Camellia sinensis* (1.5%), *Punica granatum* (1.5%), *Piper nigrum* (0.6%), *Polygonum cuspidatum* (1.5%), *Echinacea purpurea* (3.1%), *Grifola frondosa* (6.3%), and *Glycine max* (4.6%).

As previously explained, OTC is able to induce a significant IFN-γ release from human T lymphocyte and non-T cell [9] *in vitro*. Here, we demonstrated that this significant release was significantly reduced after a 24-h individual co-incubation of human T lymphocyte and non-T cells with all aforementioned extracts with the only exception of *A. vera* (**Figure 3**).

Further, we reported the same antitoxic and anti-inflammatory activity of these extracts, with the only exception of *C. papaya* and with *P. nigrum*, on canine T lymphocytes challenged with OTC (**Figure 4**).

The antitoxic and anti-inflammatory activity exerted by the extracts represents a further proof of the usefulness of the addition of selected and standardized herbal extracts within a pet food possibly free of any contaminant. In this way, it is possible to achieve a functional pet food able to support and enhance standard pharmacological treatments in the presence of infections or inflammatory diseases.

However, it is worth noting that enzyme deficiencies or different metabolic pathways make some plants, for example, onions, leeks, garlic, and chives, toxic for *dogs* and *cats* but not for humans [43]. For instance, one of the toxic effects of these plants is the oxidative hemolysis, which results from the inability of the antioxidant metabolic pathways to counteract an excess of oxidants in the erythrocytes. The toxicological mechanism is the following: (1) oxidation of the exposed β-93 cysteine residues present in the hemoglobin and consequent sulfhemoglobin formation, (2a) precipitation, aggregation, and binding of sulfhemoglobin to the cell membrane and formation of the Heinz bodies, and (2b) membrane cross-linking reactions occurring

and eccentrocyte formation, (3) erythrocyte fragility increase and consequent extravascular hemolysis, (4) decreased blood oxygen transportation capacity, and (5) impaired delivery of oxygen to the tissues [44–46].

