Food Science
Health Aspects

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Chapter 1

Health Benefits of Dietary Protein throughout the Life Cycle

Jamie I. Baum, Elisabet Børsheim, Brittany R. Allman and Samuel Walker

Abstract

Dietary protein intake and the associated health benefits continue to be a subject of great debate. The quantity of protein consumed, the quality or source of protein consumed, and the timing of protein intake throughout the day all play a role in determining the health benefits of dietary protein. Research suggests that intake of dietary protein above the dietary recommendations has health benefits throughout the lifecycle. This book chapter describes the dietary recommendations for protein intake throughout pregnancy, childhood, and adulthood and the associated health benefits with protein intake above the dietary guidelines at each stage of life.

Keywords: dietary protein, dietary guidelines, children, adults, health benefits

1. Introduction

Proteins are chains of amino acids which are involved in nearly every process in the body. Proteins function as enzymes, transcription factors, binding proteins, transmembrane transporters and channels, hormones, receptors, structural proteins, and signaling proteins [1]. However, the primary role of protein in the diet is to provide amino acids required for the synthesis of new proteins. We especially rely on dietary protein to provide the nine essential amino acids, which cannot be synthesized in the body. Protein intake greater than the dietary recommendations may prevent sarcopenia [2], help maintain energy balance [3], improve bone health

chapter focuses on the role of dietary protein, and the associated health benefits, throughout the life cycle.

2. Dietary recommendations for protein intake

The current dietary recommendations for protein intake include the estimated average requirement (EAR) [12] and the recommended dietary allowance [12]. For daily protein intake, the EAR for dietary protein is 0.66 g kg^{-1} day^{-1}, and the RDA is 0.8 g kg^{-1} day^{-1} for all adults over 18 years of age. This can become confusing when trying to make recommendations for individuals at different stages of life. Even the Food and Nutrition Board recognizes a difference between what is recommended in the RDA and the level of protein intake needed for optimal health [12]. Therefore, there is a third recommendation for protein called the acceptable daily

macronutrient range (ADMR) [13, 14]. The ADMR includes a recommendation for protein intakes ranging from 10 to 35% of daily energy (e.g., calorie intake), which makes the ADMR easier to use when developing dietary recommendations for protein [12].

3. Dietary protein intake in adults

A majority of the adult population in the United States exceeds the minimum recommendations for protein intake [15]. The current dietary protein intake in the United States is approximately 82 g d^{-1} for men and 67 g d^{-1} for women [16]. **Table 1** details the current protein intake as percent of energy intake in the United States based on sex and age. A majority of dietary protein comes from animal protein (46%), followed by plant protein (30%), dairy (16%), and mixed foods (8%) [16]. There is increasing evidence indicating that consuming dietary protein at levels above the current RDA (0.8 g dietary protein kg body $weight^{-1}$ day^{-1}) may be beneficial for children, adults, older adults, and physically active individuals [17]. For example, protein intake above the RDA may help reduce the risk of chronic diseases such as obesity, cardiovascular disease, type 2 diabetes, osteoporosis, and sarcopenia [13, 17]. However, high protein intake without a subsequent decrease in carbohydrates attenuates the beneficial effects of dietary protein [18].

Age	Total	Men	Women
Protein			
20–44 years	15.7	16.1	15.3
45–64 years	15.8	16.0	15.7
65–74 years	16.3	16.6	16.1
75 years and older	15.7	16.1	15.3

Table 1.
Percentage macronutrient intake in the United States by sex and age [19].

4. Dietary protein intake in children

Adequate dietary protein intake is essential to support cellular integrity, growth, and physical function. Although protein malnutrition is not prevalent in the United States, there is little research on optimal protein requirements for health benefits in children. Current EARs are based on the factorial method and the nitrogen balance technique. The factorial method incorporates the estimated nitrogen (protein) requirement plus the rate of protein deposition and an estimate of the efficiency of protein utilization [20] which is derived from adult dietary protein needs [12]. By using the indicator amino acid oxidation method in a group of healthy children 6–11 years old, it was found that the mean and population-safe (upper 95% CI) protein requirements were 1.3 and 1.55 g kg^{-1} day^{-1}, respectively. This is higher than the 2005 DRI for protein (0.76 and 0.95 g kg^{-1} day^{-1}, respectively) [12]. A similar study using the nitrogen balance technique also found that protein requirements in children in this age range are above current recommendations at 1.2 g kg^{-1} day^{-1} [21]. These higher estimated protein requirements in children seem to be in line with current protein consumption patterns in different pediatric age groups. For instance, children 2–3 years old are currently daily consuming ~3.6 g/kg of ideal body weight, children 4–8 years old are currently

consuming ~2.6 g kg^{-1} ideal body $weight^{-1}$, and children 9–13 years old are consuming ~1.6 g kg^{-1} ideal body $weight^{-1}$ [15]; however, the optimal protein intake for children is still under debate [22]. There are racial/ethnic differences in protein consumption in children (2–18 years old). For example, non-Hispanic black children eat about 5% below, non-Hispanic white children eat about 3% below, Hispanic children eat about 2% below, and Asian children eat less than 1% below the EAR for protein [15].

Although the currently established recommendations for protein intake in children may be lower than the requirements, the effect of diets higher in protein (e.g., 30% of total energy intake) in children is unclear [22]. Several studies have alluded to the potential benefit of higher protein intake dietary practices. For instance, diets higher in protein with a low glycemic index can be protective against obesity in children aged 5–18 years [23], and diets higher in protein can lead to smaller waist circumference, blood pressure, insulin, and serum cholesterol than lower-protein diets in children from the same age group. A recent cohort analysis found that protein intake in 8-year-olds is associated with higher fat-free mass [24], and an additional cohort analysis found that at ages 11, 15, and 22 years, protein intake is inversely associated with early adulthood BMI. However, protein intake at 2 years was positively associated with BMI and lean mass at age 22 [25], suggesting there are conflicting results regarding the benefits of increased dietary protein in children.

5. Dietary protein intake in pregnant women

Pregnancy is a period of rapid tissue growth during a short period of time. Maternal tissues, including breast, uterine, and adipose tissues, blood volume, and extracellular fluids, account for the largest amount of protein accretion during pregnancy at 60%. The remaining 40% of protein accretion occurs within the amniotic fluid, fetus, and placenta [26, 27]. In fact, protein needs to increase soon after conception to support tissue growth and development, maintenance of maternal homeostasis, and lactation preparation [27–29]. These alterations occur in an exponential way and only in response to adequate total energy intake. This means that protein deposition does not significantly change in the first trimester compared to pre-pregnancy, but increases during the second trimester and significantly increases to the highest levels of protein deposition in the third trimester. This variable period of growth makes it difficult to define recommendations regarding protein requirements. Thus, although current recommendations suggest constant protein intake throughout the duration of pregnancy, pregnancy may actually require an increase in protein intake throughout gestation to support adequate growth, although further research is needed. There are several benefits of protein intake during pregnancy including adequate maternal weight gain within recommendations, lower early pregnancy BMI, and decreased postpartum weight [30].

Although the benefits of increased protein intake during pregnancy are apparent as stated above, protein requirements during pregnancy are difficult to measure. This is due to the involved nature of some of the techniques used to measure protein requirements. Therefore, the current dietary protein recommendations during pregnancy are based on factorial estimates of recommendations for healthy, nonpregnant populations. Pregnancy protein needs have been derived from the EAR and RDA for healthy, nonpregnant populations and are set to 0.88 g kg^{-1} day^{-1} (EAR) and 1.1 g kg^{-1} day^{-1} (RDA) [12]. However, newer studies found protein needs to be 1.2 g kg^{-1} day^{-1} at 11–20 weeks, increasing to 1.52 g kg^{-1} day^{-1} at 30–38 weeks [31]. Both nonpregnant women of childbearing age (20–44 years)

and pregnant women consume at or above the current recommendations of protein intake [32, 33]. One study [31] found that pregnant women consume the same amount of protein in early pregnancy (1.44 ± 0.30 g kg^{-1} day^{-1}) as they do in late pregnancy (1.47 ± 0.53 g kg^{-1} day^{-1}), not taking fluid retention and changes in body composition into account. These findings support others that have noted little overall change in dietary protein patterns from early to late pregnancy [33]. Collectively, these findings demonstrate that pregnant women meet the recommendations for dietary protein intake. Improvements may potentially be made to increase dietary protein requirements as pregnancy progresses.

6. Protein quality versus protein quantity

An important factor to consider when incorporating protein into the diet is how the source of dietary protein (e.g., protein derived from animal or plant sources) affects nutrient intake, nutrient adequacy, and diet quality [13, 34, 35]. Proteins with differing amino acid profiles exhibit varied digestion and absorption rates [36–38], and amino acid profiles depend directly on the quality and quantity of the dietary protein [37]. For example, the digestion and absorption rates of fast- (e.g., whey) versus slow (e.g., casein)-digesting proteins need to be taken into consideration when developing protein recommendations. One study provided young, healthy subjects with either a whey protein meal (30 g) or a casein meal (43 g) (both contained the same amount of leucine [one of the BCAAs]) and measured whole-body protein synthesis. Researchers determined that the subjects consuming the whey (fast) protein meal had a high, rapid increase in plasma amino acids, while subjects consuming the casein (slow) protein meal had a prolonged plateau of EAA [39]. In addition, the chemical structure and the presence of anti-nutritional compounds such as phytic acid within the protein source can influence digestion and amino acid availability [40]. Compared to animal sources, plant proteins are shown to have a lower anabolic impact on muscle; however, the reduced ability to elicit anabolic effects can be overcome by increasing protein intake and increasing the content of leucine [41].

Whether or not the amino acid source is derived from the whole protein or a mixture of free amino acids can also influence the rate of muscle protein synthesis [42]. For example, when older subjects were given either an EAA mixture (15 g) or a whey protein supplement (13.6 g) after an overnight fast, subjects consuming the EAA mixture had higher mixed muscle fractional synthetic rate [42], which is often associated with increases in muscle mass. The differing response could be due to the differing leucine content between the supplements (EAA, 2.8 g leucine, and whey, 1.8 g leucine) or because the EAA supplement was composed of individual amino acids while the whey protein supplement was intact protein. These subtle differences could influence the rate of appearance of the amino acids into blood circulation and thus the protein synthetic response.

Another potential confounder of the protein synthetic response of various proteins is the form or texture of the protein itself, such as ground beef versus a beef steak [43]. When, older men consumed 135 g of protein as either ground beef or as a beef steak, the amino acids from the ground beef appeared more rapidly in the circulation than the amino acids from the beef steak. Whole-body protein balance was higher after consumption of the ground beef versus the beef steak. However, 6 h after the beef meals, muscle protein synthesis was not different [43]. Nonetheless, these data support that the form of the protein that is being consumed impacts digestion, absorption, and the rate of appearance of amino acids into circulation [35].

7. Timing of protein intake

The timing of dietary protein intake has received ample attention in the past several decades. Adults typically consume the majority of their protein intake at dinner (38 g) versus breakfast (13 g) [44]. However, recent research suggests that ingestion of more than 30 g of protein in a test meal does not further stimulate the effect of dietary protein on muscle protein synthesis [45]. This had led to discussion related to optimal timing of protein intake. For example, distributing protein intake throughout the day, timing of protein around nighttime eating, and protein eating at breakfast are all areas of increased interest. In general, research covering these topics is performed in young, healthy populations, or aging populations, and very few, if any, studies have been conducted in children and pregnant women.

7.1 Protein intake at breakfast

Breakfast is often recognized as the most important meal of the day [46–48]. However, there is debate as to what defines the ideal breakfast meal [47], in addition to a lack of strong evidence to define which nutrients should be represented at breakfast [47]. A recent commentary published by the American Academy of Nutrition and Dietetics suggests that protein-containing foods (e.g., eggs, lean meat, and low-fat dairy products) should be included in breakfast meals [47]. Literature supports diets higher in protein aid in the treatment of chronic, metabolic diseases such as obesity, type 2 diabetes, and heart disease and have been shown to increase EE, improve satiety, regulate glycemic control, and improve body composition (reviewed in [13, 14, 34, 49]).

7.2 Protein intake in the evening

Eating protein at night and immediately before bedtime has received substantial attention in the past decade. Although past common knowledge would claim that eating before bed precipitates negative effects on health and body composition, more recent studies show that there may be many metabolic, health, and body composition-related benefits [50]. Much of the previous research claiming the negative effects of nighttime eating was performed in shift workers [51], populations with night eating syndrome, who consume ≥50% of daily calories after dinner [52], and epidemiological data [53]. Although some of the negative effects of nighttime eating in these populations may include high BMI and abdominal obesity [54]; increased triglyceride concentration, dyslipidemia, and impaired glucose tolerance [55]; impaired kidney function [56]; and increased carbohydrate oxidation and decreased fat oxidation [57], many other factors need to be taken into consideration. For example, these populations are awake during abnormal hours and report sleep disturbances [58, 59]. In fact, the duration of sleep is inversely related to BMI [60, 61]. These populations also consume significantly more carbohydrate, protein, and fat throughout the day. Nonetheless, it is clear that eating large amounts of energy in the evening hours, in particular when the energy is carbohydrate- and fat-laden, may not be beneficial for health and body composition outcomes.

However, much more evidence has shown that eating a small protein snack (~200 kcal) before bed may elicit significant benefits. Improved muscle protein recovery, muscle mass, and strength gains mediated by enhanced overnight and next-morning muscle protein synthesis have been shown to be enhanced with 40 g of casein protein supplementation in elderly [62] and recreationally active men [63]. These effects are particularly enhanced when this dietary practice is added to the practice of resistance exercise [63]. In addition, reported hunger is lower and

satiety is higher, and resting energy expenditure is higher the following morning after a small protein snack compared to a noncaloric placebo [50, 62]. Chronically (4 weeks) there are also reports of decreased blood pressure, decreased arterial stiffness [64], and a greater decrease in body fat in overweight and obese women when consuming nighttime protein [65, 66]. Importantly, these benefits are accompanied by no significant alterations in overnight or next-morning lipolysis, fat oxidation, substrate utilization, or any blood markers in obese men or resistance-trained young women [67].

7.3 Distribution of protein intake throughout the day

Current research demonstrates that even distribution of protein intake throughout the day is more effective at stimulating a 24-h protein synthesis compared to an uneven distribution [68, 69]. This is supported by data from a longitudinal study on nutrition and aging, which found that even distribution of daily protein intake across meals is independently associated with greater muscle strength and higher muscle mass in older adult, but is not associated with loss in muscle mass [70] or mobility [71] over 2–3 years. However, there are some studies that fail to confirm the importance of spreading protein intake out over the course of the day [71, 72]. Additional studies have compared pulse feeding (72% of daily protein at lunch) versus protein being evenly distributed over four daily meals in hospitalized older patients for 6 weeks [73, 74]. These studies found that pulse feeding of protein increased postprandial amino acid bioavailability [75] and increased lean mass [74] compared to spreading protein intake throughout the day. Taken together, the optimal timing and distribution of protein intake still need to be determined.

8. Dietary protein and health

8.1 Dietary protein and obesity

Obesity is a major public health concern [76] and is associated with the development of metabolic diseases such as cardiovascular disease, nonalcoholic fatty liver disease, and type 2 diabetes mellitus in both children and adults [77, 78]. Obesity is defined as having a body mass index (BMI) (weight in kilograms divided by height in centimeters squared) greater than or equal to 30.0. In 2015–2016, the prevalence of obesity (**Table 2**) in the United States was 39.6 for adults and 18.4% for youth [76]. Obesity also impacts racial and ethnic groups differently. For example, non-Hispanic black and Hispanic adults and youth have higher rates of obesity compared to non-Hispanic white and Asian populations [79].

A primary factor in controlling and preventing obesity and associated chronic diseases is through diet, for example, diets higher in protein [13, 14, 80, 81]. Diets higher in protein (>30% of energy intake) have been shown to improve body composition [82], improve glycemic response [81, 83–85], increase satiety [85–87], and increase postprandial energy metabolism [88, 89], which are all mediating factors of weight loss.

8.2 Dietary protein and sarcopenia

Sarcopenia is the term for age-associated loss of muscle mass and function [35]. The loss of muscle function associated with sarcopenia is often referred to as dynapenia [90]. A loss or reduction in skeletal muscle function often leads to increased morbidity and mortality either directly, or indirectly, via the development of

Age group (years)	Total (percent)	Boys or men (percent)	Girls or women (percent)
Youth, 2–19	18.5	19.1	17.8
Young children, 2–5	13.9	14.3	13.5
Youth, 6–11	18.4[$]	20.4[$]	16.3
Adolescents, 12–19	20.6[$]	20.2	20.9[$]
Adults, 20+	39.6	37.9	41.1
Young adults, 20–39	35.7	34.8	36.5
Middle-aged adults, 40–59	42.8[*]	40.8[*]	44.7[*]
Older adults, 60+	41.0	38.5	43.1

[$] *Significantly different from young children.*
[*] *Significantly different from young adults.*

Table 2.
Prevalence of obesity in the United States by age group and sex [76].

secondary diseases such as diabetes, obesity, and cardiovascular disease [91]. The causes of sarcopenia include poor nutrition, diminished responsiveness to anabolic hormones and/or nutrients, and a sedentary lifestyle.

The loss in muscle mass observed with aging is often accompanied by an increase in fat mass [92], which can happen even in the absence of changes in BMI [35]. The loss in muscle mass results in a decrease in basal metabolic rate (BMR) or the amount of caloric energy we use while at rest [93]. The loss of muscle mass induces a 2–3% decrease in BMR per decade after the age of 20 and a 4% decline in BMR per decade after the age of 50 [93, 94]. Muscle loss and subsequent reduction in metabolic rate contribute to obesity that accompanies the aging process.

Several studies identify protein as a key nutrient for aging adults [2, 95]. Low protein intake is linked to a decrease in physical ability in aging adults [96]. However, protein intake greater than the dietary guidelines may prevent sarcopenia [96], help maintain BMR [3], improve bone health [4–7], and improve cardiovascular function [8–10]. These benefits of increasing protein in the diet may improve function and quality of life in healthy older adults, as well as improve the ability of older patients to recover from disease and trauma [91].

Currently, the dietary recommendations for protein intake are the same for all healthy adults above the age of 19. However, experts in the field of protein and aging recommend a protein intake between 1.2 and 2.0 g kg^{-1} day^{-1} or higher for elderly adults [91, 95, 97]. The RDA of 0.8 g kg^{-1} day^{-1} is well below these recommendations and reflects a value at the lowest end of the AMDR. It is estimated that 38% of adult men and 41% of adult women have dietary protein intakes below the RDA [16, 44].

Both protein amount and source are important to consider when recommending protein intake to older adults [34, 35]. There are three important aspects to take into consideration when recommending a protein source: (1) the characteristics of the specific protein, such as the amount of essential amino acids (EAA); (2) the food matrix in which the protein is consumed, for example, as part of a beverage or a complete meal; and (3) the characteristics of the individuals consuming the food, including health status, physiological status, and energy balance [34]. In addition, the difference in digestibility and bioavailability of a protein can impact the quantity of protein that needs to be ingested to meet metabolic needs; this is especially important in older adults since gastric motility and nutrient absorption decrease with age. The speed of protein digestion and absorption of amino acids

from the gut can influence whole-body protein building [36]. Proteins with differing amino acid profiles exhibit different digestion and absorption rates [36, 38, 98]. Amino acid availability depends directly on both the quality and quantity of the dietary protein [98].

8.3 Dietary protein and gut health

Over the past 15 years, the gut microbiome has received increased attention regarding its role in impacting overall health [99]. Interestingly, it has been shown to influence diseases associated with metabolic health [100]. The intestinal mucosa houses nearly a trillion microorganisms, and the plasticity of this environment is highly reactive to changes in diet [101]. For instance, the gut becomes an active site for protein and amino acid metabolism prior to absorption. Following enzymatic denaturation by intestinal proteases, amino acids can become fermented into various metabolites which include short-chain fatty acids and ammonia [102]. The acute microbial response and long-term adaptation associated with dietary habits have become an important area of research.

As gut assay methodologies improve, researchers have identified associations between microbial populations and their metabolite concentrations in response to dietary patterns. For instance, in vitro and human models demonstrate a potential negative link between animal protein intake and protein fermentation end products such as ammonia and trimethylamine-N-oxide [103, 104]. However, favorable outcomes associated with animal- and plant-based protein sources have been observed. For example, ingestion of both whey [105] and pea protein [106] has been shown to increase favorable gut bacterial species such as *Bifidobacterium*. In addition, supplementation with pea protein intake has been shown to increase the production of short-chain fatty acids, an important energy substrate utilized by enterocytes [106].

9. Conclusions

There is sufficient evidence that protein intake higher than the current dietary recommendations is beneficial for most healthy individuals throughout the life cycle. However, benefits of dietary protein depend on the quality, the quantity, and the timing of protein intake. Although health benefits of dietary protein have been well-established for older adults, more research is needed to determine the health benefits of increased dietary protein intake through each state of life.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Chapter 2

Stress, Natural Antioxidants and Future Perspectives

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Abstract

Stress can exist by a variety of daily challenges related to obesity, other eating disorders, long-term health issues and immune system suppression. Free radicals derived from oxygen, called reactive oxygen species, reactive nitrogen species and similarly antioxidants are part of the body's natural functioning. Oxidative stress occurs when free radicals and antioxidants are out of balance. The prooxidant-antioxidant balance is assessed by determination of both oxidant and antioxidant status, which can be measured simultaneously in blood and tissue. Dietary or natural antioxidants play an important role in helping the endogenous antioxidants in scavenging the excess of free radicals. Antioxidant supplements include several important substances such as beta carotene, lutein, phycocyanin and zeaxanthin, which are rich in vegetables, fruits and natural foods. All these contents have a key role in growth, immunity and lifetime quality. Still, high dose of the natural foods can cause the organism, not to assimilate the wastes by the mechanism. In this chapter, we'll inquire to explain the oxidative and antioxidative mechanisms and balance via importance of the natural antioxidants to life quality. For this purpose, oxidative stress, related diseases, antioxidants and their importance will be reviewed, and the correlation between natural antioxidants and health will be presented.

Keywords: stress, oxidant-antioxidant balance, diet, natural antioxidants, health problems

1. Introduction

Stress is a complex phenomenon that correlates with oxidative and antioxidative status in organism. The physiological stress responses include several biological mechanisms such as digestion, reproduction, hormone and immunity. In common, physical or psychological stresses cause stress and disrupt homeostasis. Likewise, environmental factors and diseases can be a threat of some impending conditions (malnutrition, weakness, cancer, etc.). Oxidative stress is defined as imbalance between oxidants and antioxidants, and with aging, endogenous antioxidant defenses decrease and production of reactive oxygen species increases [1]. Nevertheless, antioxidant defense system and protection mechanisms are important in maintaining the organism against the oxidative stress, and thereby homeostasis can be observed. Keeping a stable homeostasis requires, besides a better environment and gene structure, we should need to know what nutrients are needed to maintain hemostasis. Nutrition especially dietary antioxidants

decreases the adverse effects of reactive oxygen species and regulates the stress. Consequently, it is necessary to understand how antioxidants in nutrients exert its health protective effects.

Antioxidants, natural or synthetic, may protect cell damages during oxidative stress. New researches showed that natural antioxidants in foods are commonly belonged with a better health and life quality. At that place, there are several natural antioxidants, which can reduce oxidation in cell or lipid peroxidation. Several studies have been stated that natural antioxidants such as medicinal herbs, alga, ginger, curcuma, cloves and vitamins can be utilized for health maintenance. They have important biological activities which attributed to their compounds named carotenoids, polyphenols, phycocyanin and flavonoids. The biological actions of these antioxidants are anti-inflammatory, enzyme detoxification, cell damage prevention, gene regulation and antimicrobial, which have been conducted with human and animal studies [2, 3]. Besides, natural antioxidants are shown to possess the antioxidant activity in organism and maintain the normal physiological condition. Thereby, they can be applied for protective health as well as for therapeutic conditions.

Increasing world population impacts on the environmental stress like of biodiversity, air and water contamination. Physicochemical stress results from environmental agents and such effects result in chronic infections, autoimmune diseases and other physiological disorders. Because of this reason, regulation of homeostasis should be backed up by natural antioxidants. This chapter, we will attempt to explain the stress, oxidative-antioxidative balance and natural antioxidants with evaluating the association of natural antioxidants and health.

2. Stress mechanism and oxidative-antioxidative balance

Free radicals are called the reactive oxygen species (ROS), and they also include a subgroup of reactive nitrogen species (RNS) which are the products of normal cellular metabolism. Overwhelming production of these molecules leads to oxidative stress damage to lipids, proteins and DNA [4].

A balance between free radicals and antioxidants is necessary for proper function. If free radicals overwhelm the organism's ability to regulate the stress, a circumstance is known as stress. The mechanisms of stress could be explained with two parts as acute and chronic. Acute stress is termed as an emergency response of organism, which affects by short term stressors. In response to acute stress, sympathetic nervous system is triggered due to release of hormones and the response prepares the body to either fight or flight response. The sympathetic nervous system has signaled to adrenal glands for releasing epinephrine and cortisol hormones, which act on endocrine, cardiovascular, respiratory, musculoskeletal and gastrointestinal systems. All the same, the parasympathetic nervous system regulates rest and digests functions. It works without conscious control of cardiac muscle, smooth muscle and exocrine and endocrine glands, which regulate the blood pressure, glucose and thermoregulation, etc. On the other hand, chronic stress is induced by stress over a prolonged time and conducts the stress hormones to release in a long period. Also, hypothalamic-pituitary-adrenal axis is kept active by chronic stress. This can have several symptoms either physical or psychological. Chronic stress is linked the risk of certain illnesses and lower life expectancy, such as obesity, cholesterol, anxiety and depression, and so on.

Oxygen is one of the most abundant and essential elements for all the life forms on the earth. It is critical for the energy production in both prokaryotes and eukaryotes via electron transport chain [4]. As a result of stress in cellular metabolism, reactive oxygen species are produced and these molecules can damage the proteins, carbohydrates, nucleic acids and lipids, which are the important cell structures. This situation is termed oxidative stress. Oxidative stress causes to increase of free radicals production and reduction of antioxidant defense system. According to this issue, within the consumption of antioxidant, either increase or decrease of oxidant and antioxidant amounts should be assessed for determining the oxidative status [5]. The free radical effect of fatty acids is to stimulate the lipid peroxidation and thereby several damages occur. The most important molecule of lipid peroxidation is malondialdehyde (MDA), which takes in an ability to inactive the cellular proteins by forming protein linkages [6]. In additionally, MDA level increases during oxidative stress and so, in clinical studies, the measurement of the MDA on biological fluids such as plasma or tissue should be taken out for reflection oxidative stress status in vivo.

Antioxidant molecules are classified as enzymatic and nonenzymatic by structures, endogen or exogen by sources, water-soluble and lipid-soluble by resolution, and intracellular and extracellular antioxidants by placement in organism. The enzymatic antioxidants called as glutathione (GSH, GST), glutathione peroxidase (GPx), catalase (CAT) and super oxide dismutase (SOD) have a big role in eliminating free radicals. They can restrain the negative effects of free radicals on DNA, proteins and lipids [7]. The nonenzymatic antioxidants, Vitamin C and E, beta carotene and polyphenol have an efficiency of free radical chain reactions by catching the oxygen molecules [8]. Measurement antioxidant response in biological fluids should be necessary for evaluating the oxidative stress. However, besides individual oxidant and antioxidant molecules, total oxidant and antioxidant status has been important to reflect the cumulative effect of oxidative stress in the organism [9]. Endogenous and exogenous antioxidants act synergistically to maintain or reestablish the redox homeostasis, such as during regeneration of vitamin E by glutathione or vitamin C to prevent the lipid peroxidation process [10].

The oxidant-antioxidant balance is associated with increasing free radicals, inactivation or insufficiency of antioxidants and accumulation of oxidant molecules. Also, maintaining the balance between beneficial and harmful effects of reactive oxygen species is very important. Antioxidants encounter low concentrations of oxidant substances or inhibit the oxidation of target molecules [11]. They reduce the activation of oxidants or convert these molecules to weaker new molecule. Likewise, they can bind the oxidants and act on a reaction chain as in break/repair balance. Thereby, cellular prevention occurs and immunity is balanced [12]. There are both endogenous and exogenous defense against oxidative stress but endogenous defense mechanism is insufficient to completely protect against reactive oxygen species. Exogenous defense comes from the diet in the form of antioxidants, especially from fruits and vegetables [13].

3. Antioxidants in health and disease

The relationship between free radicals and antioxidants shows the unbalance of oxidant-antioxidant status. If antioxidant levels decrease, oxidant levels increase in

an organism during oxidative stress. The initial defense response can be explained with SOD, which modifies the superoxide radicals to less harmful molecular oxygen [14]. Nevertheless, GSH, GPx, and CAT have a protective role on lipid peroxidation. Although GSH and GPx can reduce the hydrogen peroxide and lipid hydrogen peroxide, CAT, which has iron, brings down the hydrogen peroxide on liver and erythrocytes [15, 16].

There are numerous studies that observe the consumption of antioxidants in tissues or blood samples, and also reviewed the correlation between balance and important diseases both for humans and animals. Uzar et al. [17] observed the lower antioxidants in tissues in brain ischemia-reperfusion damage due to the higher oxidant value. Yigiter et al. [18] determined the increase of MDA and decrease of GSH in kidney tissue damage due to increase of DNA oxidation in the kidney. Tok et al. [19] found the higher MDA and MPO and lower GSH and GST levels in oxidative situations [20, 21]. As well, some researchers reported that free radicals were the most important components for ischemia damages in several organs such as brain, heart, liver and lung [22, 23]. Atherosclerosis, hypercholesterolemia and cancer are universally accepted as important diseases due to either antioxidant depletion or unbalance of oxidant and antioxidant status [24, 25]. Generation of antioxidants in oxidative status and correlation with pulmonary, cardiovascular or nutritional diseases were reviewed [26].

The role of oxidative stress in health and disease of animals has been critiqued by some researchers [27, 28]. Metabolic diseases, heat stress and nutrition have been documented as well as performance parameters, immune defense, milk production and energy balance [29, 30]. In addition, some important biological molecules damage by oxidative stress, such as DNA, RNA, cholesterol and proteins. It was reported that high starch nutrition was resulted in an increase of oxidative stress in dairy cows [14]. In horses, it was observed that overload feeding of grains, sugar or fructans was resulted with laminitis which is associated with oxidative stress [31].

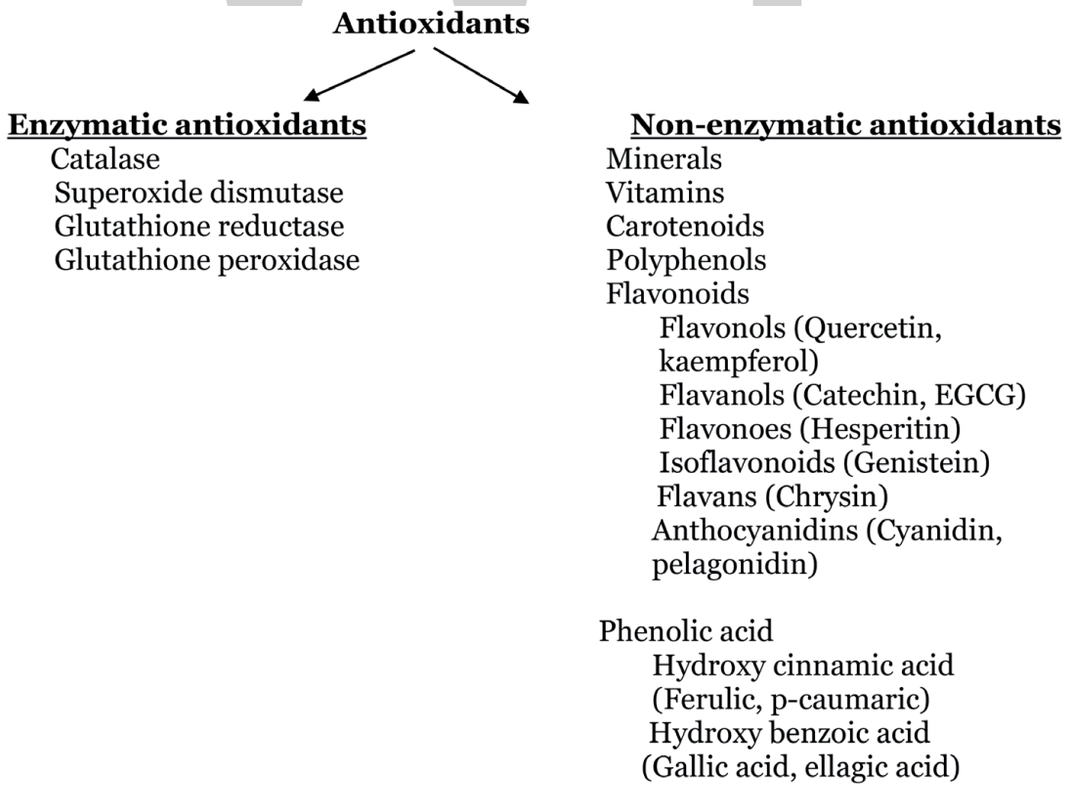

Figure 1.
Classification of antioxidants.

Besides, protein oxidation was reported important for meat quality of both ruminant and poultry [32].

Insight of this information, if the antioxidant mechanisms in organism are insufficient against oxidative stress, exogenous antioxidant supplements should be added to feed both human and animals for a better health. Antioxidants can be divided into two groups generally as natural and synthetic sources (**Figure 1**). Although synthetic antioxidant is produced from chemical processes, the important one natural antioxidant is more useful for health due to its natural contents.

4. Natural antioxidants

The relationship between food and health has addressed for many years. Diet has an essential part in maintaining our health. Natural antioxidants play decisive roles in risk reduction of so many diseases. Dietary or natural antioxidants play a persuasive role in serving the endogenous antioxidants in scavenging the excess of free radicals. Nonetheless, the dietary antioxidants can only have helpful effects in the radical scavenging if they are present in tissues or body fluids at adequate concentrations. For many dietary components, absorption is limited or metabolism into derivatives that can be easily incorporated reduces the antioxidant capacity. As well, it is important to know that some specific antioxidants have limited function because of their inability to penetrate the blood-brain barrier, poor absorption and conversion to the pro-oxidants under certain physiological conditions [33].

Natural antioxidants are widely spread in food and medicinal plants and exhibit a wide range of anti-inflammatory, anti-aging and anticancer effects. These natural antioxidants from plant materials are mainly polyphenols, carotenoids and vitamins. The most important are those coming from routinely consuming vegetables and fruits, flowers as well as traditional medicinal plant [34–37] (**Table 1**). It has been reported that medicinal plants have been used 70–80% of the world population [38]. Bioactive compounds, which mean phytonutrients as well as named natural antioxidants, are health promoting compounds that can bring down the risk of diseases.

Antioxidants contents	Natural sources
Polyphenols	Green tea, strawberries, apples, broccoli, onion, chocolate, coffee, red wine, blackberries
Flavonoids	Oranges, lemons, green tea, berries, grapes, spinach
Vitamin C	Vegetables, citrus fruits, strawberries, potatoes, green vegetables
Vitamin E	Whole grains, fish liver oil, nuts, seeds, green vegetables
Phycocyanin	Seaweed (algae)
Zeaxanthin	Egg yolks, peas, broccoli, carrots, pumpkin
Beta carotene	Tomatoes, potatoes, carrots, broccoli, peaches
Lutein	Green leafy vegetables, cooked spinach, cooked kale, egg yolks
Glutathione	Avocado, fish, meat, grapefruit, peach, broccoli, strawberries, squash
Selenium	Fish, shellfish, red meat, grains, chicken, eggs and garlic.
Cysteine	Animal protein
Peroxidase	Mango, fruit

Table 1.
Some interesting antioxidants sources.

Natural antioxidants have been valued for their contents, antioxidant activities and usage for both humans and animals feeding. Its biochemical compositions and functional attributes of these antioxidants have been important for selection criteria. It is well known that the mainly contents of the natural antioxidants are polyphenols, flavonoids, carotenoids, glutathione and some vitamins (E and C). Carotenoids and polyphenols have greater biological effects on organism such as antibacterial, anti-inflammatory, anticancer, etc. The important compounds of polyphenols are phenolic acids, lignans and flavonoids. It was proven that these contents can serve as metabolites by blocking the oxidation and clean the free radicals in the organism [39, 40]. As well, plants and spices which used for antioxidant properties have a strong hydrogen activity against oxidative stress [41, 42]. It was also reported that absorption of polyphenols in gut barrier can be linked up with increasing antioxidant efficiency [43]. In addition, although phenolic acids can be derived from apples, kiwis or cherries, flavonoids are in several common fruits and vegetables including onion, tea, citrus fruits, grapes, red pepper and broccoli [44, 45]. Carotenoids, which are also nominated as natural pigment, include beta carotene, lutein and zeaxanthin [46]. Among the carotenoids, beta carotene can be found in mango, carrot and nuts. Carotenoids can protect the protein and DNA structure of the organism against oxidative stress [47]. It was reported that carotenoids may inhibit fat oxidation [48]. Also, carotenoids have been reviewed as a health promoter from cancer due to their deactivation effect on ROS, but are not sure. It was seen that the contradictory findings have been related to the variety of carotenoids [47].

In addition, phycocyanin and zeaxanthin can be found in several plants such as microalga, broccoli and peas [46, 49]. Phycocyanin, which is an important extract of microalgae named *Spirulina platensis*, can inhibit the microsomal lipid peroxidation and hydroxyl and peroxyl radicals [47]. It was also observed that phycocyanin can improve the antioxidant activity and support the immunity and wellbeing [50, 51]. Moreover, it was reviewed that ascorbic acid (Vitamin C) and alpha-tocopherol (Vitamin E), which require for nutrition, could change the enzyme system for free radicals and protect the cellular membranes from oxidation [52–54]. Both of these vitamins can diminish the side effects of oxidative molecules with a huge amount. Vitamin E is known as a chain-breaking antioxidant, and it can protect the cell from lipid peroxidation. Also, ascorbic acid can restore the vitamin E. It was known that vitamin C is mainly rich in the peel of fruits such as orange and vitamin E is in candied orange and lemon [55]. Glutathione, which is an another antioxidant, is also produced in the body; several food resources have this important antioxidant naturally, such as melons, avocado, grapefruit, spinach, fishes and so on [56]. Especially, fish and sulfur containing amino acids are evaluated for maintaining and also increase the glutathione levels in organism.

Natural antioxidants have been extracted by several technological methods, including hot water bath and Soxhlet extraction, and different solvents have been used for the extraction of antioxidants from food and medical plants [57, 58]. Numerous works have been based on medicinal plant extraction and special antioxidant compounds. The extraction techniques, industrial applications, costs and procedures have been considered for getting more and useful extracts. The better the extraction efficiency of antioxidant components from plant materials, different methods have been developed such as ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction and electric field extraction. Still, necessity of standardization of sample collection and the analysis method has been reported [59].

5. Importance of natural antioxidants for health

The importance of natural antioxidants has been increasingly investigated for oxidative-antioxidative balance and wellness because of the consumer concern regarding the safety of using synthetic antioxidant and its low cost and strong H donating capacities. Natural antioxidants and their derivatives could be obtained from vegetables, fruits and medicinal plants. So, there have been several researches about these compounds for evaluating the effects on both humans and animals. It is known that natural antioxidants have several physiological roles on organism and actually they can act as a radical scavenger [60].

Oxidative stress can be linked to cancer, cardiovascular or respiratory diseases, immune deficiency and inflammatory conditions. Studies have shown that more antioxidant in diets being important and gets more health to the organism (**Table 2**). Nevertheless, there have been contradictory results about the effects of natural antioxidants on health. It was also reported that flavonoids, which can be metabolized by microbiota in the intestine, can be effected in the nervous system, can take down the blood pressure and reduce serum triglyceride [61]. On the other hand, antioxidant effects of polyphenols have not been awarded thus far due to its limited bioavailability in systemic circulations. It has been suggested that polyphenols may not protect oxidative damage directly, but it can be a versatile proactive rather than antioxidants [62, 63]. It was reported that polyphenols in green tea can protect the cardiovascular diseases [64–66], reduce cholesterol [67] and glucose [68], and as well it can be a cardiovascular and an anticancer medicine [69–71] in humans. Phenols have been read widely for human health as well as animals especially flavonoid compound. Researchers reported the increase in villus height [72] and improvement of duodenum health [73] in broiler belong to polyphenol rich feeding. Polyphenols and flavonoids can affect positively on intestinal health due to inhibition of pathogenic bacteria, and thereby can stimulate the animal performance such as monogastric animals, chicken and pigs [73–76]. It was observed that flavonoids (*Ginkgo biloba*) could improve the immune system parameters via expression of the constituents of interleukins and cytokines [77–79].

It was proven that the beta carotene in food could reduce the risk of cardiovascular diseases, although vitamin C could avoid the cardiovascular mortality [80, 81]. It was conducted that beta carotene, vitamin E and vitamin C may improve the mortality ratio [82]. Even so, it was reported by the National Institutes of Health (NIH) that Vitamin C, vitamin E or beta carotene has no effect on cancer and some cardiovascular diseases as heart attack or stroke. This place has been associated with several reasons such as insufficient antioxidants consumed in foods, not given long enough time, lower doses, individual differences and differences in the chemical compounds of antioxidants [83]. Even so, it was determined that vitamin C additive had a great role on germs and bugs in resting mice due to the reduced effect of vitamin C on stress hormones' amounts [84]. Additionally, vitamin E additive in sows showed the similar results in fertility and mating success compared to animals in feeding with polyphenols [85]. Another work, the SOD, GPx and total antioxidant capacity parameters were found higher in chickens fed by either polyphonic or vitamin E [86]. It was indicated that vitamin C additive in animals is related to improvement of osteoclast formation and bone health [87]. Also, in fishes vitamin C helps with proper health was reported by researchers [88, 89].