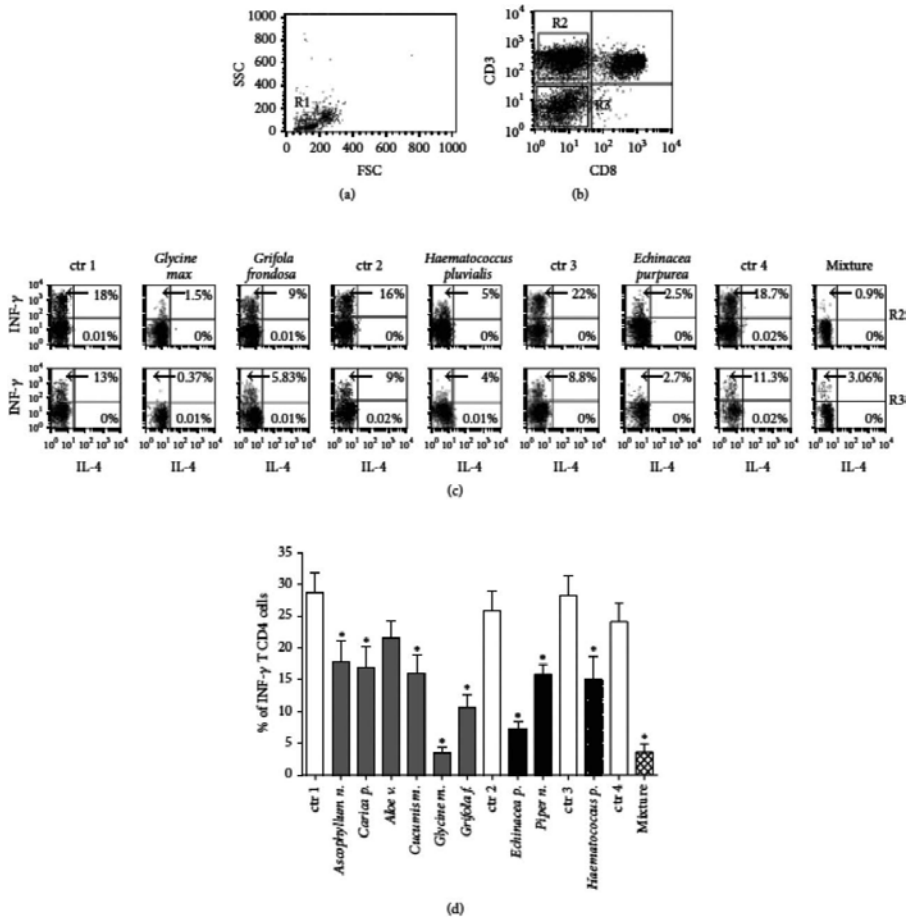

Figure 3. The effects of botanicals on cytokine production by human PBMCs. (a) shows the gating on viable lymphocytes (R1 in dot plot graph) based on FSC and SSC parameters (see Section 2); (b) represents the gating on TH lymphocytes ($CD3^+$ $CD8^-$ as R2 in the dot plot graph) and on non-T cells ($CD3^-$ $CD8^-$ cells as R3 in the dot plot graph); and (c) shows the IFN-γ and IL-4 production in human TH lymphocytes and non-T cells incubated with ad hoc medium derived from botanicals or from mixture (see Section 2). Cytokine production was evaluated as percentage of IFN-γ (*y*-axis) and IL-4 (*x*-axis)-producing cells. The percentage of IFN-γ (upper left quadrant inside the dot plots) and IL-4 (lower right quadrant inside the dot plots)-producing CD4 T (R2) and non-T (R3) cells is reported. The different cell incubations with ad hoc medium derived from botanicals or from mixture (see Section 2) are indicated on top of each graph. (d) reports the statistic representation of 10 experiments on human $CD4^+$ T lymphocytes evaluated as percentage of IFN-γ-producing cells, $^*p < 0.05$. The different cell incubations with ad hoc medium derived from botanicals or from mixture (see Section 2) are indicated on top of each column. The abbreviation "ctr" in (c) and (d) indicates the basal cytokine production by PMBCs stimulated by PMA and ionomycin and in the presence of the ad hoc medium based on the same solubilizing vehicle but free from the botanicals (see Section 2); specifically, ctr 1 (*Ascophyllum n.*, *Carica p.*, *Aloe v.*, *Cucumis m.*, *Glycine m.*, and *Grifola f.*), ctr 2 (*Echinacea p.*, *Piper n.*), ctr 3 (*Haematococcus p.*), and ctr 4 (the mixture of all the botanicals) (with the permission of Hindawi) [42].