Natural antioxidants and their products have a vast potential for both human and animal feeding and health [90–94]. Understanding of natural antioxidants

Natural antioxidants	Functional properties	Reference
Polyphenols	Antioxidant parameters↑ MDA↑ Minimize the adverse effects of lipid peroxidation	Lipinski et al. [86]
Flavonoids	Anticancer Triglyceride↓	Gengatharan et al. [2], Lipinski et al. [86]
Vitamins (C-E)	Total antioxidant↑ GSH↑	Lipinski et al. [86]
Phycocyanin	Anticancer Regression of leukoplakia Antioxidant parameters↑ Induces apoptosis	Pinero et al. [50], Karkos et al. [51]
Zeaxanthin	Protect DNA structure	Mezzomo and Ferrira [46], Seyidoglu et al. [49]
Beta carotene	Protect DNA structure Anticancer Anti-inflammatory	Mezzomo and Ferrira [46], Pinero et al. [50]
Lutein	Antioxidant Reduction of cataract and macular degeneration related to age	Mezzomo and Ferrira [46]
Glutathione	Antioxidant Protects cells from free radicals	Ashadevi and Gotmare [56]
Selenium	Anticancer Antioxidant Reduce cancer incidence and mortality	Ashadevi and Gotmare [56], Helzlsouer et al. [90]
Cysteine	Antioxidant Blocks oxidants of the free radical	Ashadevi and Gotmare [56]
Garlic	Antioxidant Prophylactic and therapeutic medicinal agent	Elosta et al. [91]
Ginger	Antioxidant Reduce or delay the progression of diseases Extracts of ginger have different antioxidant capacities	Tohma et al. [92]
Curcumin	Antioxidant, Anti-inflammatory ROS scavenger Exert chemopreventive effects on carcinogenesis	Menon and Sudheer [93]
Saffron	Antioxidant Antimicrobial agent	Kakouri et al. [94]

Table 2.
Functional properties of some natural antioxidants.

in the context of coordinated oxidative stress and antioxidants and translation of this knowledge to improve animal and human health is a large challenge. In order to attain the health benefits, molecular mechanism of protective effects of fruits and vegetable has been enlightened. Future efforts should be addressed to explain in detail the mechanism of the natural antioxidants health promoting effects, increase in public attention and their utilization in animal and human foods and their recommended dosages, thereby achieving their health advantage and reducing health care expense.

6. Conclusions

Stress has been the most important problem in life for years. Nutrition, unhealthy environmental conditions, genetic factors and physiological insufficiency may create the stress. Although oxidative stress is related to diseases, antioxidant strategies or use has been still questionable.

Today, there is an increasing intake of the antioxidants, especially natural ones, to maintain the antioxidative status in both humans and animals. Natural antioxidants have several beneficial effects, which are considered to protect the homeostasis of the organism. Assessment of natural antioxidants, extracts and functional properties are summed in this chapter. At that place, several studies include oxidative stress mechanism and natural antioxidant consumption in both humans and animals. These findings enrich our knowledge of natural antioxidants in both humans and animals, and the scientific evidence suggests that a well-balanced homeostasis should be associated with a good balanced diet that is rich in antioxidants. Besides, future direction studies in oxidative stress and natural antioxidants should be correlated with intake of antioxidants and impression of oxidative stress markers.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

ROS	reactive oxygen species
RNS	reactive nitrogen species
MDA	malondialdehyde
GPx	glutathione peroxidase
CAT	catalase
SOD	super oxide dismutase
Vitamin C	ascorbic acid
Vitamin E	alpha-tocopherol
DNA	deoxyribonucleic acid
Spirulina	algae
Ginger	*Zingiber officinale*
Curcuma	*Curcuma longa*
Cloves	*Syzygium aromaticum*
Carotenoids	tetraterpenoids
Vitamins	organic compounds
Polyphenols	micronutrients
Phycocyanin	pigment of plants
Flavonoids	a class of plant and fungus secondary metabolites

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Chapter 3

Tree-Borne Edible Oilseeds as Sources of Essential Omega Fatty Acids for Human Health

Bithika Chaliha, Debajit Saikia and Siddhartha Proteem Saikia

Abstract

Certain positional isomers of polyunsaturated omega-3 and omega-6 fatty acids are the essential fatty acids that the human body needs for metabolic functioning but cannot produce themselves and therefore must be acquired from the diet. The beneficial effects of omega-3 fatty acids are related to brain development, coronary heart disease (CHD), cancer, inflammatory bowel disease, rheumatoid arthritis, psoriasis, mental health, and neurodegenerative disorders. The essential omega-3 fatty acid is α-linolenic acid (ALA; 18:3ω3), found in green leafy vegetables and in the seeds of flax, rape, chia, and walnuts. The essential omega-6 fatty acid, linoleic acid (LA; 18:2ω6), is plentiful in nature and being found in the seeds of many edible plants. There are at least hundred species of plants occurring in wild or cultivated from forest areas that may be a source of vegetable oil. These vegetable oils are rich in polyunsaturated fatty acids, which are highly beneficial for human health.

Keywords: fatty acids, oilseeds, tree-borne, PUFA, prostaglandins, human health

1. Introduction

1.1 Tree-borne oilseeds

Triglycerides constitute a vital part of human nutrition, and 90% of the global production from plant, animal, and aquatic sources is used as edibles or as an ingredient in edible products. A major portion of the dietary energy comes from triacylgycerols which contain more than twice the value of identical amount of carbohydrate. Tree-borne seed oil can be defined as a vegetable oil that is obtained from the seed (endosperm) of some trees, rather than the fruit (pericarp).

Vegetable oil production and bioenergy generation from high oil-yielding tree-borne oilseeds have been a topic of interest [1]. The popular tree-borne oilseed (TBO) species include *Azadirachta indica* (neem), *Calophyllum inophyllum* (Undi), *Garcinia indica* (Kokum), *Jatropha curcas* (Ratanjot), *Madhuca longifolia* and *M. indica* (mahua), *Pongamia pinnata* (Karanj), and *Simarouba glauca* (*Simarouba*). Tree-borne seeds rich in non-edible oils, mostly produced by perennial species, are referred to as tree-borne oilseed species. *Simarouba*, which is not a familiar species in India, was studied to standardize various aspects of its cultivation [2]. *Simarouba glauca*, an exotic species belonging to family Simaroubaceae, is indigenous to North

America and commonly known as "American bitter wood." The species is native to places near the equatorial region having rain forest like Florida, Mexico, Cuba, Lesser Antilles, and other Central American counties like El Salvador, etc. It was first introduced in India from Brazil in the year 1966 under the plant introduction scheme of Indian Council of Agricultural Research (ICAR), New Delhi, in two places: NBPGR Maharashtra and the other at Akola in 1970 for its consumable acetone oil or aceituno [3]. Oil obtained from *S. glauca* is edible and probably requires comparatively better growing conditions. Kokum has recently become popular as edible oil with various benefits. *M. indica* oil is used by the local tribes as vegetable oil, but it is a slow-growing species hence may not fit into an agroforestry system. *C. inophyllum* seeds yield maximum amount of oil among these species but due to its restricted growth in sandy soil with humid environment, it is n0t widely available. *Azadirachta indica* has more insecticidal properties and less seed oil uses. Therefore, it can be summarized that only Simarouba and Kokum can be further processed for high production values as tree-borne edible vegetable oils.

About 80% of the global vegetable oil production is edible, while the remaining 20% is used in animal and chemical industries [4]. Bio-oils from oilseeds are used as straight vegetable oil (SVO) or as biomass fuel (transesterified oil) depending on the type of engine and level of blend of the oil; this also includes soya bean oil [5].

1.2 Definition of fatty acids

1.2.1 SFA

Single-bond containing fatty acids are termed as saturated fatty acids (SFA). Foods high in saturated fats include butter, whole milk, chocolates, cream, eggs, lard, red meat, and solid shortenings. An excess intake of saturated fat can raise blood cholesterol and increase the risk of developing coronary heart disease [6, 7].

1.2.2 MUFA

Fatty acids (FAs) having double bonds are called unsaturated fatty acids. Fatty acids with only one double bond are termed as monounsaturated fatty acids (MUFAs). Avocados and nuts and olive, canola, and peanut oils are good sources of MUFAs. Increased consumption of MUFA is beneficial in lowering LDL cholesterol, and it also lowers the risk of coronary heart disease, especially if monounsaturated fats are used as substitute for saturated fats and refined sugars [8]. Omega-9 fatty acids are monounsaturated fatty acids.

1.2.3 PUFA

Unsaturated fatty acids with more than one double bond are polyunsaturated fatty acids. Polyunsaturated fatty acids are important constituents of the phospholipids of all cell membranes. Corn and soy are rich sources of PUFA. PUFAs are essential fatty acids that a human body needs for metabolic functioning but cannot produce on their own, so they have to be included in their diet. PUFAs are basically of two classes: omega-3 and omega-6 fatty acids.

Ω-3 fatty acids are a category of key PUFAs characterized by the presence of a double bond positioned in the third carbon away from the terminal methyl group. Flaxseed oil, canola oil, walnut, salmon, mackerel, trout, albacore herring, halibut, and sardines are the foods rich in omega-3 fatty acids. Spinach, catfish, light chunk tuna, clams, shrimp, etc. also contain some amount of ω-3. Omega-6, another group of essential PUFAs, has a carbon double bond in the sixth position counting from

the methyl end. Ω-6-rich food includes cottonseed, safflower, sunflower, corn, and soybean oils. Trans-fatty acids undergo hydrogenation to solidify liquid oils. Heating vegetable oils at high temperature produces trans fats which increases the shelf life, hence providing stability to foods like vegetable shortenings, fried foods, few margarines, cookies, crackers, and packaged snacks. The intake of trans-fatty acids raises blood LDL-cholesterol (bad cholesterol) and lowers HDL cholesterol (good cholesterol) and that in turn increases the risk of coronary heart disease [9].

There are many types of omega-3 fatty acids, which differ based on their chemical structure and size. Here are the three most common:

Alpha-linolenic acid (ALA): This 18-carbon fatty acid can be converted into DHA and EPA, although the process is not very efficient. ALA mainly provides energy to the body [10].

Eicosapentaenoic acid (EPA): It is a 20-carbon fatty acid and mainly produces eicosanoids, responsible for reducing inflammation and symptoms of depression [11, 12].

Docosahexaenoic acid (DHA): It is a 22-carbon fatty acid which makes up about 8% of brain weight. It is essential for normal development and functioning of the brain [13].

N-6 fatty acids are another class of essential PUFA, which must be obtained from the diet. The most common omega-6 fatty acid is linoleic acid, which can be converted into longer omega-6 fats such as arachidonic acid (ARA) [14]. Like EPA, ARA is used to produce eicosanoids. However, the eicosanoids produced by ARA are more proinflammatory [15]. Proinflammatory eicosanoids are important chemicals in the immune system. However, when too many of them are produced, they can increase inflammation and inflammatory disease [16]. The recommended ratio of omega-6 to omega-3 fatty acids in the diet is 4:1 or less. However, the American diet has a ratio of 10:1 which is more than necessary. Therefore, although omega-6 fats are essential in the right quantities, most people in the developed world should aim to reduce their omega-6 intake [17]. Besides the drawbacks of overconsumption, omega-6 fatty acids are beneficial in treating symptoms of chronic disease. Gamma-linolenic acid (GLA), an omega-6 fatty acid, is found in evening primrose oil (EPO) and borage oil. When consumed, much of it is converted to another fatty acid called dihomo-gamma-linolenic acid (DGLA). An interesting study proved that taking GLA supplements along with a breast cancer drug was more effective at treating breast cancer than the drug alone [18]. Conjugated linoleic acid (CLA) is another form of omega-6 fat with some health benefits. For example, one large study found that intake of 3.2 grams of CLA supplements per day effectively reduced body fat mass in humans [19].

N-9 is the only class of monounsaturated fatty acids having a number of health benefits. Oleic acid is the most prevalent omega-9 fatty acid in the diet. A study reported that consumption of high monounsaturated fats could decrease triacylglycerol (TAG) of plasm by 19% and very-low-density lipoprotein (VLDL) cholesterol by 22% in diabetic patients. Another study in mice stated that high dose of monounsaturated fats improved insulin sensitivity and reduced inflammation. This study further reported that human diet rich in monounsaturated fatty acids suffered less inflammation and better sensitivity to insulin than diets with high saturated fats [20, 21].

There has been a raise in demand for purified PUFA lipids due to their numerous applications, but due to the scarcity of present plant, fish, and mammal sources there has been an urge to explore alternatives such as bacterial, algal, and fungal production systems. Hence it is important to screen unexploited tree-borne oilseeds for polyunsaturated fatty acid-rich oil production as the country is not producing sufficient quantities of PUFA-rich oils particularly enriched with alpha-linolenic, gamma-linolenic, eicosapentaenoic, and docosahexaenoic acids. These omega3 fatty acids are nutritionally important and are needed by infants for development of the brain, retina, etc., as well as by geriatric adults, both of whom cannot synthesize them.

2. Importance of essential fatty acids

Balancing fatty acids is all about decreasing intake of the "inflammatory" omega-6 fatty acids versus "anti-inflammatory"omega-3 fatty acids in order to have a better omega-6/omega-3 ratio.

A typical Western diet includes a lot of heavily processed and fried oils, so their intake of ALA (omega-3) is lower than LA (omega-6) as per the permissible limits. ALA is a plant-based fatty acid, so dietary sources include flaxseed oil, canola, walnuts, chia seeds, perilla seed oil, pumpkin seeds, tofu, spinach, mustard green, etc. Only a small amount, i.e., 5–10%, of these fats can be converted into EPA and DHA by the body. EPA and DHA content in the body can be increased by consuming cold-water fishes such as red salmon, mackerel, anchovies, and sardines. Some algae also contain DHA [22].

By altering the kinds of fatty acids that a person eats to more alpha-linolenic acid (omega-3) and less linoleic acid (omega-6), it is quite possible to effectively produce more anti-inflammatory prostaglandins than inflammatory prostaglandins which can help reduce the pain caused by PMS [23–25].

Prostaglandin production, which is the primary function of EFA, regulates body functions such as pulse, blood pressure, coagulation of blood, fertility, and conception and also plays a role in the immune system. Deficiency of EFAs and imbalance in ω-6/ω-3 cause serious health conditions such as heart attacks, asthma, depression, insulin resistance, schizophrenia, cancer, early aging, obesity, stroke, diabetes, Alzheimer's disease, ADHD, and arthritis, among others [26, 27].

Acute psychological stress in humans generates the production of proinflammatory cytokines, such as interferon gamma, tumor necrosis factor-a (TNFa), IL-6, and IL10. Overproduction of proinflammatory cytokines in the peripheral blood is caused by an imbalance of omega-6 and omega-3 polyunsaturated fatty acid. Evidences reveal that alteration in composition of fatty acids is involved in the pathophysiology of major depression. Theoretically, changes in PUFA cause changes in serotonin (5-HT) receptor number and function, thus resulting in the current receptor and neurotransmitter theories of depression [28–30]. Increased production of proinflammatory cytokines and eicosanoids might increase C20:406/C20:503 ratio and omega6/omega3 imbalance in major depressions [28]. DHA and EPA reduce the risk of relapse in patients with manic depression [31, 32].

Omega-3 fatty acids are a crucial part of human cell membranes. They also have a number of other important functions, including:

Improving heart health: Daily intake of omega-3 fatty acids can increase HDL cholesterol which is good cholesterol for the body. It can also reduce blood pressure and the formation of arterial plaques [33, 34].

Reducing weight and waist size: Omega-3 fats play an important role in weight management and can help reduce waist circumference [35].

Decreasing liver fat: Addition of omega-3 fatty acids in the diet can help decrease the amount of fat in the human liver [36].

Supporting infant brain development: Omega-3 fatty acids are extremely important for brain development in infants [37].

Fighting inflammation: Omega-3 fats are anti-inflammatory, meaning they can reduce the inflammation in the body which contributes to a number of chronic diseases [38].

Preventing dementia: People who eat more fish, which is high in omega-3 fats, tend to have a slower decline in brain function in old age. Thus, omega-3 s may also help to improve memory in older people [39].

Promoting bone health: People with higher omega-3 intake and blood levels tend to have better bone mineral density [40].

Preventing asthma: Omega-3 intake can help reduce symptoms of asthma, especially in young age [41].

Omega-6 fatty acids also play a crucial role in brain function, normal growth, and development along with omega-3 fatty acids. Ω-6 helps in stimulating skin and hair growth; it also maintains bone health, regulates metabolism, and also maintains the reproductive system. The roles of omega-6 in certain diseases are given below:

Diabetic neuropathy: Keen et al. showed that taking gamma-linolenic acid for 6 months or more may reduce nerve pain in people suffering from diabetic neuropathy. People with good blood sugar control found GLA more effective than other community [42].

Rheumatoid arthritis: There have been mixed results related to the role of evening primrose oil (EPO) in Rheumatoid arthritis. Initial evidences suggest EPO to be helpful in reducing pain, swelling, and morning stiffness, while other studies have found no effect. GLA requires 1–3 months for benefits to appear in patients, but it is unlikely that EPO would help stop progression of the disease [43].

Breast cancer: Consumption of tamoxifen (drug for treatment of estrogen-sensitive breast cancer) along with GLA resulted in commendable improvement in breast cancer patients than those who consumed it alone. Some investigation also suggested that omega-6-rich diet might promote development of breast cancer [18].

3. Process of extraction of oils from oilseeds

Oil extraction process undergoes two basic steps: solid–liquid extraction and solvent extraction. Prior to these processes, mechanical and thermal pretreatments are carried out which enhances their performance. The standard pretreatments for oilseeds include de-husking, size reduction, breaking, grinding, as well as hydro-thermal treatment, cooling, or steaming. De-husking separates the oil-rich seeds from hulls and eliminates the antinutritional factors unattractive to consumption. Crushing and grinding changes the cake permeability and thus promotes solvent extraction. Moisture conditioning of seeds, oil viscosity reduction, increasing plasticity of seed, breaking of cell walls, protein clotting by denaturation, sterilization and deactivation of thermosensitive enzymes, and destruction of thermolabile toxic components are several of the benefits provided by cooking [44–47]. Enzymatic hydrolysis opens up the oilseed cell walls through biodegradation and thus provides an alternative for pretreatment. It also breaks up the complex lipoprotein and lipopolysaccharide molecules into simple molecules releasing extra oil for extraction [48, 49]. The increase in demand for vegetable oils both for human consumption and industrial application has prompted and encouraged the evolution and optimization of procedures leading to efficient production of oil of high quality and purity [50, 51].

Oil yield from an oleaginous seed material is generally dependent on the quality of oilseeds. However, certain factors like moisture content of material, particle size, and temperature can be controlled during pretreatment in order to increase the oil yield. However, according to Olaniyan [52], oilseed pretreatment prior to oil extraction normally affects oil yield and quality. Similarly, Faugno et al. [53] concluded that the combination of seed preheating and high extraction temperature, among others, had a significant effect on oil yield. Thus, oilseed processing or pretreatment provides a platform for manipulating key parameters and conditions for enhanced oil yield and quality [54].

Nowadays, several promising technologies are available for extraction of vegetable oils such as ultrasonic processing, mechanochemical processing (MCP), etc. UAE is a new innovation which makes use of the ultrasonic sound waves to increase

vibration and heat, resulting in the destruction of rigid plant cell walls, thereby enhancing contact between the solvent and the plant material [55]. When coupled with solvent extraction, the UAE method represents an innovative way of increasing extracted oil yield by making plant cell walls thinner, thus enhancing the interaction of the solvent. Samaram et al. [56] analyzed oil production from papaya seeds by both UAE and solvent extraction. They reported that conventional solvent extraction lasted 12 hours, whereas the UAE method lasted only 30 minutes. Thus UAE is more timesaving and gives better yield. UAE plays a significant role in edible oil industry due to its potential to modify efficiency and decrease production time [57].

Mechanochemical processing activates chemical reactions and structural changes by using mechanical energy. The field of mechanochemistry has vivid applications ranging from waste management to the production of advanced materials with novel microcomponents and enhanced mechanical properties. MCP has similar potential to screw expelling as high shear force can act on the cell wall of seeds resulting in its breaking and subsequent oil expulsion [58]. The extraction medium used in supercritical extraction (SFE) is predominantly environmentally benign carbon dioxide (CO2). The extraction of specific lipid components, like cholesterol, can be achieved with SC-CO2 [59–61]. SFE is also applied to determine the fat-soluble vitamins in food. Bruhl and Matthaus [62] reported that the highest yield of lipids and tocopherol content were achieved with the SFE method. Thus it was summarized that SFE-based methods have a promising future in analytical lipid chemistry [63].

4. Oilseeds as source of omega fatty acids

Triacylglycerols together with carbohydrates, proteins, vitamins and minerals are the important nutrients of the body. Triglycerides contain two and a half times the calories of carbohydrate (per unit weight), hence being a rich source of energy. TAGs are not only sources of vitamins A, D, E, and K but also contain EFAs. These EFAs must be included in diet as they cannot be created by the body [64].

Recently, oilseed crops were genetically engineered (GE) to produce two new bioactive omega-3 long-chain fatty acids (eicosapentaenoic acid [EPA, 20:5n-3] and docosahexaenoic acid [DHA, 22:6n-3]) which significantly enhance the nutritional value of the seeds. These GE oilseed plants represent a new type of crop because these fatty acids cannot be manufactured naturally by terrestrial crop plants. These two bioactive compounds are known to have critical involvement in key physiological activities in chordates and non-chordates particularly for their positive effects on chordate cardiovascular and neurological health [16, 65, 66]. Together, DHA and EPA reduce inflammation and the risk of heart disease. Recommend daily intake of EPA and DHA depending on age, gender, reproductive status, health status, various institutes like World Health Organization, American Heart Association, etc. recommend daily intake of EPA and DHA [67]. Algae in aquatic environment produces both EPA and DHA naturally [68–70] while retained y upper trophic level organisms. Both EPA and DHA can be obtained in bulk by consuming seafood, fish, or algal oil spills [16, 71]. Though aquaculture is a major source of sea food, but dietary source of EPA and DHA required by farmed fishes is obtained from the oil derived from wild fisheries [72]. Nowadays, limited exploitation 0f wild fishes are insufficient to support the increasing demand of fish oil necessary for aquaculture and other industries like pharmaceutical, livestock, and food fisheries [73]. Therefore, a feasible source of EPA and DHA would definitely reduce dependency on wild fisheries. Thus these GE crops will provide an alternative source of DHA and EPA for various industries including human utilization. The functional genes introduced

into these new crops were primarily extracted from marine algae, a marine fungus, and a moss [74–76]. These genes and the enzymatic activities they encode together represent a tool by which biological engineers can reconstruct the capacity to synthesize EPA and DHA in a crop plant species [77]. The seed oil thus obtained resembled fish oil when compared to the wild-type cultivar, because it contained similar levels of EPA and DHA as fish oil. Thus, two oilseed crops have been identified so far as prospective host for the omega-3 LC-PUFA biosynthetic trait: canola (*Brassica napus L.*) and *Camelina* (*Camelina sativa*). The transgenic lines developed from these two species have enabled the manufacture of nearly 30% DHA + EPA of the total FAs [77] or 12% DHA only in *Camelina* [74] and 4% of both (EPA + DHA) in canola [76].

Among these only *Camelina* has undergone field trials [78, 79]. Technology for commercial development of transgenic canola have been patented by Cargill and BASF [80], Dow AgroSciences, and DSM Nutritional Products [76, 81]. Nevertheless, this commercialization has not received much public acclaim [82]. While nutritionally improved crop traits intend to prMcGlouovide health benefits but these improvements are due to conventional plant breeding and selection [83].

EPA and DHA are considered to be the main drivers of the difference in fatty acid content observed between aquatic and terrestrial primary producers. Introduction of higher amount of EPA and DHA to terrestrial ecosystems would be unique as they would provide the opportunity for these bioactive FAs to be consumed and metabolized for the first time by consumers in agroecosystem. Since these new GE-oilseed crops are not equivalent to other GE crops, so it warrants careful regulatory consideration of them.

Scientists working on oils and fats have recently reported about a potential oil crop that can match fish oils in nutritional value. This oil crop called corn gromwell or field gromwell (*Buglossoides arvensis*) is abundant in the wild in the higher elevations of the Jammu and Kashmir such as Pampore but can be adapted to other agroclimatic conditions in the country. The seeds of this plant are rich in polyunsaturated fatty acids, including nutritionally important omega-3 fatty acids. More importantly, they contain stearidonic acid (SDA), which is generally absent in regular oilseed crops. Omega-3 content of the oil is found to be 18–20% in lab studies. SDA is a key precursor in the biosynthesis of those omega-3 acids that are commonly found in fish oils. While health benefits of fish oils are well-accepted, those who do not eat fish are often deprived of these benefits from their diet [84].

According to Sreedhar et al., though oils extracted from chia and flax seeds, too, are rich in omega-3 fatty acid, they contain only alpha-linolenic acid, one of the three types of omega-3 fatty acids. "Consumption of *B. arvensis* seed oil has been reported to increase the circulating omega-3 PUFA levels in a dose-dependent manner and associated with anti-inflammatory phenotype in healthy people." This oil needs to be consumed in the form of softgel capsule, salad dressing oil, powder or protein/cereal bars, he said, adding that it may not be an ideal cooking medium as it has low smoke point and degrades upon heating, losing its nutritional qualities [84].

Vegetable oils are a major source of low-cost dietary fatty acids in modern diets. Considerable research has been undertaken in the public and private sectors into the feasibility of producing of omega-3 LC-PUFAs in oilseed crops. The first reports of engineering oilseed plants to produce EPA in seed oils were published in 2004, and subsequent work has achieved levels of up to 20% EPA in *Glycine max* and *Brassica carinata*. Though it has proven to be challenging to produce DHA in plants; however hindrance in pathways have been identified and surpassed expanding the list of useful transgenes. The outcome of this has been recently the production of 4–15% of DHA in seeds of the model oilseed plant *Arabidopsis*. These studies present striking proof of principle that oilseed plants can surely be engineered to synthesize

and store "ω-3 fish oils" in oilseeds [85]. Production of oilseed crops rich in omega 3 LC-PUFA have technically provided a new answer to meet the increasing consumer demands for convenient, low-cost, and sustainable sources of these healthy dietary fatty acids. LC-PUFAs can be manufactured in oilseed plants by two indigenous approaches:

i. Omega-3 LC-PUFAs can be formed by modifying existing plant fatty acids with novel elongases and desaturases.

ii. Introduction of a self-contained microalgal PUFA synthase enzyme system for synthesis of LC-PUFA de novo [86].

Although these processes have their own characteristics and flaws, it would be exciting to see the impact of these technologies in providing omega-3 supplements for improvement of human health.

However, no such attempts have been made to produce tree-borne oilseeds which are enriched with omega acids. Oilseed trees are easily and widely available throughout the forest of the globe both in wild and cultivated forms. There are many trees like *Garcinia, Cinnamomum, Litsea*, etc. which can be utilized as oilseed sources. *Garcinia morella* and *Sapindus mukorossi* are nutritionally considered as a few of the best salad vegetable oils due to the highest content of MUFA (75–79.8%) [87]. If these resources are properly analyzed and collected for the production of omega-rich oils, then we can surely overcome the crisis of vegetable oils rich in omega acids and replace the non-vegetarian sources of omega-rich oils which will be beneficial for both the vegetarian and non-vegetarian communities of the world. Therefore it has become a necessity to search for alternative sources of omega fatty acids in the nature that are available and unexplored. In this regard the tree-borne oilseeds are the best potential source as they have not been exploited much. In this way we will not only discover new sources of omega rich oils but also improve India's sustainability in this sector.

5. Conclusion

- Fats and oils form an important part of the human diet, though less-exploited tree-borne oilseeds have untapped potentialities as sources of vegetable oil.

- Omega-3 and omega-6 are essential polyunsaturated fatty acids (EFAs) which the body cannot produce in adequate amount and have to be obtained from other sources. The recommended ratio of omega-6 to omega-3 fatty acids in the diet is 4:1 or less.

- The primary function of EFAs is the production of prostaglandins which regulate various functions of the body like the immune system, vision, and cell structure.

- Reliable food sources for these EFAs include vegetable oils present in mayonnaise and salad dressings. Other ω-3 fatty acids like eicosapentaenoic acid and docosahexaenoic acid are also important for the immune system, cellular processes, and neural responses.

- The intake of more alpha-linolenic acid (omega-3) and less linoleic acid (omega-6) effectively produces more anti-inflammatory prostaglandins than inflammatory prostaglandins.

- Hence it has become a necessity to screen unexploited tree-borne oilseeds for production of polyunsaturated fatty acid-rich oils as the country is not producing sufficient quantities of PUFA-rich oils particularly enriched with alpha-linolenic, gamma-linolenic, eicosapentaenoic, and docosahexaenoic acids.

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Chapter 4

Nutrients for Money: The Relationship between Portion Size, Nutrient Density and Consumer Choices

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Abstract

Overweight and obesity are major risk factors for chronic disease and in the past 40–50 years portion sizes of offered foods, especially energy-dense, nutrient poor varieties, have dramatically increased along with global rates of overweight and obesity. Studies have shown that offering larger portion sizes result in increased food intake, known as the 'portion size effect'. This is likely due to consumption norms, the expected satiation and satiety of larger portions and the effect of unit bias. In addition, inconsistencies between serving sizes on nutrition information labelling compared to national dietary guidelines, makes it difficult for consumers to estimate and select appropriate portion sizes. Consumers find larger portion sizes more appealing due to their perceived value for money however, the nutritive value of the food is most often not acknowledged. Nutrient profiling models, which classify foods based on their nutrient density per unit cost may help consumers make healthier food choices. This narrative review aims to provide an overview of the portion size effect and discusses the application of nutrient profile score-based labels as a means of promoting nutrient density as value for money to influence consumer choices.

Keywords: energy density, nutrient density, portion size, portion size effect, serving size

1. Introduction

Globally, non-communicable diseases are the leading cause of mortality and morbidity, contributing to 73% of total deaths and 62% of disability adjusted-life years (DALYs) [1]. Overweight and obesity are a leading risk factor for the development of non-communicable diseases [2]. It is widely accepted that diet is a major contributor to an energy imbalance by which energy intake exceeds energy expenditure over an accumulative period of time, leading to the development of overweight and obesity [3]. Limiting the consumption of energy-dense, nutrient poor foods to manage energy intake is a strategy recommended for regulating body

weight and preventing non-communicable diseases [4, 5]. The energy density of food refers to the proportion of energy compared to the total mass weight (i.e. kilojoules per grams), which is influenced by the macronutrient and water content of the food [6].

Over the past 40–50 years, offered portion-sizes have significantly increased in food retail, restaurants and cookbook recipes [7–13]. Young et al. [7] reported that the portion size of energy-dense, nutrient-poor ready-to-eat foods exceeded government recommended standard serve sizes by up to 700% [14, 15]. This increase in offered portion sizes is driven by consumers seeking value for money. After taste, consumers regard price as the most important factor determining food choices. Larger portions appear more attractive by offering more food for a lower unit price [13]. From a producer's perspective, offering larger portions is therefore profitable. The cost of the extra food product is often negligible compared to the cost of the food packaging and offering a bigger unit may only slightly increase production costs. In addition, by offering a larger product, a producer can increase consumer satisfaction and is likely to have an advantage compared to a competitor offering smaller units. Therefore, in many settings, prices per gram are lower for large packages compared to small packages. This phenomenon is known as value size pricing.

Offering larger portions of foods to adults and children has been shown to increase the amount of food consumed and total energy intake [16, 17]. This relationship between offered portion size and amount of food consumed is known as the 'portion-size effect' [16–18]. Kling et al. [19] found that doubling the meal portion size offered to children aged 3–5 years increased energy intake by 24%. This study also found that increasing the energy density of the meal did not reduce amount of food consumed [19]. Therefore, serving larger portions of food, especially energy-dense, nutrient-poor varieties, in the long-term may be an important mediator for overweight and obesity and non-communicable diseases. The mechanisms underlying the portion size effect are unclear [20], however value for money has been identified as an incentive for consumers to choose larger portion sizes, which drives the marketing of larger packet sizes by food producers [13, 21]. Additional contributing factors such as appropriateness, unit bias, expected satiation and satiety, visual cues and bite size have also been identified and will be discussed later in this narrative review.

Consumers lack nutritional knowledge and skills to identify appropriate portion sizes and make healthy food choices [22, 23]. To overcome these barriers the European Commission proposed the concept of nutrition profiling, which categorises foods based on their nutritional composition [24]. Nutrient profiling has been used in a number of educational and regulatory strategies including translating nutrition information to consumers via front-of-pack labelling systems [25], identifying foods for re-formulation to improve nutrient density, directing food advertising to specific sub-populations, regulating where specific foods are distributed and informing tax policies of unhealthy foods [25, 26]. Nutrient profiling can also help consumers identify nutrient-dense foods for their unit price [27]. This application may help mitigate the portion size effect by shifting value to nutrients for money, rather than size for money [28].

The scope of this narrative review is to define the portion size effect, discuss the underlying mechanisms of the phenomena and identify the limitations of using a portion size approach when making food choices. This review will define nutrient profiling and its application for consumers, with a particular emphasis on the use of Nutrient Profile models in identifying nutrient-dense foods for their unit price.

2. Defining the 'Portion Size Effect': offered and consumed amounts of food

Understanding the definition of a 'portion size' and where the term sits in relation to other health terminology is a key challenge for consumers and food manufacturers [29, 30]. Definitions on what is considered a 'portion size' oscillate between the amount of food consumed at a single eating occasion and the amount of food served by an individual, food-outlet or manufacturer [31]. The distinction between a 'portion size', a 'serving size' and a 'serve' is also unclear, with the terms found to be used interchangeably on food labels to describe the recommended amount of product to eat [32]. For the purpose of clarity, this review will discuss 'portion size' as defined by Benton et al. [31] as the amount of food offered to consumers (of all ages) as well the amount of food selected and consumed. Portion size is then clearly distinguished from a 'serving size' which is defined as 'the amount (e.g. grams, millilitres) of a food or beverage item listed on the nutrition information label and specified in national dietary guidelines for consumers' [31].

Evidence indicates that serving larger portions increases the amount of food consumed in a specific meal and also subsequent energy intake [20]. This association has been termed the portion size effect [20]. Evidence indicates that offered food portion sizes can contribute to a difference in energy intake [33] however, the relationship is curvilinear. Doubling the amount of food offered can lead to a 35% increase in consumption but as portions continue to increase the portion size effect decreases [16]. This indicates that when conservative and excessive portion sizes of food are offered, additional factors such as physiological satiety cues and consumption norms may be stronger predictors for the amount of food consumed [16]. Contributing factors to the portion size effect will be discussed below.

2.1 Contributing factors to the portion size effect (PSE)

2.1.1 Appropriateness or consumption norms

The concept 'appropriateness' is a widely cited explanation for the portion size effect [13, 34]. This concept explains that portion sizes perceived as 'appropriate' or normal provide an important cue for determining how much food will be consumed [35, 36]. Lewis et al. [37] examined food portion sizes in relation to social and personal norms using 12 food computer-based images presented in 17 different portion sizes. Adults (aged 18–60 years) (n = 60) responded more or less to each image to indicate their portion size preference or perceived portion sizes of others [37]. Overall, this study found that portion sizes for personal norms exceeded social norms for most foods [37]. Personal norms for portion size were found to be significantly larger in obese individuals compared to lean individuals ($\beta = 0.076$, $p = 0.026$), especially in males ($\beta = 0.177$, $p < 0.001$) [37]. Personal norms were also larger for foods with a higher liking rating ($\beta = 0.142$, $p < 0.001$) [37]. Other studies have also confirmed that portion size norms are influenced by weight status and gender, as well as socio-demographics, childhood experiences and personal motivational factors including dietary restraint [38–40]. Further evidence suggests that individuals perceive a wide range of portion sizes related to a particular food to be the 'norm', which suggests that significant confusion exist around estimating appropriate portion sizes [23].

2.1.2 Unit bias

Herman et al. [36] suggested that the amount of food consumed may not only be influenced by the portion size, but also by the number of units or single servings

presented by food packaging (e.g. 1 can of soft drink, 1 packet of chips). Studies have shown that individuals consume smaller amounts when food is divided into several smaller units rather than fewer larger units [41]. Geier et al. [42] described these phenomena as 'unit bias'. Other factors may also drive the amount of food consumed including cost, availability and convenience of the food unit size [42].

2.1.3 Expected satiation and satiety

Expected satiation may also be an important determinant of the portion size selected [20]. Expected satiation is defined as the feeling of fullness that a food or meal is expected to provide immediately after consumption by an individual [20]. Expected satiety is influenced by learnt behaviours and macro-nutrient content of the food [43] and is directly related to food familiarity, whereby familiar foods are expected to be more filling [43]. Expected satiation also varied across food groups (e.g. vegetables, fruit, dairy) with energy-dense nutrient-poor foods being perceived to have a lower expected satiation ratio [43]. Foods with a lower expected satiation are often served in larger portions [44].

2.1.4 Visual cue

It has been suggested that visual cues such as dishware size, are used as a reference point for judging the amount of food to be consumed. Therefore, larger dishware might promote larger portion size selection and greater food consumption [45]. A meta-analysis (8 publications and 9 experiments) indicated there is some evidence to suggest that larger dishware is associated with greater food consumption, however, this relationship was not statistically significant ($p = 0.28$, 95% CI -0.35, -0.00) and a high level of heterogeneity was present across the studies [46]. Furthermore, the rim width of the plate may also impact on an individual's ability to estimate the portion size ($p < 0.01$) [47]. Currently, there is insufficient evidence to determine the impact of visual cues on portion size and food consumption.

2.1.5 Bite size

Emerging evidence suggests that larger portion sizes increases the amount of food consumed per bite [48–50]. It is hypothesised that larger bite sizes may result in reduced oral exposure time (i.e. an amount of food has less exposure time in the mouth) and less responsiveness to physiological satiety signals and therefore contribute to greater food consumption [51].

3. The ambiguity of nutrition labelling and serving sizes

Food product labelling provides consumers with nutritional information to help them make informed choices. A systematic review, including 36 studies showed that different types of food labels on packages influence consumed portion sizes with effects varying from increased to decreased intake (34).

Worldwide regulations for nutrition labelling on foods products differ considerably. In some countries (e.g. member states of the EU), nutrients listed on the nutrition label must be provided per 100 grams or millilitres, whereas other countries (e.g. US, Brazil) require the nutrient content per serving and some countries require both (e.g. New Zealand, Thailand) [52]. Furthermore, in some countries (e.g. US, Canada), standard serving sizes are defined for specific foods by regulatory bodies, whilst in others (e.g. Australia, New Zealand) food manufacturers define their own

serving sizes [52]. Evidence suggests that portion sizes are altered by food manufacturers to present a more favourable nutrition profile for their product, especially for 'unhealthy foods' that are energy-dense and nutrient-poor [53–55].

Some national dietary guidelines (e.g. Japan, Austria) specify standard serve sizes for specific foods within a food group on one eating occasion, as well as the total number of standard serves to be consumed per food group per day [56]. An important point of confusion for consumers is that the labelled serving size of packaged food can vary significantly to the standard serve sizes defined by national dietary guidelines [29]. For example, Yang et al. [57] analysed the nutrition labels of 4046 packaged foods in Australian supermarkets and found that only 24% adopted serving sizes that were similar with the standard serve sizes specified in the Australian Dietary Guidelines. Furthermore, Chan et al. [58] reported that at least 80% of Canadian packaged food (n = 1406) did not adopt the Canada's Food Guide Recommended Serving Sizes. These inconsistencies and confusing terminology prevent consumers from correctly interpreting nutrition labelling and making informed choices about appropriate portion sizes [57]. A systematic scoping review of studies conducted between 2010 and 2019 has found that consumers have a poor understanding of the labelled serving size [59]. Consumers frequently interpreted the labelled serving size as the recommended standard serve sizes specified within dietary guidelines for healthy eating rather than a typical consumption unit that is set by the manufacturer or other regulatory authority. A detailed discussion and review how consumers interpret the labelled serving size on food packages and how this information influences consumption behaviour was provided in the studies by Van der Horst et al. [59] and Bucher et al. [60].

Most national dietary guidelines do not provide standard serve size recommendations in weight or metric cups for 'unhealthy' energy-dense, nutrient-poor foods [61]. Furthermore, the definition of energy-dense, nutrient-poor foods is often ambiguous. For example, the Eat Well Guide, describes energy-dense, nutrient-poor foods, as foods 'high in' fat, salt and sugar without providing quantitative criteria [56]. Consequently, these factors prevent consumers from clearly distinguishing foods of high and low nutrient density as well as estimating appropriate portion sizes.

4. Nutrient profiling

Consumers often perceive larger portion sizes to be of greater value for money, without considering the nutritive value of the foods in relation to cost [62, 63]. In 1894, the nutrition scientist, Wilbur Atwater, was the pioneer for recognising the need to educate consumers about choosing cost-effective nutrient-dense foods and provided a legacy of studies which contributed to the development of nutrient profiling models [64].

Nutrient profiling is an emerging field of nutrition research that aims to classify foods based on their nutrient density using numerical scores or qualitative classifications [65, 66]. Nutrient profiling models calculate the energy and macro and micronutrient content per specified unit [67]. Nutrients typically chosen for nutrient profiling models include protein, dietary fibre, calcium, iron, vitamin A, C and D, which are defined as shortfall nutrients, while saturated fatty acids, total sugars and sodium are identified as nutrients to limit [67]. Foods which contain a higher proportion of shortfall nutrients compared to energy are defined as nutrient-dense, while foods that contain a higher proportion of nutrients to limit compared to energy, are defined as energy-dense, nutrient- poor foods [65].

Depending on the nutrient profiling model used, the nutrient content of a food may be expressed using standard units, which include per 100 g, 100 kcal or per

serve. The standard unit chosen for a model will affect the nutrient density classification [66]. For example, using the standard unit per 100Kcal for foods low in energy such as fruit and vegetables, may result in the nutrient density being classified as disproportionately high in relation to the amounts typically consumed [66]. Another challenge in the field is the validation of nutrient profiling models [65, 68]. A recent systematic review of 78 profiling models identified that only 58% had performed validity testing [67]. The World Health Organisation (WHO) has developed and tested a draft guideline which specifies a series of tests that should be completed for the validation of nutrient profiling models [69]. However, these guidelines are not yet publicly accessible.