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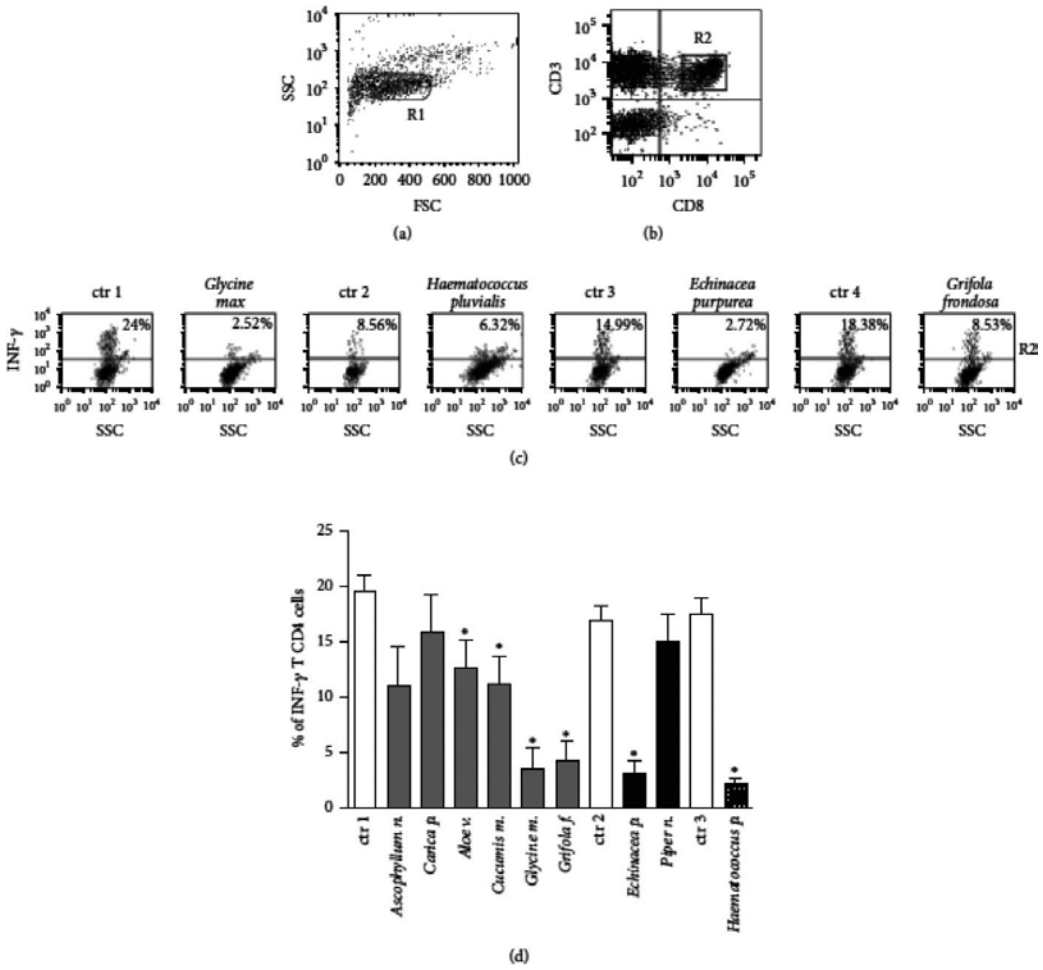

Figure 4. The effects of botanicals on IFN-γ production by canine PBMCs. (a) shows the gating on viable lymphocytes (R1 in dot plot graph) based on FSC and SSC parameters (see Section 2). (b) represents the gating on $CD4^+$ T lymphocytes ($CD3^+$ $CD8^-$ as R2 in the dot plot graph). (c) reports the results from one representative experiment showing the percentage (the number in upper quadrant) of IFN-γ-producing canine CD4+ T lymphocytes gated on R2 (*y*-axis); *x*-axis indicates the SSC parameter (see Section 2). The different co-incubations of cells with ad hoc medium or mixture (see Section 2) are indicated on the top. (d) shows the statistic representation of the IFN-γ production by canine $CD4^+$ T lymphocytes evaluated as percentage of IFN-γ-producing cells in 10 representative experiments, $^*p < 0.05$. The abbreviation "ctr" in (c) and (d) indicates the basal IFN-γ production by PMBCs stimulated by PMA and ionomycin and in the presence of the ad hoc medium based on the same solubilizing vehicle but free from the botanicals (see Section 2): specifically, ctr 1 (*Ascophyllum n., Carica p., Aloe v., Cucumis v., Glycine m.*, and *Grifola f.*), ctr 2 (*Echinacea p., Piper n.*), and ctr 3 (*Haematococcus p.*) (with the permission of Hindawi) [42].

This phenomenon can be obviously exacerbated in the presence of heritable high erythrocyte-reduced glutathione and potassium concentrations or glucose-6-phosphate dehydrogenase deficiency or zinc deficiency [47, 48].

Thus, dietary supplements, home-made or commercially available pet food containing some plants or herbal extracts, might transform a functional food into a poisoning food.

4. Pet diets and animal well-being

Some scientific evidence has pointed out the efficacy of selected ingredients, as part of a commercially available diet, in relieving inflammatory conditions in pets by means of an immune modulatory and antioxidant activity [49–57].

Pasquini et al. [53] demonstrated that *dogs* fed a specific diet (F10 Maxi Maintenance®) based on maize, fish meal (20%), maize oil, fish oil, brewer's yeast, beet pulp, minerals, MOS, FOS, *Elaeis guineensis*, *Yucca schidigera*, *C. papaya*, *Ananas* spp., *P. granatum*, *Panax ginseng*, and *Rosmarinus officinalis* was able to influence gender, age, and breed-derived lipid metabolism alterations in healthy *dogs* by significantly decreasing C-tot, C-high-density lipoprotein (HDL), and C-low-density lipoprotein (LDL) ($^{*}p < 0.05$) [57].

Further studies have then clearly demonstrated the synergic efficacy of selected ingredients in modulating several inflammatory conditions, which commonly affect pets, especially *dogs* [49–52, 56, 58].

An inflammatory condition can also occur during food allergy reactions, which usually takes place after the intake of a harmless dietary component [59]. Generally, food-allergic reactions in pets include cutaneous (flush, itching, dandruff, skin malodor, dry fur, and skin lesions) and gastrointestinal manifestations (dehydration, appetite loss, regurgitation, emesis, abdominal pain, flatulence, borborygma, diarrhea, weight loss, stool consistency, blood, and mucus presence in the stool) [56].

Based on these observations, we conducted two different clinical evaluations aimed to validate two different commercially available formulas for aforementioned dermatological and gastrointestinal issues. For instance, a mixture of fish, potato, *A. vera, Arctium lappa, Malva sylvestris,* and *Ribes nigrum* (FORZA10 Dermo Active™) resulted particularly effective in halving the intensity of cutaneous symptoms (flush, itch, dandruff, skin malodor, dry fur, and skin lesions) in 71 *dogs* affected by atopic dermatitis ($^{***}p < 0.001$) [56]. On the other hand, a specific diet consisting of a mixture of milk enzymes, *Origanum vulgare*, chestnut, *Plantago psyllium*, MOS, FOS, electrolytes, and *Rosa canina*, significantly reduced the intensity of symptoms (dehydration, appetite loss, regurgitation, emesis, abdominal pain, flatulence, borborygma, diarrhea, weight loss, stool consistency, blood, and mucus presence in the stool) in 60 *dogs* with evident gastrointestinal issues ($^{***}p < 0.001$) [56]. Obviously, an inflammatory condition may also occur in other segments of the gastrointestinal apparatus, such as the mouth. In this case, the main clinical manifestation is halitosis, which is generally the sum of metabolic anomalies, poor oral hygiene, hyposalivation, dental appliances, as well as gingival, mucosal, and periodontal disease [60]. In this regard, pets can be affected by halitosis and a correct dietary approach becomes fundamental to manage and treat such complaints [50, 61]. We compared the efficacy of a specific nutraceutical diet and a standard diet in significantly reducing the concentration of three specific volatile sulfur compounds, hydrogen sulfide, methyl mercaptans, and dimethyl sulfide, in 16 *dogs* suffering from chronic halitosis [50]. More in detail, the diet consisted of a mixture of fish meal, rice carbohydrates, propolis, *Salvia officinalis*, lysozyme, bioflavonoids, *Thymus vulgaris*, *R. nigrum,* and an Omega 3/6 ratio of 1:4.

Then, by means of a portable gas chromatograph (OralChroma™), a syringe to collect the breath and specific software, a significant reduction in halitosis, were observed after 30 days from the beginning of the nutraceutical diet supplementation ($^{*}p < 0.05$). Further, a long-lasting effect was still observed even 20 days after the diet interruption.