4.1 Application of nutrient profiling for consumers

Nutrient profiling has a wide range of applications related to public health including both educational and regulatory strategies [65]. Nutrient profiling can been used to help consumers make healthier food choices by translating nutrition information via front-of-pack labelling systems on food packaging, supermarket shelf labels and through smart-phone applications [25]. Regulatory applications of nutrient profiling have been analysed by Raynor et al., [26] using the '4Ps' of Marketing Theory; Product, promotion, place and price of foods. In applying this theory, nutrient profiling can be used to; identify foods for re-formulation to improve the nutrient-density (product), direct food advertising to suitable sub-populations (promotion), regulate where specific foods are distributed (place) and taxation of unhealthy food (price) [26]. A systematic review indicated that the most common regulatory applications for nutrient profiling were for school food standards or guidelines (n = 27), food labelling (n = 12) and the regulation of food marketing to children (n = 10) [67]. More recently, nutrient profiling has been used as a criteria for the taxation of energy-dense, nutrient-poor foods [70]. For example, in Mexico an 8% taxation has been enforced for foods with an energy density of >1151 KJ (275 kcal)/100 g such as cakes, pies, cookies, chips and snacks [71]. Further development and analysis of these applications in the future will be important for optimising their impact on diet quality of consumers.

Research reports positive findings in regard to the effectiveness of nutrient profile scores for helping consumers make healthier food choices. As an example, the recent 5-year review of the Australian Health Star Rating reported that 70% of Australian consumers agreed that this voluntary front of pack nutrient profile logo helped them to identify healthier options within the same food category [72]. It was also found that two thirds reported that the label influenced purchasing decisions and that the label was driving product reformulation [72]. Furthermore, a randomised controlled trial of adults (n = 11,981) indicated that the use of the Five-Colour Nutrition Label enabled participants to choose foods of higher nutritional quality, including less saturated fat and sodium ($p < 0.05$) [73]. Although, significant challenges remain, nutrient profile scores could be used to promote the sales and consumption of healthier foods by consumer education and regulation. Nutrient Profiling Indices could also help identify foods that are both healthy and affordable [28, 63, 65]. Drewnowski et al. [28] demonstrated this by cross-referencing the Nutrient Rich Food Index with the US Department of Agriculture (USDA) nutrient composition and food prices data sets. The study demonstrated that foods could be characterised according to nutrients per dollar, helping consumers identify affordable, nutrient-dense foods [28], highlighting an area whereby nutrient profiling may contribute to the mitigation of the portion size effect by educating consumers on the nutritive value of foods and shifting preference for large portion sizes to high nutrient-density (**Figure 1**).

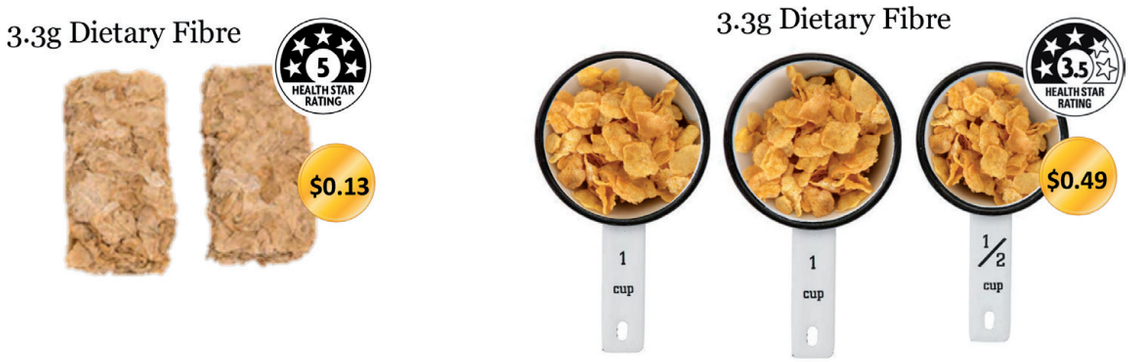

Figure 1.
Fibre for money. This figure visually represents the volume and cost of two different cereal types of providing 3.3 g dietary fibre each. It demonstrates that to match the amount of fibre in a single serve (30 g) of Weet-Bix, consumers must eat approximately 2.5 cups (82.5 g) of corn flakes to reach the equivalent amount of dietary fibre. This larger portion would cost consumers 3.7 times more, demonstrating the value in emphasising nutrients for money rather than volume for money.

5. Conclusion

It is clear that larger portion sizes contribute to greater food consumption and higher energy intake, known as the portion size effect. However, consumers have difficulty in identifying appropriate portion sizes due to inconsistencies between the serving sizes of packaged foods compared to standard serving sizes defined by national dietary guidelines. In addition, consumers find larger portion sizes more appealing due to greater perceived value for money but often do not consider the nutritive value of the food. Pricing strategies were suggested to be an innovative way to counteract the portion size effect [21]. However, experimental research suggests that equalising unit prices alone may not be sufficient to counteract the effect of larger offered portions [74].

Nutrition profiling has been implemented for public health initiatives including food labelling, food standards and guidelines and the regulation of food marketing. Front of pack labels that are based on nutrient profile scores such as the Health Star Rating help consumers to identify healthier foods. However, these labels could be developed further to better assist consumers in identifying foods of high nutrient-density per unit cost. Further development of food labelling, consumer education and public health efforts are needed to promote nutrient density as the value for money, which should be driving product development. Specifically, future research is needed to evaluate the long-term impact of nutrient profile scores in real-life contexts (e.g. purchasing behaviour in supermarket) rather a controlled laboratory setting. The ability of nutrient profiling initiatives to effectively communicate nutrition messages to different target groups warrants further investigation. This body of evidence will be important for informing global industry reformulation and food policy development, which has the greatest potential to impact on consumer food choices and dietary intake.

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Chapter 5

Recombinant Probiotics and Microbiota Modulation as a Good Therapy for Diseases Related to the GIT

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Abstract

Many diseases that affect the gastrointestinal tract (GIT) have great influence on the quality of life of the majority of patients. Many probiotic strains are being highly studied as a promising candidate due to their beneficial effect reported in the GIT. With the purpose of increasing the beneficial characteristics of some probiotics strains and, consequently, to improve further the reported results, many probiotic strains expressing or encoding different proteins, with anti-inflammatory activities, have been developed. These recombinant strains have been reported as good candidates for the treatment of different pathological conditions, especially colitis and mucositis disease since they have been shown to have positive results and good perspectives for GIT inflammation. Thus, this chapter will first address the aspects of the gastrointestinal tract in humans as well as its microbiota. In a second moment, it will discuss about chronic diseases, mainly the intestinal ones. Finally, it will discuss about probiotics, especially concerning on lactic acid bacteria (LAB), and its action in the prevention and treatment of these diseases. At the final part, we will point out aspects on the development of recombinant strains and the results found in the literature on disease models.

Keywords: *L. lactis*, *Lactobacillus*, DNA vaccine, heterologous protein

1. The human gastrointestinal tract

The human gastrointestinal tract is formed by a complex ecosystem which includes the gastrointestinal epithelium, immune cells, and resident microbiota [1] and comprehends one of the biggest existent interfaces between the host, environmental factors, and antigens in the human body.

The intestine encompasses a broad variety of microorganisms (bacteria, archaea, eukarya, and viruses) [2] from more than 3500 different species [3, 4] that coevolved with the host in a mutually beneficial relationship [5, 6]. The composition and density of bacterial populations in adult individuals differ considerably over the GIT. The area

of the GIT that has highest microorganism abundance is the colon (10^{14}) followed by dental plaque (10^{12}), ileum (10^{11}), saliva (10^{11}), and skin (10^{11}) [7]. However, low concentrations (up to 10^2–10^7 cells/mL) and bacterial diversity are found in the upper GIT (stomach, duodenum, jejunum) [3, 4], since the presence of acid, bile salts, and pancreatic secretions hinders the bacterial colonization [8], so that there is no nutritional competition between the microbiota and the host [9]. Thus, both function and structure of microbial communities are significant and are closely related. However, function could be the more important measure of microbiome health, since bacterial ecology suggests that analogous ecosystems have similar function although they have moderately diverse composition [10, 11].

2. Gut microbiota

The importance and the specific functions that gut microbiota has in human nutrition and health are well settled. The attributed functions can be classified in three classes: metabolic, protective, and trophic [12]. The gene diversity of the microbial community provides a variety of enzymes and biochemical pathways, specific to the host, able to contribute to short-chain fatty acid (SCFA) production by carbohydrate fermentation and production of some vitamins such as K, B12, biotin, folic acid, and pantothenate. These factors added to synthesis of amino acids from ammonia or urea contributing to the metabolic function of the microbiota [13, 14].

The gut microbiota's protective function is related to barrier effect, once the resident bacteria generate a resistance line which avoid pathogens/opportunistic bacteria and maintain normal mucosal function. The activity of some bacteria to secrete antimicrobial substances, such as bacteriocins, is able to inhibit the growth of other bacteria and nutrient competition [15, 16].

Regarding trophic functions of gut microbiota, the interaction between resident microorganisms has influence in differentiation and proliferation of epithelial cells [17], as well as in the development and regulation of the immune system by numerous and varied interactions between microbes, epithelium, and gut lymphoid tissues [18].

It is important to highlight that the interactions between the gut microbiota and the host immune system are required to preserve the gut homeostasis [19–21]. When this relationship is affected, alterations in bacterial function and diversity lead to the imbalance in the composition of the resident microbiota, favoring either the growing of pathogenic bacteria or the decreasing in beneficial bacteria in a process known as dysbiosis [22], which appoint a great threat to gut integrity and is intrinsically related to the development and progression of several diseases, such as inflammatory bowel diseases.

3. Chronic inflammatory diseases

One of the most well-characterized chronic inflammatory diseases that mainly affect the digestive tract is inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD). The exact etiology of IBD is still unclear, but the strict relation between genetic and the environmental factors, such as enteric immune dysregulation and alterations in the intestinal microbiome [23, 24], is broadly known. Besides, these diseases generate substantial morbidity and have a high prevalence in developed countries (5 in 1000 individual are affected) they remain to increase in developing nations [25].

Both diseases, UC and CD, present different pathogenesis, symptomatology, inflammatory profiles, and gut microbiota composition. CD is characterized by the irregular transmural inflammation (extending deeply into the submucosal regions) which can

affect any portion of the GIT and often made difficult by strictures, abscesses, and fistulae. On the other hand, the inflammation presented in UC is restricted to the superficial layers of the intestinal mucosa characterized by mucosa erosion and/or ulcer, generally localized in the region of the gut most colonized by bacteria, the colon [26, 27]. In addition, regarding the immune response associated with these diseases, it is possible to relate CD with an increased IL-12, IL-23, IL-27, interferon γ (IFN-γ), and tumor necrosis factor-α (TNF-α) production, all associated with Th1 and Th17 immune responses, different from UC which is correlated with a Th2 immune response, with high levels of IL-5 and transforming growth factor-β (TGF-β) production [28].

4. IBD complications and microbiota manipulation

It is important to highlight that the principal cause of death in IBD patients is colorectal cancer (CRC) [29]. Frequent episodes of inflammatory process in the intestinal mucosa are related to development of this disease, which is the second most frequently identified cancer in females and the third in males.

There are increased evidences that environmental factors such as lifestyle and diet alterations have effect in CRC incidence [30]. This effect has been documented because there is evidence showing an essential relationship between dietary antigens and antigens of commensal bacteria with the regulatory T cells (Tregs), which maintain the immune tolerance and, consequently, reduce the risk of tumorigenesis associated with inflammation [31].

In this context, it was reported that the higher consumption of diet rich in grains and vegetables decreases the incidence of CRC. This effect involves different mechanisms such as the diminution in the fecal transit time due to the increase in the stool bulk, and consequently, it reduces the contact of carcinogen with colon cells and the fermentation of these fibers of colonic components [14, 32]. In addition, significant reduction in concentration of acetate, propionate, and butyrate with increase in fecal pH [33] and the decrease in the number of obligate anaerobe microorganisms have been reported in individuals with colon cancer [34] when compared with healthy people. Thus, intestinal environmental alterations are the keys to evolution toward adenoma and afterward to CRC progression [35].

It has been also reported that up to 30% of patients with UC need surgical management such as the restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) [36]. This procedure removes the entire colon and rectum while preserving the anal sphincter and, hence, normal bowel function and fecal continence, therefore acting as an internal pelvic place for intestinal contents [37]. Around 50–60% of UC patients with following IPAA develop inflammation in the ileal pouch, generating the condition called "pouchitis." The reported incidence of pouchitis is variable, generally because of the diagnostic criteria that have been used to define this syndrome [38, 39]. In addition, although its pathogenesis is uncertain, the main hypothesis for the mechanism by which the disease occurs is the break in the mucosal barrier generated by dysbiotic microbiome in susceptible patients, generating an unusual mucosal immune activation [40]; still the disease typically responds to antibiotics.

Corresponding to the increased attention given to the role of the intestinal microbiota in a variety of diseases, there has been an intense exploration of potential means to manipulate the intestinal microbiome either by probiotic administration or fecal microbiota transplant (FMT) for therapeutic effect [41].

In this context, a randomized clinical trial based on a 1-week treatment with anaerobically prepared donor FMT, compared with autologous FMT, resulted in a higher probability of remission in 8 weeks for patients with UC, revealing that stool administration from healthy donors to UC or CD patients is an intervention that seeks to restore a healthier balance of gut microbes and control IBD [42]. Data on FMT for

Crohn's disease is rather more limited than for UC, but it has been shown that single standardized FMT resulted in a clinical remission sustained for more than 9 months in CD patients [43]. However, the authors suggest that further studies are needed to enhance the knowledge about the use of stool transplantation for IBD treatment.

Alteration in the gut microbiome composition with increase in some groups of microorganisms, such as *Clostridium* and *Fusobacterium*, was also reported in patients with pouchitis [44, 45]. In this context, literature evidences indicate that the probiotic administration such as VSL#3 is effective in the chronic pouchitis prevention [46]. On the other hand, FMT to pouchitis treatment did not report the same beneficial results. Only three reports with this approach [47–49] exposed that neither clinical remission nor any adequate response was observed in the evaluated patients suggesting that the efficacy of FMT for pouchitis after proctocolectomy is limited [49]. The importance of standardization of this procedure needs to be highlighted to improve its efficacy, since frequency, route of administration (e.g., endoscopy, nasogastric tube, colonoscopy), and the criteria of choice of healthy donor are very important parameters to be considered.

5. Intestinal mucositis

Different chemotherapy regimens such as FOLFOX (5-fluorouracil and oxaliplatin), FOLFIRI (5-fluorouracil and irinotecan), and triple FOLFOXIRI regimen (5-fluorouracil, oxaliplatin, and irinotecan) [50, 51] are adopted for different types of cancer but with a broad range of collateral effects.

Mucositis is the most common side effect in patients undergoing chemotherapy/radiotherapy treatments, which consist in an inflammation and/or ulcers in the gastrointestinal tract [52] with consequent loss of cells from the epithelial barrier of the GIT. Many symptoms are related to gastrointestinal mucositis, such as diarrhea, severe abdominal pain, bleeding, fatigue, malnutrition, dehydration, electrolyte imbalance, and infections, with potential fatal complications which can conduce to reduction or interruption of antitumor treatment [53] and consequently leads to longer hospitalization.

This pathology occurs due to cytotoxic effects of anticancer drugs/radiotherapy that cause damage at the DNA of stem cell (epithelial cell progenitors) with intense oxidative stress and consequent cell death. This apoptotic process is exacerbated affecting the absorption by shortening the villi structure of enterocytes and causing the loss of epithelial barrier with an invasion of inflammatory cells (neutrophils, eosinophils, and macrophages) leading to an increased production of inflammatory mediators at the mucosal area with consequent epithelial erosion and ulceration. The progressive destruction of mucosal integrity causes the rupture of the *tight junctions* proteins, leading to an increase in the intestinal permeability with subsequent penetration of commensal microbiota to the submucosal layer generating bacteria translocation which exacerbates the inflammatory process and intensifies the symptoms [53–57]. Besides, the intestinal microbiota composition is also modified by the chemotherapeutic drugs and radiotherapy action [54, 58, 59] resulting in dysbiosis. After the end of treatment, recovery and restoration of the GIT structure occur [60].

6. Metabolic syndrome

Besides IBD and mucositis, it has been reported that intestinal microbiota has an intrinsic effect on metabolism, potentially contributing to several features of the pathophysiology of metabolic syndrome [61, 62]. The metabolic syndrome is an accumulation of various risk factors (glucose intolerance, hyperinsulinemia,

hypertension, as well as dyslipidemia) which can often be associated with insulin resistance, hypertension with abdominal fat accumulation, and obesity [63–65].

The etiology of metabolic syndrome is not well-defined; however there are evident characteristics and life habits that could contribute to its development such as unbalanced diet, smoking, lack of physical activity, and the genetic predisposition [66]. These factors directly increase the risk of cardiovascular disease and chronic diseases as type 2 diabetes mellitus and obesity, and the interaction between components of both the clinical and biological phenotypes of the syndrome contributes to the development of a pro-inflammatory state [67].

The inflammatory process observed in MS is directly associated with increased oxidative stress. The reactive oxygen species (ROS) are capable of mediating symptoms of diabetes mellitus, such as insulin resistance and decrease in insulin secretion, and attend as precursors for the formation of LDLox (oxidized low-density lipoproteins), responsible for a large part of the development of atherosclerotic lesions, and the increase in circulating cholesterol fractions and glucose [68, 69]. In addition, chronic diseases are directly related to changes in the intestinal microbiome [70, 71], and they are also associated with elevated circulating levels of pro-inflammatory cytokines such as TNF and IL-6 [72].

The probiotic use in attenuating symptoms of different inflammatory diseases is widely reported in the literature. Among the commercial probiotics studied for treatment of these diseases, only a few products have been extensively tested in clinical trials in patients with MS, in order to demonstrate an effective result on weight loss, lipid metabolism, and reduction of inflammatory markers.

Studies performed with *Lactobacillus* strains have shown the ability of these probiotics in reducing the lipid accumulation in adipose tissues, as well as in inducing the subexpression of lipogenic genes [73, 74]. Animals that received diets with high concentrations of lipids and then treated with *L. gasseri* SBT2050 had shown lower intestinal permeability and bacterial translocation, as well as reduction of inflammatory parameters, suggesting that this strain improves the intestinal barrier function [75–78]. In addition, *L gasseri* BRN17 was studied to treat animals with MS caused by the carbohydrate-rich diet consumption. This strain reduced the accumulation of adipose tissue in mice, and it has a beneficial effect on weight loss [79–81]. Another important approach with associated probiotics (*Bifidobacterium*, *Lactobacillus*, and *S. thermophilus*) for treatment of overweight patients has shown an improvement in lipid profile, as well as insulin sensitivity [82]. Besides, recently Hsieh e collaborators [83] demonstrated that administration of live *Lactobacillus reuteri* ADR-1 and killed *Lactobacillus reuteri* ADR-3 strain ameliorated type 2 diabetes mellitus in a clinical trial. The results indicated that the consumption of ADR-1 displayed a reduction effect on serum glycated hemoglobin (HbA1c), triglyceride, and cholesterol levels. On the other hand, the intake of ADR-3 showed a beneficial effect on blood pressure reduction. Besides, a reduction in the levels of pro-inflammatory cytokines (IL-1β), increase in antioxidant enzyme (superoxide dismutase), and the changes in intestinal microflora composition (increase in intestinal level of *Lactobacillus* spp. and *Bifidobacterium* spp. and decrease in *Bacteroidetes*) were observed. Thus, these strategies highlight the beneficial and potential effect of interventions targeting gut microbiota modulation by the use of probiotic strains to treat components or complications of metabolic syndrome.

7. Functional foods

The human being for more than 4000 years has been consuming fermented products, by the fermentation process. At the beginning this practice was done

to preserve foods from either physical, chemical, or microbial alterations. The microorganisms participating in this process are the lactic acid bacteria, extensively widespread in nature and also belong to the GIT communities, able to convert the sugar in lactic acid as well as produce other metabolites which contribute to food modifications, either sensorial or nutritional value. Thus, the terminology "functional food" was attributed to food with health benefits to the consumer including nutritional and physiological function [84–86].

During the fermentation, these bacteria can contribute to improving the digestion of nutrients (lactose, proteins, small peptides, and polysaccharides); providing essential micronutrients (vitamins) as well as bioactive compounds (metabolites) with potential health benefits to the host, such as prevention against enteric inflammation [87, 88]; providing antimicrobial, antihypertensive, hypocholesterolemic, immunomodulatory, antioxidant, and anticancer effects [46, 85, 89–92]; showing ability to regulate the immunity; and, consequently, improving host quality of life [93].

Therefore, the gut communities and the microbial-derived molecules present in the gut lumen have been strongly influenced, either qualitatively or quantitatively, by consumption of dairy products [94] such as yogurts, cheeses, and fermented milk, among other fermented products using probiotic bacteria. Thus, the microbiota manipulation by functional food, probiotics, and prebiotics are evaluated as a beneficial option for treatment of GIT diseases [95].

8. Lactic acid bacteria: the largest group of probiotic bacteria

There is a constant interaction between the host and the bowel commensal bacterial community in order to maintain the homeostasis [3, 96–98]. However, when this mutualist relationship is compromised, the intestinal microbiota may cause and/or contribute to either the establishment or the progression of inflammatory diseases [96–99]. In this context, the search for therapeutic strategies that minimize the development and progression of pathologies caused directly and indirectly by the unbalance of the commensal microbiota has grown. The consumption of probiotic bacteria is one of these strategies, as they present several effects, such as ability to improve the intestinal barrier, stimulate the systemic and mucosal immune system, regulate the composition of the intestinal microbiota, and provide essential micronutrients (such as vitamins and SCFAs) and other bioactive compounds (metabolites) with potential health benefits for the host [100–103].