As previously stated, *cats* and *dogs* can be affected by adverse food reactions, which can involve apparatuses including the gastroenteric and cutaneous but can be extended also to otological, ocular, urinary, and respiratory systems [49, 62]. We recently ascertained the effectiveness of a combined use of a nutraceutical diet and current pharmacological therapy in 15 adult *dogs* affected by chronic bilateral otitis externa [49]. The diet, which was composed of fish proteins, rice carbohydrates, *Melaleuca alternifolia, Tilia platyphyllos scapoli et cordata, Allium sativum L., Rosa canina L.* and Zinc and an Omega 3/6 ratio of 1:4, and the drug (Otomax®) significantly reduced the mean intensity of all clinical symptoms (occlusion of ear canal, erythema, discharge quantity, and odor) within 90 days ($^{***}p < 0.001$). This study can be considered a further example of the importance of the selection of substances endowed with anti-inflammatory and antioxidant activity in a pet food diet.

In some cases, substances endowed with anti-inflammatory as well as immune-modulatory activity can drastically influence the clinical outcome of lethal pathologies, that is, *Leishmania* [63].

A study conducted by Cortese et al. investigated the effect of an immune-modulating diet, based on fish- and vegetable-hydrolyzed proteins, minerals, *A. nodosum, C. melo, C. papaya, A. vera, Astaxanthin, C. longa, C. sinensis, P. granatum, P. nigrum, Poligonum* spp., *E. purpurea, G. frondosa, G. max* and an Omega 3/6 ratio of 1:1 along with an anti-*Leishmania* pharmacological therapy (meglumine antimoniate, and allopurinol) in 20 naturally infected *dogs* over a period of 12 months [63]. The diet results were particularly effective in restoring regulatory T cells and decreasing T helper cell percentage ($^{***}p < 0.001$).

In other cases, the selection of substances with a remarkable antioxidant activity also acquires a pivotal role in other clinical conditions, which are not strictly related to adverse food reactions, that is, cognitive impairment, as a consequence of aging or pathologies such as Alzheimer's and Parkinson's disease [52, 64, 65].

In this regard, we studied the effect of a nutraceutical diet based on fish proteins, rice carbohydrates, *G. frondosa, C. longa, C. papaya, P. granatum, A. vera, P. cuspidatum, Solanum lycopersicum, Vitis vinifera, R. officinalis*, and an Omega 3/6 ratio of 1:0.8 on cognitive decline of nine elderly *dogs* over a period of 6 months [52]. Specifically, derivatives of reactive oxygen metabolites, biological antioxidant potential levels, and brain-derived neurotrophic factor were evaluated in *dogs*' plasma samples at the beginning and at the end of the dietary regime. Results showed a significant decrease of dROMs ($^{*}p < 0.05$) and a significant increase in brain-derived neurotropic factor (BDNF) ($^{*}p < 0.05$) serum levels.

A recent study has also raised the possible key role of some selected ingredients (fish proteins, rice carbohydrates, *P. granatum, Valeriana officinalis, R. officinalis, Tilia* spp., tea extract, and L-tryptophan, with an Omega-3:-6 ratio of 1:0.8) in modulating behavioral disturbances in 12 *dogs* with chronic anxiety and stress caused by intense and restless activity over a period of

only 10 days [58]. By means of a sophisticated and extremely sensitive sensor, a mobile phone app, and a wireless router, it was possible to induce and monitor significant improvements in the time spent in activity and at rest ($^{**}p < 0.01$ and $^{*}p < 0.05$, respectively). Last but not least, all *dogs* also showed an overall significant improvement in clinical (dandruff, itchiness, flush, seborrhea, fur opacity, vomiting, diarrhea, flatulence, lachrymation, and anal sac repletion) and behavioral (marking, anxiety, diffidence, irregular biorhythm, reactivity, activation, irritability, alertness, environmental exploration, and attention requirement) symptoms.

5. The "market stand" of functional pet foods

The interest into the adequacy and safety of commercially available pet foods has been growing worldwide [66]. Functional foods such as prebiotics, for example, inulin, gluco-oligosaccharides, and galacto-oligosaccharides have shown to induce beneficial effects on biochemical parameters improving satiety and reducing postprandial glucose and insulin concentrations, thus reducing diabetes-related disorders [67–69]. Inulin and oligofructose, but also dietary fibers, can also modify the intestinal microflora in pets and humans by promoting commensal bacteria growth [70–72]. However, many *in vitro* studies highlighted other hidden properties of dietary fibers such as gastric emptying, gastric transit time and decrease in blood cholesterol concentrations, increase in satiety, glucose uptake rate, and fecal excretion as well as dilution in diet calorie density [73–76]. Another valuable fiber source is represented by corn fiber due to the lack of detrimental effects on palatability and nutrient digestibility, and the glycemic response lowering in adult *dogs* [71, 77]. Based on these novel and unexpected activities of dietary fibers, many commercially available pet diets moved to an accurate use of these along with novel sources of carbohydrates including cereal grains, which represents almost 90% of animal diet content, and whole grains [78]. These latter, whose main source are wheat, corn, oats, barley, and rye [79], are rich in dietary fibers, trace minerals, and vitamins B and E [80]. Furthermore, whole grains have bioactive compounds, for example, tocotrienols, lignans and polyphenols, lipotropes and methyl donors, such as choline, methionine, betaine, inositol and folate and antinutrients, that is, compounds that interfere with the absorption of nutrients such as phytic acid, tannins, and saponins endowed with antioxidant and anti-carcinogenic effect [79–82]. As to rice bran, the vitamin-rich outer layer that surrounds the endosperm of whole grain brown rice has bioactive molecules such as tocopherols, tocotrienols, polyphenols including ferulic acid and α-lipoic acid, phytoesterols, γ-oryzanol and carotenoids such as carotene, lycopene, lutein, and zeaxanthin, which was endowed with antioxidant, anti-inflammatory, and chemopreventive activity [83]. Rice bran is also an excellent source of essential amino acids (especially sulfur-containing amino acids) and micronutrients such as magnesium, manganese, and B-vitamins (especially B9 and B12) [83, 84]. It is worth noting that during pet food heat processing, known as the Maillard reaction, that is, a nonenzymatic browning and flavoring reaction, a reduction of essential amino acids, such as lysine, bioavailability occurs [85, 86]. Therefore, many pet diets might be at the risk of supplying less lysine than the animal may require. Hence, the understanding of nutritional benefits of functional foods currently available is of key importance for the owners to provide their pets with the

correct diet. Nevertheless, great attention has to be paid to pet food palatability along with adequacy and safety. For instance, Spears and coworkers examined the palatability and its effect on digestion of stabilized rice bran in a dry canine diet determining fecal characteristics, food intake, selected immune mediators, and blood lipid characteristics [87]. They observed that dry pet food containing 12% stabilized rice bran was well tolerated by *dogs* with no detrimental effect on nutrient digestibility, fecal characteristics, and changes in inflammatory/immune mediators. Moreover, the rice bran diet presented greater palatability compared to the defatted rice bran diet. Vitamin A (retinol), whose safe upper limit in complete diets for *dogs* ranges from 5.24 to 104.80 mmol, is an essential fat-soluble vitamin at the center of investigations in *dogs* in the context of immune stimulation, vision-supporting functions, reproduction, bone growth, and cellular differentiation [88–90]. In *dogs*, unlike humans where retinyl esters are only detected in plasma in cases of intoxication or following a vitamin A-rich meal [91], vitamin A is present in the plasma predominantly in the form of retinyl esters, in both adequate and vitamin A-deprived states [92]. Moreover, in *dogs* [93], which excrete vitamin A in the urine [91] along with retinyl esters [94], retinol concentrations are unaffected by dietary vitamin A intake (1.2 ± 0.03 vs. 1.0 ± 0.03 mg/l, respectively), whereas serum retinyl esters parallel the concentrations of vitamin A in the diet [91].