Probiotics are defined as "live microorganisms that offer host health benefits when administered in adequate amounts" [104, 105]. The majority of the studied probiotics belongs to the group of lactic acid bacteria. However, other microorganisms with probiotic properties also deserve attention, such as yeasts (*Saccharomyces* spp.) and bacteria of the genus *Bifidobacterium* and *Faecalibacterium*, among others [106–108].

LAB, which include, mainly, species from the genus *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Streptococcus*, constitute a group of Gram-positive, anaerobic or aerotolerant, nonspore-forming, nonmobile, and highly low pH-tolerant microorganisms. However, the main characteristic of this group is its ability to produce lactic acid as the final product of the fermentation of carbohydrates [109–111].

9. Probiotic effects in gastrointestinal inflammation

LAB are often present in the human gut but also can be introduced by the ingestion of fermented foods, such as yogurt and other fermented milk products and fermented cured meat by-products [103], having the generally recognized as

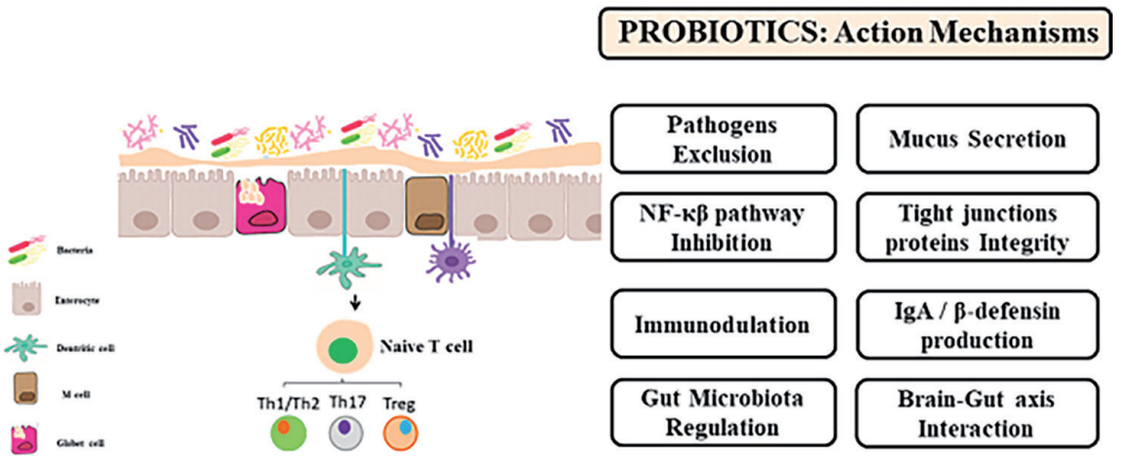

Figure 1.
A schematic diagram about potential action mechanisms of probiotic bacteria.

safe (GRAS) status by the Food and Drug Administration (FDA). *Lactobacillus* spp., *Streptococcus* spp., and *Lactococcus* spp. are the major LAB species with probiotic effects, and they have been used in therapeutic applications for treatment and prevention of various intestinal disorders [112, 113].

Scientific evidence reveals that the mechanisms by which probiotic bacteria ameliorate inflammatory bowel damage are heterogeneous, strain specific, and dependent on the number of available bacteria. Thus, administration of probiotic bacteria, specially LAB, improves intestinal inflammatory responses by (i) modulation and normalization of perturbed intestinal microbial communities; (ii) competitive exclusion of pathogens such as *Staphylococcus aureus* and *Salmonella typhimurium*, among others; (iii) bacteriocin and SCFA production; (iv) enzymatic activities related to metabolization of a number of carcinogens and other toxic substances; (v) adhesion to mucosal cells, cell antagonism, and mucin production; (vi) intestinal permeability reduction by tight junctions protein modulation (e.g., zonulin, claudin, occludin, junctional adhesion molecule); (vii) modulation of the immune system by stimulating Tregs cells, IgA production by B cells, and NF-kβ signaling pathway inhibition; and (viii) interaction with the brain-gut axis via the generation of bacterial metabolites (**Figure 1**) [103, 114–118].

10. Recombinant LAB probiotics

In order to potentialize the beneficial effects of probiotic strains, research has been conducted over the last decades, based on genetic engineering techniques, especially those related to DNA manipulation. Thus, modern methods of genetic engineering open the new opportunities to design and create genetically modified probiotic strains with the desired characteristics or to exclusively target a specific pathogen or toxin to be used either as a vaccine or for drug delivery [119, 120]. Since most of the probiotic strains are part of the LAB group, most of the genetic manipulation studies are carried out with species that belong to this group, such as *Lactococcus* and *Lactobacillus* genera. Consequently, recombinant probiotics have been created for mucosal delivery of therapeutic and/or prophylactic molecules comprising DNA, peptides, single-chain variable fragments, cytokines, enzymes, and allergens [121, 122], leading to the concept of "biodrug" for the prevention and treatment of various diseases [123]. Thus, researches have emphasized the use of species of these genera in two different approaches: the first as producers of heterologous protein and the second as vehicle for delivery of DNA vaccines [124].

10.1 LAB as producers of heterologous protein

Many studies are carried out with *Lactococcus lactis* due to its economic importance in the production of cheese and its easy growth and manipulation. In addition, it was the first species of LAB to have its genome completely sequenced, which allowed a greater understanding of its genetic and physiological mechanisms, aiding in the development of technological packages for its genetic manipulation in a laboratory environment [124–128].

There are several ways to make LAB produce heterologous proteins, and the most used form is through the insertion of a plasmid into its cytoplasm. Plasmids are elements of extrachromosomal DNA that are naturally found in prokaryotes. With the advent of the recombinant DNA technique, these elements have been manipulated to act as molecular vehicles that allow the production of proteins of interest by the bacterium [129].

The first heterologous protein production system based on plasmid insertion in LAB was developed for *L. lactis.* These systems included both inducible and constitutive promoters, which ensure efficient expression of the antigen of interest under different conditions [130, 131]. Although it is possible to choose the type of promoter to be used in the vector, the vast majority of expression vectors present inducible promoters that allow controlled expression of the protein of interest by protecting against aggregation and protein degradation in the bacterial cytoplasm. On the other hand, these vectors present safety issues that need to be analyzed since it is necessary to introduce chemical compounds into the culture medium to induce protein expression prior to animal administration [132–134].

With the improvement of cloning and expression techniques, several production systems were developed, specifically for LAB, allowing the production of different molecules of interest, including pathogen antigens, by a large number of LAB species [135–139]. The most commonly used regulation systems in LAB are the following:

10.1.1 Nisin-controlled gene expression (NICE)

Among the heterologous production systems, the most widely studied is the nisin-controlled gene expression system. This system is based on the expression of three genes (nisA, nisF, and nisR) that are involved in the production and regulation of the antimicrobial peptide nisin, which is naturally secreted by different strains of *L. lactis*. In this system the membrane-located histidine kinase NisK senses the signal inducer nisin and autophosphorylates and then transfers the phosphorous group to the intracellular response regulator protein NisR which acts as a transcription activator of nisA/nisF and induces gene expression under pNis promoter. Depending on the presence or absence of the corresponding targeting signals, the protein is either expressed into the cytoplasm or the cell envelope or secreted into the external medium [140]. Thus, it has already been successfully used for the expression of different proteins of medical and biotechnological interest [141, 142].

10.1.2 Xylose-inducible expression system (XIES)

In 2004, Miyoshi and colleagues [143] developed the xylose-inducible expression system whose promoter is the xylose permease gene (pxylT) found in *L. lactis* NCDO2118. This system produces either cytoplasmic or secreted proteins being activated in the presence of xylose and strongly repressed in the presence of glucose, fructose, or mannose [143].

10.1.3 Stress-inducible controlled expression system (SICE)

More recently, the stress-inducible controlled expression system was developed using the *L. lactis* groESL promoter [134]. This system induces expression of proteins of interest via stress stimuli such as those found in the GIT (e.g., bile salt, acid pH, antimicrobial peptide, and heat shock proteins) [134, 144]. This system does not require the induction of bacterial culture or the presence of regulatory genes, being a good alternative in the delivery and production of therapeutic proteins at mucosal surfaces.

10.2 LAB as a live vehicle to deliver DNA vaccine plasmids to eukaryotic cells

Among the available approaches to stimulate efficient mucosal responses, the use of bacterial system for DNA delivery and its expression using the eukaryotic cell machinery have been extensively explored. Unlike the production of heterologous protein, in which the bacterium is responsible for the synthesis of the protein of interest, in the DNA vaccine platform, the bacteria only act as a delivery vehicle for prophylactic and therapeutic purposes [109, 145].

New vectors had been developed to approach the DNA vaccine using LAB as live delivery vehicles [146, 147, 148–150]. These vectors present a series of common characteristics such as the presence of a eukaryotic promoter, which allows protein expression by eukaryotic cells; a prokaryotic region, which has a selection marker (usually antibiotic resistance); a multiple cloning site, where the open reading frame (ORF) of interest will be inserted; and a prokaryotic origin of replication, which ensures that the plasmid replicates only in prokaryotic cells [151]. Some molecules (IL-10, IL-4, and HSP65) have been cloned in these vectors to evaluate their effect, especially as a treatment approach in diseases related to the bowel [152, 153], as well as reporters (GFP and Cherry) which allowed the understanding of this platform in the mammalian body [148, 154]. Although further studies need to be conducted in order to elucidate whether the cloning of ORFs of interest in these vectors is really effective pointing to disease prevention and treatment, this approach is undoubtedly an important tool for the development of new techniques with potential in the medical clinic.

11. Next-generation recombinants: using CRISPR-Cas system

Among the different techniques used to construct recombinant LAB strains, the most recent is associated with the use of the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system, based on the use of a system present in several bacterial strains that works as part of the adaptive immune system of bacteria and archaea against the presence of external DNA, such as plasmids and bacteriophages [155–159].

Although this system has been studied for more than 30 years [160], it was only in 2013 that the first experiments were carried out emphasizing its use as a tool for genome editing [161, 162]. Evaluating the CRISPR databases, it is possible to observe that about 46% of all bacterial genomes presents the CRISPR-Cas system, and this percentage reaches approximately 63% of the sequenced *Lactobacillus* genomes [163]. The natural presence of this system in most of the LAB strains expands the possibilities of genetic manipulation of microorganisms of this group, including probiotic ones [164].

The first gene editing experiment in LAB based on the CRISPR-Cas system was conducted by Oh and van Pijkeren [165] where they were able to edit three different

regions of the genome, with efficiency up to 100% in the selected clones. After this pioneering work, few others were published focusing on LAB gene editing [166–168].

Therefore, the use of this technology is presented as a widely viable strategy to be applied in LAB, enabling the development of food-grade recombinant strains in order to allow their future use in the clinic [169].

12. Use of recombinant LAB to treat GIT-related disorders

The use of recombinant *L. lactis* strains, as well as others recombinant LAB strains, using different systems has shown promising results in many studies as an alternative therapy to treat, especially, GIT inflammation and other diseases (**Table 1**).

To arrive at mucosa in sufficient quantities to exert their therapeutic effects, many LAB strains must survive, during their passage through the GIT, stressor factors such as pH, temperature, bile salt concentration, and the presence of antimicrobial peptides [170–172]. In this context, an interest approach was recently developed by Coelho-Rocha and colleagues [154] using an encapsulated recombinant strain (*L. lactis* pExu:*mcherry*) and tested it through the GIT at different times post-administration. They have shown that the microencapsulation process is an effective method to improve DNA delivery, guaranteeing a greater number of viable bacteria able to reach different sections of the bowel [154].

The use of recombinant probiotics to improve therapeutic approaches has been widely studied using different systems with different molecules. As IBDs are a serious clinical topic, many strategies have been tested trying to improve previous results found with wild type strains.

L. lactis MG1363 strain carrying the pTREX1 vector expressing the mouse IL-27 protected mice against the inflammatory effects of dextran sulfate sodium (DSS)-induced colitis. This recombinant strain was able to reduce disease activity scores and pathology features of the large and small bowels and also led to reduced levels of inflammatory cytokines IL-1β, TNF-α, and IFN-γ in colonic tissue. In addition, reduction in the number of $CD4^+$ and $IL\text{-}17^+$ T cells in gut-associated lymphoid tissue and increase in IL-10 production were observed [173].

Besides, it was also demonstrated in a DSS-induced colitis mouse model that the oral administration of *L. lactis* NZ900 strain harboring the NICE system expressing either the anti-inflammatory cytokine IL-10, TGF-β1, secretory leukocyte protease inhibitor (SLPI), or elafin was able to ameliorate some clinical parameters in inflamed mice. Even though it was possible to observe the reduction of weight loss and diarrhea, microscopic colonic damage scores, colon thickness, and myeloperoxidase (MPO) activity, the authors reported that treatments with recombinant *L. lactis* strain delivering either SLPI or elafin were more efficient to reduce signs of colitis than treatments with anti-inflammatory cytokines. Altogether these recombinant strains display anti-inflammatory effects in inflamed mice [174].

Approaches using the invasive *L. lactis* MG1363 $FnBPA^+$, by expressing the FnBpA protein at their surface and carrying the pValac eukaryotic expression vector coding either the IL-10 cytokine [r*L. lactis* $FnPBA^+$ (pValac:*il-10*)] or the IL-4 cytokine [r*L. lactis* $FnPBA^+$ (pValac:*il-4*)] in DSS or trinitrobenzenesulfonic acid (TNBS)-induced acute model of colitis, respectively, were also investigated. The administration of *L. lactis* $FnPBA^+$ (pValac:*il-10*) recombinant strain was capable to reduce the intestinal inflammation by increasing IL-10 levels and sIgA production, accompanied by decreasing IL-6, as well as the restoration of intestinal architecture of mice colon [153]. Besides, the engineered *L. lactis* $FnPBA^+$ (pValac:*il-4*) was able to slump the level of pro-inflammatory cytokine (IL-12, IL-6) and myeloperoxidase activity and increase levels of IL-4 and IL-10, consequently decreasing the colitis harshness [153].

Microorganism	Gene	Expression System	Inflamation Condition	Anti-Inflamatory Properties	References
L. lactis MG1363	Mouse IL-10	SICE	Mouse model of DNBS-induced colitis	Restoration of intestinal architecture; IgA production and IL-6 reduction; Reduced tissue damage	[134]
L. lactis MG1363	Mouse IL-10 and IL-4	pValac vector	Mouse model of DSS/TNBS-induced colitis	Decreased IL-6, IL-12 and MPO activity Reduced tissue damage	[152-153]
L. lactis NZ9000	Mouse TGF-β1; IL-10 and leukocyte protease inhibitor Human Elafin	NICE	Mouse model of DSS-induced colitis	Reduced tissue damage Decreased pro-inflammatory cytokines	[174]
L. lactis NCDO 2118	Human 15-lipoxygenase-1	XIES	Mouse model of DSS-induced colitis	Reduced tissue damage	[175]
L. lactis NCDO 2118	*M. leprae* Hsp65 protein	XIES	Mouse model of DSS-induced colitis	Restoration of intestinal architecture CD4+Foxp3+ and CD4+LAP+ regulatory T cells production	[176]
B. bifidum BS42	Mouse IL-10	BEST	Mouse model of DNBS-induced colitis	Reduced tissue damage	[177]
L. casei BL23	Superoxide dismutase A from *L.lactis* MG1363 Catalase from *L.plantarum* ATCC	pLEM415 vector	Mouse model of TNBS-induced Crohn´s disease	Reduced tissue damage Reduced microbial translocation Increase IL-10/ INF-γ reduction	[180]
S. thermophilus CLR807	Superoxide dismutase A from *L.lactis* MG1363 Catalase from *L.plantarum* ATCC	pIL253 vector	Mouse model of TNBS-induced colitis	Reduced tissue damage Reduced microbial translocation IL-17 reduction	[181]
L.lactis AG013	Human Trefoil Factor 1 (Htff-1)	ThyA native promoter of *L.lactis*	Hamster model of radiation-induced oral mucositis	Reduced clicnical scores of oral mucositis	[186]
L. lactis NZ9000	Human pancreatitis associated protein (Reg3A)	NICE	Mouse model of 5-FU-induced intestinal mucositis	Microbiota Regulation Villus architecture preservation Increased Paneth cells activity	[185, 187]

Microorganism	Gene	Expression System	Inflamation Condition	Anti-Inflamatory Properties	References
L.lactis NCDO2118	*M. leprae* Hsp65 protein	XIES	Mice model of experimental encephalomyelitis	Increased CD4+Foxp3+ regulatory T cells Reduced encephalytogenic CD4+ T cells	[184]
L.lactis MG1363	Mouse IL-17	SICE	Mice model HPV-induced cancer	Reduced tumor size Induced IL-6 and IL-17 secretion	[182]
L.lactis NZ9000	*M. leprae* Hsp65 protein and peptide derived of human Hsp60 protein	NICE	Mice model of diabetes type 1	Reduction of insulitis Inhibition of T cell proliferation	[183]

Table 1.
Protein with anti-inflammatory properties produced in different strains of bacteria.

The human 15-lipoxygenase-1-producing *L. lactis* NCDO2118 harboring the xylose-inducible expression system (pXylt:CYT:*15-LOX-1*) was also effective in attenuating the symptoms of DSS-induced colitis in a murine model [175]. Its oral administration improved the body weight, decreased pro-inflammatory cytokines (IFN-γ and IL-4) while increasing the anti-inflammatory cytokine IL-10, and, consequently, ameliorated the macroscopic damage scores associated with the inflammation.

The oral pretreatment with genetically modified *L. lactis* NCDO2118 able to secrete HSP65 protein from *Mycobacterium leprae*, using XIES system (pXylt:SEC:*hsp65*), prevented DSS-induced colitis in C57BL/6 mice [176]. This protection was associated with reduced pro-inflammatory cytokines, such as IFN-γ, IL-6, and TNF-α; it also increased IL-10 production in colonic tissue and expansion of $CD4^+FoxP3^+$ and $CD4^+$ latency-associated peptide (LAP^+) regulatory T cells in the spleen and mesenteric lymph nodes. Besides, the authors showed that this effect was dependent on IL-10 and toll-like receptor 2 (TLR-2) [176].

Although *L. lactis* represents an excellent candidate for a live mucosal vector delivery system, other bacteria have also been explored as promising live vehicles for molecule expression with therapeutic properties, such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*. In this context, Mauras et al. [177] using the new *Bifidobacteria* Expression SysTem (BEST) allowing the production of IL-10 in *Bifidobacterium bifidum* BS42(pBESTExp4:*il-10* and pBESTBL1181:*il-10*) demonstrated that the use of these recombinant strains in a DNBS-induced colitis model showed its ability to decrease local inflammation and confirmed therefore its potential for delivery of therapeutic molecules in the colon.

It is well known that IBD is associated with oxidative stress by the increase in concentration of reactive oxygen species in the GIT and impaired antioxidant defenses [178, 179]. In this context, it has been shown that some probiotic LAB strains may play a protective role in IBD by expressing antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [180, 181].

LeBlanc et al. and Del Carmen et al. [180, 181] showed, respectively, that *L. casei* BL23 and *S. thermophilus* CRL807 transformed with two different plasmids (pLEM415:*mnkat*; pLEM415:*sodA*) (pIL253:*sodA* and pIL253:*mnkat*) harboring

the genes encoding catalase (CAT) or superoxide dismutase (SOD) antioxidant enzymes exhibited anti-inflammatory activities in a mouse model of Crohn's and colitis disease induced by trinitrobenzenesulfonic acid (TNBS). The authors observed a reduction in weight loss, fewer liver microbial translocation, lower macroscopic and microscopic damage scores, and modulation of the IFN-γ/IL-10 [180] and IL-10/IL-17 [181] cytokine production in the large intestines of mice treated with either CAT- or SOD-producing lactobacilli/streptococci.

The stress-inducible controlled expression (SICE) system represented by *L. lactis* MG1363 strain harboring the pLB333 plasmid was developed to avoid the external induction of culture before the host administration [134]. Several interesting molecules were cloned in this system such as IL-10 [134] and IL-17 [182], and the effect of *L. lactis* secreting them was evaluated in mice models. *L. lactis* (pSICE:*il-10*) was tested in a DNBS-induced colitis mice model, resulting in a significant reduction in colitis parameters with improvement in weight loss and a decrease in macroscopic scores [134]. The intranasal administration with *L. lactis* secreting IL-17A (pSICE:*il-17*), in a mice model of human papilloma virus (HPV)-induced cancer, was able to reduce tumor size and induce IL-6 and IL-17 secretion in reactivated splenocytes from mice challenged with the tumoral cell line [182]. Both works confirmed the potential use of *L. lactis* harboring the SICE system to deliver interesting molecules either to colitis or colon cancer patients [134, 182].

Although many studies have focused on the use of recombinant bacteria for the treatment of IBDs, as was previously discussed, the use of recombinant probiotic strains expressing/delivering therapeutic molecules has been explored for treatment or prevention of other diseases such as mucositis, cancer, obesity, multiple sclerosis, and diabetes [182–185].