5.1. The role of microbiota

Pet's well-being and health also depend on gut microbiota, whose composition and activity is correlated to several diseases [95–97]. *Cats* and *dogs* harbor several bacterial species (with *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria,* and *Eubacterium* as the predominant phyla [98–102]), which differ from each other but also from the same species [103]. As shown in human studies, gut microbiota also plays a key role in immune and food intake regulation [104, 105]. However, only a few studies have examined the impact of the diet on canine gut microbial population [106–108]. For example, Kerr and coworkers evidenced the lack of negative alterations of the microbiome in healthy *dogs* of different species (*Golden Retriever, Hound Mix, Pitbull Mix, Mixed, St. Bernard, Australian Cattle, Dalmatian, Pointer, Standard Poodle, Terrier*) fed on a cooked navy bean powder [109]. Cooked beans decreased *Actinobacteria* and *Fusobacteria* and increased *Firmicutes*. Therefore, it is reasonable to think that future dietary products for *dogs* will modulate the gut microbial population in order to treat or prevent some food-related diseases (dysbiosis, leaky gut, intestinal bowel disease, irritable bowel syndrome, coeliac disease, sepsis, renal failure, autoimmune disease, peritonitis, and intestinal obstruction). Recently, Park and coworkers monitored healthy *dogs* for 6 months [110]. The first group was fed *ad libitum* on commercial food, while the second was fed on a restricted amount of the same commercial food. Animals fed *ad libitum* resulted in obesity with high levels of triglycerides and cholesterol. The microbiota presented differences between the two groups of animals, while *Actinobacteria* and *Bacteroidetes* were the predominant microflora in animals receiving a restricted amount of food, animals fed ad libitum presented *Firmicutes*, *Fusobacteria,* and *Actinobacteria*. Thus, a targeted diet may promote changes in gut microbiota affecting the activity of specific beneficial microbes resulting in benefits for the overall dog's health. In this sense, a targeted diet might also be based on the use of prebiotics, for example, chicory, fructooligosaccharides, pectin, and polydextrose. Zentek and coworkers demonstrated that

nine adult healthy *beagles* fed a diet supplemented with 3% chicory had more consistent stools with increased levels of *bifidobacteria* and decreased *Clostridium perfringens* and a lower fecal pH with respect to a protein-rich diet [111]. Further, *cats* fed diet supplemented with FOS (4% of diet) showed increased concentrations of *bifidobacteria* and reduced count of *Escherichia coli*, while pectins (4% of diet) increased *C. perfringens* and *lactobacilli* concentrations [112]. Conversely, *dogs* fed a diet with low level of dietary fiber (beet pulp) for 2 weeks decreased *Fusobacteria* and increased *Firmicutes* [107]. Interestingly, feline diets, particularly rich in animal proteins and low-carbohydrate plant-based additives, promoted fecal *Clostridium*, *Faecalibacterium*, *Ruminococcus*, *Blautia*, and *Eubacterium* growth affecting circulating hormones and metabolites of key importance in satiety and host metabolism [113, 114]. As to polydextrose, its consumption by *dogs* resulted in an increase of fecal acetate, propionate, and total SCFA concentrations, while fecal pH, indole, and *C. perfringens* population decreased [106]. Thus, incorporating prebiotics in pets' diet may beneficially modulate gut microbiota and intestinal health and possibly protect the animals from enteric infections.

6. Conclusions

Recent advances in pet food production have raised the potential of some functional ingredients to be useful in preventing and treating disease in pets. Although further work is required to better characterize the long-term effect of these substances on biological mechanisms, we reported some *in vivo* and *in vitro* examples of the synergic efficacy of selected ingredients, present in some commercially available nutraceutical diets, as a valid and reliable natural approach for the management of some pet diseases. Functional food development can be considered a promising new research area, which surely will be able to improve the health and quality of life of *dogs* and *cats* in the near future. However, great attention should be paid on pet food production and ingredient selection since, without a correct R&D process, diets might shift from the primary source of feeding to primary source of poisoning.

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This book focuses on the usage and application of plant- and animal-based food products with significant functional properties and health benefits as well as their development into processed food. Many chapters in this book contain overviews on superfood and functional food from South America. Details on the functional properties of apiculture products are also included herein. Additionally, an area that is not widely discussed in academia - pet food with functional properties - is also covered. It is hoped that this book will serve as a source of knowledge and information to make better choices in food consumption and alterations to dietary patterns.

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