An in vivo study reported by Caluwaerts et al. [186] showed that recombinant *L. lactis* AG013 secreting human trefoil factor 1(hTFF-1) was able to reduce the severity and course of radiation-induced oral mucositis. Carvalho et al. [187] also demonstrated that a recombinant strain of *L. lactis* NZ9000 using the inducible NICE system to express the human pancreatitis-associated protein (PAP) was able to prevent 5-FU-induced intestinal mucositis in a murine model. It was observed that this protein preserved villous architecture, increased Paneth cell activity [187], and suppressed the growth of *Enterobacteriaceae* during inflammation [185].

It also has been shown that oral administration of a recombinant *L. lactis* NCDO2118 strain (pXylT:SEC:*hsp65*) prevented the development of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice [184]. Mice fed daily with this recombinant strain increased the number of natural and inducible $CD4^+FoxP3^+$ and $CD4^+$ latency-associated peptide (LAP^+) regulatory T cells in the spleen, inguinal and mesenteric lymph nodes, as well as in the spinal cord. In addition, a reduction in the recruitment of encephalitogenic $CD4^+$ T cells to the spinal cord was observed, which decreased IgG response against HSP65 and induced an anti-inflammatory cytokine profile (IL-17 reduction and IL-10 increase) during EAE development.

The oral administration of recombinant *L. lactis* expressing HSP65 and tandemly repeated P277 (pCYT:*HSP65-6P277*) was also analyzed in a model of type 1 diabetes mellitus (DM1) [183]. The authors observed that oral administration of recombinant *L. Lactis* resulted in the prevention of hyperglycemia, improved glucose tolerance and reduced insulitis, and induced HSP65- and P277-specific T-cell immunotolerance, as well as antigen-specific proliferation of splenocytes, demonstrating to be an effective therapeutic approach in preventing DM1 [183].

Another study using the *E. coli* Nissle 1917 strain engineered to secrete N-acylphosphatidylethanolamines (NAPEs) (pDEST-At1g78690 expression

plasmid) demonstrated that this strain was able to reduce the obesity of mice fed with a high-fat diet when added to drinking water. N-acyl phosphatidylethanolamines are precursors to the N-acylethanolamine (NAE) family of lipids, which are synthesized in the small intestine in response to feeding and reducing food intake and obesity. Mice that received modified bacteria had dramatically lower food intake, adiposity, insulin resistance, and hepatosteatosis than mice receiving standard water or control bacteria [188]. In addition, it was observed that changes on intestinal microbiota significantly decreased the abundance of *Firmicutes* and increased the abundance of *Proteobacteria*. Thus, these results provide evidence of the potential efficacy of this approach to inhibit the development of metabolic disorders and related diseases.

13. Conclusion

Currently the association between disease progression, especially chronic inflammatory diseases, and intestinal dysbiosis has been more frequently observed. As a clinical strategy, the use of probiotic bacteria, which naturally benefit the host, has been increasingly used on the treatment of diseases related to the GIT. In view of the good results obtained with this approach, researchers have sought through bacterial genetic modification to increase the beneficial potential of probiotics, either through their use for heterologous protein production or as a vehicle for vaccinal plasmid delivery, by developing recombinant bacterial strains and by testing their action in different disease models. And while there are still a number of questions that need to be answered about the use of genetically modified organisms for health care, especially in human, the use of these strains has proven to be a potentially effective therapeutic alternative, so much so that clinical trials using recombinant lineages have already been authorized and conducted in humans.

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Chapter 5

Valuable Food Molecules with Potential Benefits for Human Health

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Abstract

The rapid development in the food supply chain has led to increased interest for quality in the food sector. In the last two decades, the human health and food safety have become essential. Health problems are highly related to diet and nutritional habits. The connection between nutrition and the development of various health problems is even more noticeable when close attention is given to every age group. Regarding the chemical composition of foods, a large number of bioactive compounds present in plants, fruits, vegetables, dairy products, meat, and fish are currently known. Bioactive compounds from food play an important role in prevention of illnesses. Covering essential aspects of health benefits of foods, the present chapter underlies without being exhaustive, the potential of valuable compounds such as soy isoflavones, phytochemicals, polysaccharides, probiotics, prebiotics, lipids, and marine proteins to be used as an effective prevention strategy for developing various human cancers, cardiovascular diseases, diabetes, and metabolic disorders.

Keywords: bioactive compounds, functional food, probiotics and prebiotics, phytochemicals, soy isoflavones, polysaccharides, seafood products

1. Introduction

The modifications occurred during globalization and the fast development of food production had led to new expectations from consumers regarding food and healthy diets [1]. Nowadays, as life expectancy has substantially extended, there is an acute demand for special foods that fulfill all the nutritional needs and help us maintain a balanced diet—a key role in sustaining human health [2]. Therefore, the food sector companies need to keep up with the consumers' interests and needs while designing novel products. Moreover, health authorities, food engineers, scientists, health insurers, and customers seem to highlight an increased interest in illnesses prevention.

Today, food market is richer than ever, but the demand and the challenges seem to increase even more. Due to the globalization of the food market, an increasing number of special diets such as vegetarian or vegan, various food allergies, and intolerances have gained attention. Thus, scientists, food engineers, and health authorities seem to look for foods that bring added value and may even prevent diseases. These desiderates have brought the concept of "functional food" [3]. Thus, the consumers can correlate this category of foods with recognized health benefits [4].

Functional foods are known as healthy foods, medicinal foods, regulatory foods, fortified foods, nutraceuticals, and pharmacological foods [5]. A main statement or a clear definition of functional foods does not exist since all foods (conventional foods) provide energy and nutritional functions. Beyond these, functional foods contain elements that have the potential to sustain human health or reduce the risk for certain diseases [6, 7].

According to the potential medical benefits and properties of their ingredients, functional foods can be classified in several groups: dietary fiber, sugar alcohols, amino acids, oligosaccharides, glycosides, peptides and proteins, vitamins, cholines, lactic acid bacteria, minerals, polyunsaturated fatty acids, and others (e.g., phytochemicals and antioxidants) [8].

Studies have shown that an individual health status is influenced by diet even from very early stages. Special attention should be given so consumers have a well-balanced diet in order to avoid undernutrition and functional decline. In this light, specific recommendations are comprised in a 2014 report of Joint Research Centre, the European Commission's in-house science service. The report summarizes the evidence on key micronutrient supplementations for preventing age-related diseases and draws attention on nutrition as a crucial element in healthy aging [9].

1.1 Consumer behavior: general overview

The quality of food is perceived by the consumers depending on intrinsic factors (freshness, organoleptic properties, health and hygiene safety) and external factors (origin, traceability, geographical indications and certification, labeling, health claims, production processes, etc.) [10].

In some countries, legislation policies have scrutinized the health claims labeling, as it represents a strategy that positively influences the purchase decision [11]. The European Union introduced important quality (origin) labels, in order to help consumers, namely Protected Designation of Origin (PDO) or Protected Geographical Identification (PGI) and Traditional Specialty Guaranteed (TSG). Moreover, the products' quality and safety are regulated by the ISO 9001 (International Organization for Standardization) standards, which define the requirements of a quality system in order to ensure control throughout the production process and to prevent or detect any non-conformities. As an operational tool, the Hazard Analysis Critical Control Points (HACCP) system has been implemented, whose purpose is to achieve self-checking objectives [12].

Broadly, functional food refers to any food or food ingredient that may provide a health benefit beyond the nutritional ones. The market of functional foods covers foods that contain bioactive molecules such as polyunsaturated fatty acids [13], plants' primary or secondary metabolites (polyphenols, anticipants, carotenoids, phytosterols [14–20], probiotics and/or prebiotics [21, 22] and others (**Figure 1**)).

The consumers' perception regarding functional food is highly influenced by various factors such as food attributes (e.g., taste and flavor), the consumption behavior, the consumer's knowledge, the potential benefits, purposes, diet preferences or restrictions, the consumer's health problems, advertising, the label

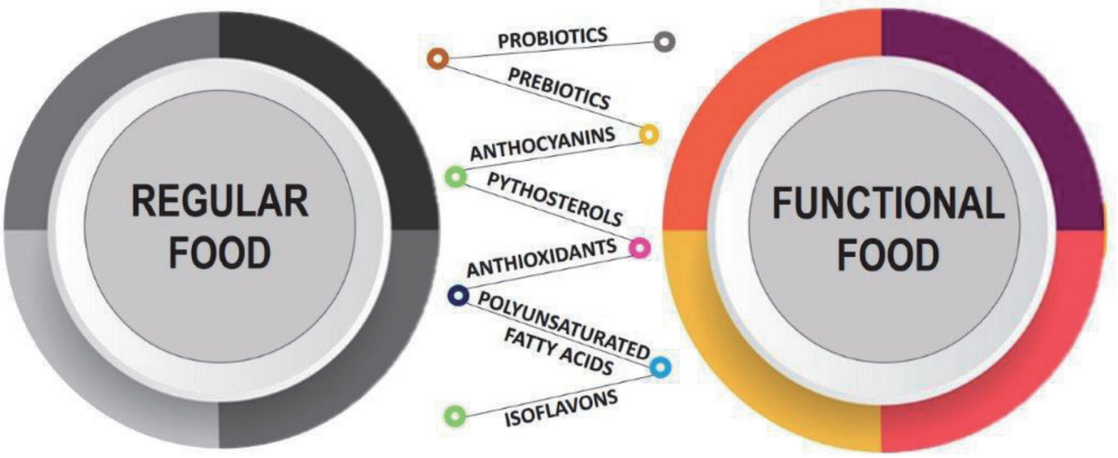


Figure 1.
The bioactive molecules which add a supplemental value to regular food and provide functional benefits to human health.

information, the packaging or the appearance, the brand and, not least, the product's price [23–26]. On the other hand, the food quality and safety play an important role in building the image of a product [27]. At the same time, attributes such as food palatability and healthiness are key elements in the consumer choices [28]. A recent research of Haasova and Florac has shown that manufactures need to focus on increasing the consumers' orientation toward health goals and on reducing the consumers' beliefs that healthy food equals tasteless food [29]. In another study, conducted in 2019 in the U.S., focused on consumers' perceptions on natural and healthy foods, it was shown that consumers buy food products according to the several factors such as taste, price, healthiness, safety, and naturalness. Also, young consumers valued the food naturalness more compared to older consumers [30].

In most cases, functional foods have attractive forms and are certainly not commercialized as tablets or capsules [31]. Food producers constantly analyze the consumers' behavior in relation to engineered foods, projected to maintain their health and prevent illnesses. The consumer's keen interest in consuming natural products has challenged food industry to create innovative food products, developed for functional applications, but to keep the product as natural as possible. Due to their special indications, these products are consumed for a limited period of time. Thus, the main objective is to obtain functional foods using natural compounds and with minimal processing. A 2013 report [32] has estimated that sales of these special products will double by 2020. Similar to any food products, the market life and attractiveness of functional foods are following a Gaussian curve. Scientists report [33, 34] that a large number of functional foods get recalled soon after they enter the market, in most cases due to the reliance on technology, coupled with the absence of a thorough market research [35]. In order to explain what triggers the market failure of functional foods, it is mandatory to understand the consumer's desire for products considered healthy.

In our opinion, the consumer's attitude toward the potential benefits of these products for disease prevention and health improvement is the rhyme factor which dictates the consumption behavior. Thus, the consumer's perception on the beneficial health effect of functional foods can essentially affect the purchasing process. These perceptions are, in turn, influenced by various factors such as involvement degree, lifestyle [36], food product characteristics (i.e., sensorial, nutritional, price, etc.) [37], package information, and package design [38]. In any case, the consumers' trust in the functional claims is decisive in the purchase decision [39]. Information about food products and healthy diets is known and available to

customers, and they evaluate all this information. Therefore, understanding the customer's reasoning in evaluating the healthfulness of a functional product represents an important step in the process of designing functional foods. The big challenge is making the health benefits as credible as possible [40].

However, health claims are not the only factors that convince a customer to purchase functional foods. Other important factors are the product design, the cultural influences, the food matrix used as carrier for the active molecules, and the price. Among all the elements that influence the customer's decision to buy functional foods, the confidence of obtaining well-being effects weighs the most. The customer's certainty must represent the cornerstone for food products developers, stakeholders, and marketers.

1.2 Diet and health problems

Unhealthy diet is associated with regular consumption of foods and beverages rich in saturated fat, transfats, and polyunsaturated fats [41].

Worldwide, obesity is one of the major public health issues with multiple consequences: chronic diseases, including type 2 diabetes, coronary heart disease, and several cancers [42]. Besides the main factors that influence obesity, such as genetics, lack of physical activity, social and psychological factors, diet remains one of the major determinants that influences body weight. In the last 50 years, the prevalence of obesity has dramatically increased, reaching pandemic proportions, with more than 1 billion adults being overweight, of which at least 300 million are clinically obese. As a result, the consumption of functional foods has gradually expanded and gains more and more interest [43, 44].

In a recent study on health impact of dietary risks in 195 countries, authors have concluded that dietary habits are correlated with coronary heart sickness and other chronic non-communicable diseases, and urgent strategies to ameliorate the diet quality are required [45].

The diseases that prevail most in developed countries, the so-called "diseases of civilization," are affecting the life quality of 100 times more people than mortal accidents [46]. In preventing overweight, obesity, or any chronic diseases (cardiovascular illnesses, various cancers, depression, and type 2 diabetes), the customer's food choice can have a great impact on all related industries (i.e., food, pharmacy, etc.) [2, 47]. A first change in the consumers' behavior can start by limiting the excessive food quantities and adjustment to a controlled, moderate food amount.

The maternal nutrition both before and during pregnancy, as well as the child's nutrition after birth, will critically impact her or his later development and future health status [48]. The most common child health problems caused by an unbalanced diet are iron deficiency anemia, vitamin D deficiency, obesity, dental caries, and faltering growth [49]. In toddlers, the prevalence of iron deficiency anemia and severe obesity is each approximately 2%. Later in life, iron deficiency has been associated with potential neurodevelopmental impairments, while obesity in childhood may continue into adulthood, accompanied with adverse cardiometabolic outcomes [50].

Malnutrition, in all its forms, includes undernutrition (including wasting, stunting and micronutrient deficiency/insufficiency) and overweight and obesity. Acknowledging the need of accelerated actions against malnutrition and the need to promote a healthier maternal, infant and child nutrition, WHO has elaborated, in 2012, a group of six general nutrition targets that by 2025 aim to: (1) attain a 40% reduction in the number of children under 5 who are stunted; (2) achieve a 50% reduction of anemia in women of reproductive age; (3) achieve a 30% reduction in low birth weight; (4) ensure that there is no increase in childhood overweight; (5) increase in the rate of exclusive breastfeeding in the first 6 months up to at least

50%; and (6) reduce and maintain childhood wasting to less than 5% [51]. Some of these targets have been extended to 2030.

In adults, half of the total chronic disease deaths are attributable to cardiovascular diseases. Accelerating trends can be seen also for obesity and diabetes as more patients are diagnosed at an early age. The list of most common chronic diseases in adults and elderly is completed by hypertension, hypercholesterolemia, arthritis, depression, Alzheimer's disease, osteoporosis, and chronic obstructive pulmonary disease [52]. However, these chronic diseases are largely preventable and diet plays a key role in preventing or promoting chronic illnesses.

According to a standardized case-control study, eight of nine modifiable risk factors associated with acute myocardial infarction are influenced by diet. Most of these factors act by promoting atherogenesis, by modulating vasodilation, prothrombotic and pro-inflammatory processes that trigger the endothelial dysfunction, an early predictor of atherosclerosis [53, 54]. Apparently, vascular inflammation is associated with unhealthy dietary patterns, overweight and obesity, smoking habit, alcohol consumption and sedentarism [54].

In a population-based prospective cohort study, higher dietary intake of vitamin E, but not vitamin C, beta carotene, or flavonoids was modestly associated with lower long-term risk of dementia. Vitamin E antioxidant mechanisms are not fully understood, but it could improve cognitive performance by diminishing the effects of β-amyloid, as it was shown in experimental studies [55].

1.3 Health benefits of functional food

With the emergence of the term FOSHU (Foods for Specific Health Use), in the early 80s in Japan, the impact of food on health has gained much attention in the recent decades. However, the scientific community has not fully agreed on what is covered by the term "functional foods." The FUFOSE (Functional Food Science in Europe), for its part, has defined them as follows: "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, thus either improving, the general physical condition or/and decreasing the risk of the generation of disease" [56]. However, the study of food, focused on therapeutics, is not something new and if we go back to the past, the classification of the use of food and plants with therapeutic properties goes back to the Mid Paleolithic age [57]. Also, during the history of humankind, the health benefits of food have been capitalized in traditional medicines such as Unani, Traditional Chinese Medicine, and Ayurveda. In this same sense, Persian Medicine (PM) in the Islamic era (980 AD) already considered the classification of certain foods as potential drugs with a significant role in health, not only as mere energy providers, but also as being able to affect the human body by changing even the temperament and personality of individuals. Faced with a disease, PM first considered a special diet for the patient using specific foods, and if the patient's health did not improve, drug therapy was the second option [58]. Already at that time, there was a classification for food and drugs very similar to the classification currently available for functional foods, nutraceuticals, and pharmaconutrients.

Foods are classified as functional after their effects have been demonstrated in well-designed and properly executed intervention studies in humans [8]. Functional food can play an essential role in disease treatment and prevention.

The beneficial health properties of various foods have been scientifically recognized thanks to the analytical development in identifying and characterizing their chemical composition and due to the clinical studies that have assessed their role in various pathologies. Studies based on *in vitro* and cell-culture systems, preclinical

interventions using animals, and clinical trials have investigated the potential of functional foods to combat various human cancers, cardiovascular disease, diabetes, metabolic disorders, inflammation, high blood pressure, microbial, viral and parasitic infections, mental diseases, spasmodic disorders, ulcers, etc. [59].

The relationship between food and human health is extremely tight, and World Health Organization has stated that "A healthy diet helps to protect against malnutrition in all its forms, as well as noncommunicable diseases (NCDs), including such as diabetes, heart disease, stroke, and cancer. Unhealthy diet and lack of physical activity are leading global risks to health." Regarding the chemical composition of foods, a large number of bioactive compounds present in plants, fruits, and vegetables are currently known, such as polyphenols or carotenoids and nowadays it is known that the concentration of these bioactive compounds is variable depending on the parts of the plant, season, climate, and the particular growth phase [60].

The Global Burden of Disease study has reported that unhealthy diets represent a risk factor for disease, morbidity, and disability both in Canada and worldwide [61]. Certain diets, such as vegetarian diet (rich in antioxidants), the Mediterranean diet (high in olive oil with monounsaturated fatty acids), and the Okinawan diet (high in fruits, vegetables, and omega-3 fatty acids in fish) can impede the development of age-related diseases. More specifically, Everitt et al. reported in their study that epidemiological research consistently demonstrates that the intake of certain foods is correlated with cardiovascular disease risk [62].

Daily diet can be improved by including essential fatty acids, minerals, vitamins, and proteins from fish and meats and increasing the intake of calories and proteins from nutritionally appropriate sources (nuts and seeds, pulses, soy products, dairy products) [63].

2. How probiotics and prebiotics sustain human health: a correlation with functional food intake

A worldwide known fact is that the gut microbiota composition has a strong influence on the host health and metabolism [64, 65]. More than 500 species of bacteria coexist in the human gastrointestinal tract (GI), and among them, about 95% of the total number of cells in the colon [66]. Many of the indigenous GI habitants are probiotic bacteria cells that pertain to the genera *Lactobacillus*, *Bifidobacterium*, or *Enterococcus*. Notably utilized and commercialized probiotics are belonging to the first two genera. An extremely studied topic for the past two decades was the gut microflora, being in the spotlight of food and medicine scientists, food engineers, authorities, and customers as well. In the functional food market, the pathway has two lanes, namely how functional foods influence gut microbiota and how common foods can incorporate these valuable cells and become functional foods with important characteristics (**Figure 2**).

Even though the presence of probiotics in different functional food matrices has been widely studied, the amount of documented therapeutic applications and clinical trials is very modest. The influence of different functional foods on gut microbiota is now in the spotlight of many researchers. Most of the studies regarding the effect of functional foods on gut microbiota fail to prove the causative role. In most of the cases, the disease is treated with certain functional food and, afterward, the gut microbiota composition is tested and correlated, correct or not, with the cause of the disease alleviation. But, stating this may be as true as the affirmation "the consequences of diseases alleviation were the registered changes in the gut microbiota." Today, the concept of functional food (i.e., foods containing dietary fibers, polyunsaturated fatty acids, polyphenols, etc.) utilization in order to obtain

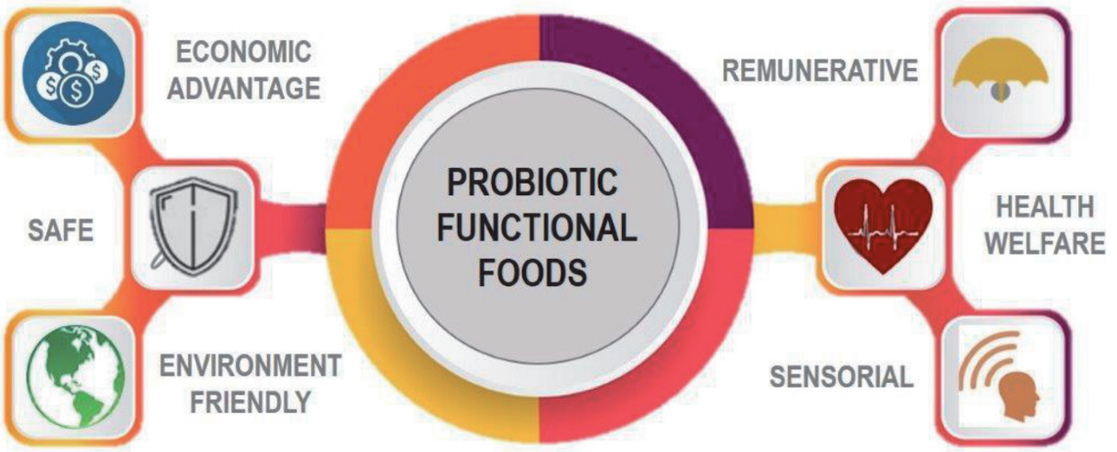


Figure 2.
Probiotic functional foods characteristics.

a certain response with respect to a disease is embraced by many customers, but the modulation of gut microbiota by changing eating patterns and/or diet composition is still a novel practice [67]. In the attempt to evaluate the changes in the gut microbiota composition in different diseases and after ingestion of functional foods, the literature reports two approaches for the *in vivo* tests on mice. The first one is the mice treatment with antibiotics to see whether disorder of the gut microflora could cut off the curative effects of functional foods on a certain illness. The limitations of this method arise from the fact that antibiotics could not entirely eradicate the effect of gut microbes on the evolution of the diseases, as they could not completely eliminate microbes from the gut. A different approach is the utilization of germ-free mice [68, 69]. Thus, it tests if a specific functional food product may be unsuccessful in the treatment of the targeted disease in germ-free mice. This fact reveals that the target of the ingested functional food may not be the gut microbiota. However, in these times when microbiota-targeted nutrition has become a frequently proposed technique, we predicted more studies that can establish a causal connection between functional foods ingredients, host health, and gut microbiota.

Another approach, in order to modulate the gut microflora, is to ingest functional foods, which have as active ingredient viable probiotic cells [70], prebiotics (the probiotic food) [71], and/or synbiotics (pro and prebiotics) [72]. Nowadays, probiotic cells are included in more and more products, extending the size of functional food market. Not long ago, probiotic cells were associated mostly with dairy and fermented foods, but things have been considerably changed. There are several studies that have demonstrated good viability cells in products such as chocolate [73, 74], bread [75], juices [76], jelly [77], meat [78, 79] and even edible films or coatings [80]. Results of these studies demonstrate, besides the good viability of the probiotics during processing, shelf life and even gastrointestinal passage, no negative changes regarding the food structure or sensorial properties [81]. However, reports regarding the concrete influence of functional foods probiotics on the consumer health are modest. Health benefits of probiotics intake are multiple and based on scientific evidence. Among the multiple well-being advantages related to probiotic consumption, we will discuss the most studied and known ones.

One of the health benefits of probiotic cell intake is its capacity to regulate the host's intestinal microflora and the general well-being. An individual's intestinal microflora composition is rather well balanced after early infancy, in healthy subjects, even though variations exist among subjects [82]. One of the most important actions of probiotics is to positively influence intestinal microbiota. Evidence shows that Lactobacilli intake ensures a reduction of pathogenic gram-negative anaerobes,

such as *Clostridia* or *Enterobacteriaceae* [83]. Postsurgical period predisposes the individual to infections with gram-negative anaerobe bacteria or with sulfite-reducing *Clostridia*, leading to inflammatory response due to the secreted endotoxins. The mentioned pathogens are inhibited by *Lactobacillus* due to their capacity to produce antimicrobial metabolites (i.e., bacteriocins, hydrogen peroxide) and other valuable substances such as short-chain fatty acids. Beside *Lactobacillus*, *Bifidobacterium* intake may reduce the butyrate-producing anaerobes [84]. Another study [85] revealed the efficacy of *Lactobacillus* in *Rotavirus* infections. Probiotics proved to have a positive effect in reducing the duration of acute rotavirus diarrhea in children in comparison with control. Thus, we can conclude that the consumption of viable probiotic cells, trough functional foods or as supplements, can considerably improve the composition of intestinal microbiota, leading to health benefits that can confer a better life to the consumer.

Treatment or amelioration of diarrhea is a promising and extensively studied technique. Diarrhea causes may be multiple and need to be approached with different types of probiotic species, in single or multiple formulations. A review, published by Marteau et al., pointed out that these valuable cells have good results in treating diarrhea induced by antibiotic treatment, *Rotavirus* or/and *Gastroenteritis*, but they are less efficient for traveler's diarrhea [86]. Another appreciated health benefit induced by probiotic ingestion is the amelioration of irritable bowel syndrome (IBS) symptoms. Even if the cause of this disorder is still unknown, the symptoms are very clear, namely abdominal pain, constipation or diarrhea, and infrequent nausea, fluid retention, tiredness, and bloating. For most of the patients suffering from IBS, an imbalance in the intestinal microbiota can be found. In most of the cases, a decreased number of bifidobacteria can be observed, with an increase in the facultative anaerobes pathogens [87]. Many of the mentioned intestinal disorders may lead, in time, to carcinomas.

3. Phytochemicals: general overview, potential applications, and health benefits

Fruits are a source of active compounds, such as vitamins (C and A), minerals (electrolytes), and more recently phytochemicals, especially with antioxidant properties, which include phenolic compounds, flavonoids, lignins, tocopherol (vitamin E), carotenoids, betaine, colin, saponins, and phthalates [88]. Because of their importance, we are going to focus on polyphenols.

Polyphenols are natural compounds characterized by the presence of phenol, catechol, and resorcinol (benzene rings with several hydroxyl groups in *o*, *m,* and *p* positions). In phenolic acids, the presence of a carbonyl group, such as aromatic acid, ester, or lactone, enhanced its antioxidant activity as well as when its carbonyl group is separated from the aromatic ring [89, 90]. Phenolic acids are derivatives of hydroxycinnamic acid such as *p*-hydroxybenzoic, 3,4-dihydroxybenzoic, vanillic, syringic, *p*-coumaric, caffeic, ferulic, sinapic, chlorogenic acid, and rosmarinic acid. The derivatives of cinnamic acid are more active antioxidants than the derivatives of benzoic acid derivatives [90, 91]. There are three structure groups responsible for the determination of free-radical scavenging and antioxidant activities of flavonoids: a catechol moiety of the B-ring, the 2,3-double bond in conjugation with a 4-oxo function of a carbonyl group in the C-ring, and presence of hydroxyl groups at the 3 and 5 positions [90]. They are classified as flavonols, flavons, flavanons, flavanols, flavandiols, isoflavonoids, cathechins, chalcones, dihydrochalcones, anthocyanidins, leucoanthocyanidins, proanthocyanidins, or condensate tannins [92].

Some drinks such as coffee, tea, beer, and wine; fruits such as apple, orange, guava, papaya, and grape; vegetables such as, zucchini, beet, avocado, watercress, chili, and tomato; dry fruits as nuts; and cacao are important sources of polyphenols and other antioxidants [93–95]. Polyphenols possess several pharmacological activities and are characterized by their antioxidant activity, which depends on the substitution in either *ortho*- or *para*-position, while the substitution in *meta*-position has a rather limited effect. It is known that the polyphenols consumption could be related with the decrement of chronic and degenerative diseases such as diabetes, cancer, cardiovascular, neurodegenerative, and antitumoral diseases, promoter of the immune system, anti-inflammatory, skin protective effect from UV radiation, antibacterial and antifungal activities [96–100].

Free radicals are generated as a result of normal cell metabolism. These reactive oxygen species (ROS) cause lipids, proteins, and nucleic acids damage in cells and modulate several signaling pathways [101]. As a consequence, different pathologies such as chronic and degenerative diseases can develop. All aerobic organisms have antioxidant defenses, including antioxidant enzymes and antioxidant constituents to remove or repair the damaged molecules. Also, the natural antioxidants from certain foods can be beneficially used to remove oxygen and reactive oxygen species [90, 102].

Polyphenols are involved in cell cycle regulation and may inhibit the progression of cancer in many organs or even block latent tumors due to their anti-angiogenic and anti-inflammatory properties. As for the origins and causes of various cancer types, they are not yet well established. However, it is known that high levels of free radicals such as reactive oxygen species (ROS) produce lipid peroxidation that induces various cell injuries. These injuries can later lead to cancer development [103]. In this context, polyphenols studies have shown that they can regulate cell proliferation and specific protein modulators associated with cell cycle [104]. Likewise, polyphenols can control cancer cell progression in many organs [105]. They also have the capacity to block latent tumors by direct inhibition of tumor cells or by anti-angiogenic and anti-inflammatory properties and protect deoxyribonucleic acid (DNA) from lesions caused by reactive oxygen species. Using these mechanisms, cancer progression can be hindered and pro-apoptotic mechanisms are then triggered [105].

Flavonoids possess antioxidant activity due to their structures, which is capable of donating an electron or chelate metal ions. Some foods such as blueberry, red wine, green tea, and cocoa have been studied for their antioxidant properties in order to prevent diseases [106]. The group of antioxidants includes mainly vitamins (ascorbic acid, tocopherol) and flavonoids such as quercetin, pycnogenol, and flavan-3-ol monomers and oligomers [100, 107, 108]. Flavonoids and other phenolic compounds showed cardiovascular protection by improving the endothelial function, reducing the oxidative stress, lowering arterial pressure, improving the elasticity of the internal blood vessels' walls, or by inhibiting platelet agglutination. These properties could prevent blood clot formation in the arteries, and can positively influence blood lipid balance and insulin sensitivity [109, 110].

Specific components of fruits may also show protective effect in human organism. Quantitatively, the most important carotenoids in the human diet are β-carotene, lycopene, lutein, β-cryptoxanthin, zeaxanthin, and astaxanthin [111, 112]. Lycopene is a carotenoid found in brightly colored fruits and vegetables, and research suggests that foods containing carotenoids may protect against lung, mouth, and throat cancer [113, 114]. In addition, a study suggests that lycopene may help protect men against prostate cancer, especially in aggressive forms [115].

Finally, the consumption of fruits rich in polyphenols and carotenoids could reduce the incidence of developing chronic degenerative diseases, stroke, and

cancer [106, 116–118]. Nowadays, the use of extracts and secondary metabolites of plants and foods in different pathologies is extremely well documented [105]. **Table 1** presents some pharmacological activities of several secondary metabolites.

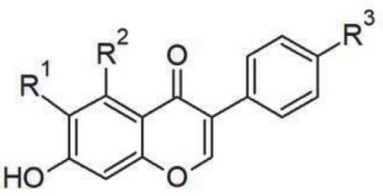
Cardioprotective activity	
Kaempferol, rutin, luteolin, quercetin, resveratrol	Activity against doxorubicin-induced cardiotoxicity [119]
Gallic, ellagic, syringic, ferulic, cinnamic acids, and quercetin	Attenuate oxidative stress in H9c2 cardiomyoblasts [120]
Aspalathin and phenylpyruvic acid-2-*O*-β-D-glucoside	Protect myocardial infarction caused by chronic hyperglycemia [121]
Puerarin	Protects myocardium from ischemia and reperfusion damage (Ca^{2+}-K^+ channel and the protein kinase C activated) [122]
Naringenin-7-O-glucoside	Activity against doxorubicin-induced cardiotoxicity, protects against cardiomyocyte apoptosis [123]
Isorhamnetin	Effect against cardiotoxicity of doxorubicin [124]
Antibacterial activity	
Gliricidin 7-*O*-hexoside and quercetin-7-*O*-rutinoside	*Proteus mirabilis, P. vulgaris,* and *Pseudomonas aeruginosa* [125]
3,4,7-trihydroxyflavone	*Providencia stuartii* and *Escherichia coli* [126]
Pseudarflavone A and 6-prenylpinocembrin	*E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus faecalis,* and *Staphylococcus aureus* [127]
2-(3,4 dihydroxy-phenyl) 3,5,7-trihydroxy-chromen-4-one	*Pseudomonas aeruginosa* [128]
Kaempferol	*P. acnes* [129]
Strictinin	*Propionibacterium acnes* and *Staphylococcus epidermidis* [130]
Anticancer activity	
Flavopiridol	Treatment of lymphomas and leukemia [131]
Quercetin	Induces apoptosis of each one of these cell lines: acute lymphoblastic leukemia MOLT-4 T-cells, human myeloma U266B1 cells, human lymphoid Raji cells Prostate adenocarcinoma LNCaP cells, human prostate PC3 cells Colon carcinoma CT-26 cells, Pheochromocytoma PC12 cells, Estrogen receptor-positive breast cancer MCF-7 cells, ovarian cancer CHO cells [132] Induces microRNAs involved in Notch signaling/cell-fate determination of the tested pancreatic cancer (primary pancreatic cancer cell line ASANPaCa, AsPC1, and PANC1) [133]
Gliricidin7-O-hexoside and Quercetin 7-O-rutinoside	Human hepatoma HepG2 and carcinoma HeLa cells [125]
Genistein	Inhibits the activation of Nuclear factor kappa B (NF-kB) involved in balance of cell survival and apoptosis on prostate cancer [134]
Curcumin	Acts as a pro-apoptotic agent in skin cancers [98] Inhibits melanoma cell proliferation related to epigenetic integrator UHRF1 [135] Inhibits the proliferation of human prostate cancer cell lines such as LNCaP and 22Rv1 cells [136]

Anti-inflammatory activity	
Apigenin, C-rhamnosyl flavones, and luteolin	Reduce nitric oxide levels in macrophages, inhibit the activity of phospholipase A2 [137]
Quercetin, apigenin, hesperidin, and luteolin	Anti-inflammatory effects [138]
Astilbin	Anti-inflammatory effects observed *in vitro* after lipopolysaccharide-induced inflammation suppresses nitric oxide production, tumor necrosis factor-α (TNF-α), mRNA expression of inducible nitric oxide synthase [139]
Acteoside	Inhibits inflammation in LPS-induced lung injury in mice, inhibits inflammation in lung epithelial cells A549, inhibits NF-kB activation in LPS-induced mice and lung epithelial cells A549 [140]
Skin protective effect from UV radiation	
Apigenin	Skin protective effect of damage caused by UV light [141]
Quercetin	Inhibits UVB-induced skin damage in hairless mice [142]
Silybin	Prevention of apoptosis in UVB-exposed human epidermal keratinocytes [143]
Genistein	Photoprotective activity in human skin against photocarcinogenesis by inhibiting UV-induced DNA damage [144]
Equol	Prevents damage from UV-induced erythema-associated edema, inhibits DNA photodamage [145]
Metabolic syndrome	
Genistein	Improves factors of risk for diabetes and cardiovascular disease in postmenopausal women with MetS [146]
Acteoside	Antihypertensive activity in lowering systolic blood pressure (SBP) and diastolic blood pressure (DBP) [147]

Table 1.
Pharmacological activities of secondary metabolites from food and plants.

4. Soy isoflavones: evidence-based health benefits and official recommendations

Isoflavones are polyphenolic plant metabolites produced almost exclusively by the members of the *Fabaceae* family. More specifically, isoflavones are part of the flavonoids group, which share the 2-phenylchromen-4-one backbone. Due to the structural similarity with 17-β-estradiol, the primary female sex hormone, isoflavones have been included in the wide family of phytoestrogens. The main sources of the natural isoflavones are soy beans, chickpeas, fava beans, pistachios, and peanuts. Of these, physiologically relevant amounts are found only in soybeans and soy-derived foods, and raw soybeans containing 1.2–4.2 mg/g dry weight isoflavones. Besides isoflavones, soy is also an excellent protein source and contains vitamins, minerals, and insoluble fibers. Genistein, daidzein, and glycitein (**Figure 3**) are the biologically active aglycones from soy-based foods and red clover. Other isoflavones present in various plants are biochanin A and formononetin, which can be converted through 4′-O-demethylation to genistein and daidzein, respectively. Generally, the soy aglycones are conjugated with a glucose moiety through the -OH group from position C7 (7-O-glucoside) and form the β-glycosides, genistin, daidzin, and glycitin [148, 149].



Isoflavone aglycone	R^1	R^2	R^3
Genistein	H	OH	OH
Daidzein	H	H	OH
Glycitein	OCH_3	H	OH
Biochanin A	H	OH	OCH_3
Formononetin	H	H	OCH_3

Figure 3.
The chemical structures of soy isoflavones.

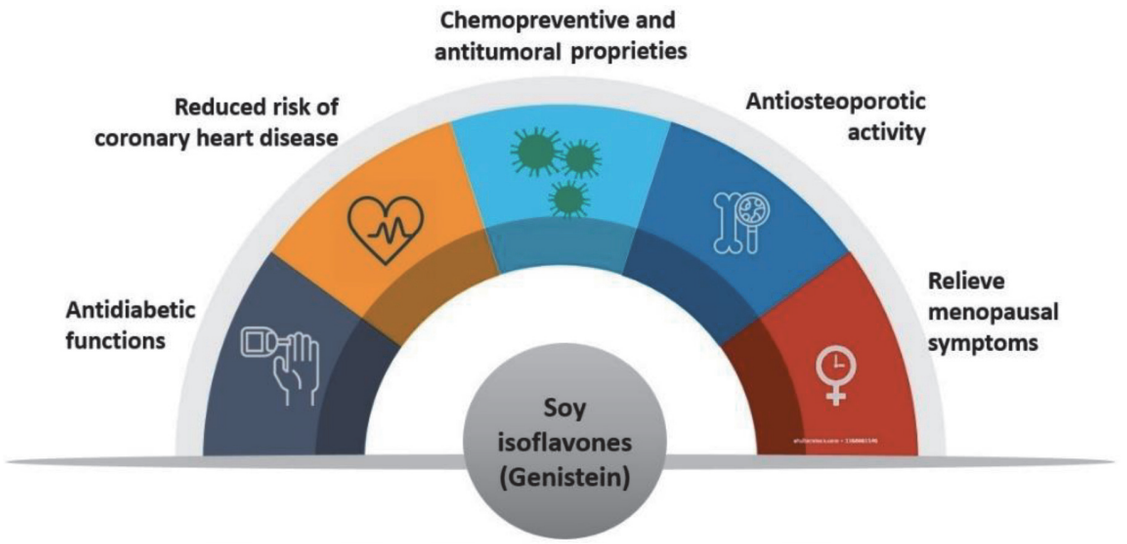


Figure 4.
The health benefits of soy isoflavones.

The aglycones are rapidly absorbed in the small intestine by spontaneous, passive diffusion. The free aglycone forms are only merely present, as soy beans and non-fermented soy foods mostly contain the glycosylated forms. These conjugates need to be prior converted to the bioactive aglycones. Their hydrolysis is completed by β-glucosidases from intestinal bacteria, or an enzyme in the intestinal mucosa. Daidzein and genistein aglycones can also derive from formononetin and biochanin A hydrolysis [150]. Soy isoflavones have drawn the attention of the scientific community due to their multiple medicinal and therapeutic proprieties. The health benefits provided by soy isoflavones are presented in **Figure 4** [151]. These potential benefits will be presented herein.

4.1 Soy isoflavones and cancer

Perhaps the most controversial aspect related to soy isoflavones is linked to their potential role in chemoprevention. Most studies have investigated the correlation between soy consumption and breast or prostate cancer, but several studies have also investigated the role of soy isoflavones in other malignancies, such as: colon cancer, liver adenocarcinoma, bladder cancer, or brain tumor [151].

The perception that soy isoflavones possess anticancer proprieties derives from epidemiological data, which showed that Asian women who regularly consume soy or soy-based foods have a significant lower breast cancer risk than Caucasian women who do not consume soy as part of their daily diet [152]. These epidemiological studies have indicated an inverse correlation between polyphenol-rich dietary intake and cancer development.

The differences between the daily isoflavone intake in the US, Europe, and Asia are significant: non-Asian populations consume less than 3 mg isoflavones per day, whereas Japanese and Shanghai Chinese populations consume approximately 40 mg or more [153]. Moreover, the prostate cancer incidence in Asian immigrants moved to Western countries was found to be higher compared to the one from their countries of birth [154]. Also, when Asian women migrate to the West, their daughters born in the West have a higher risk for developing breast cancer compared to their mothers [152]. This growth can be attributed to dietary patterns and underlines, once more, the importance of nutritional factors in cancer development.

Concerns related to the potential proliferative effects of soy isoflavone on breast cancer cells have emerged with *in vitro* data, when the effects of isoflavones were tested on estrogen receptor positive breast cancer cell lines. According to several cell-based studies, isoflavones, and, especially genistein, exhibited a dose-dependent effect. When estrogen-dependent cells were exposed to relatively low concentrations of genistein (0.01–10 μM), the cell growth and proliferation were promoted, while higher concentrations of genistein (>20 μM) have displayed inhibitory effects. In contrast, in estrogen-independent breast cancer cells, this twofold effect was not observed. In these cells, isoflavones have induced only antiproliferative effects, particularly after high dose exposure [155–158]. These observations have concluded that genistein can induce estrogenic and antiestrogenic effects (depending on the dose), but other cytotoxic mechanisms might be involved as well.

The dual effect of genistein observed in *in vitro* studies was confirmed using a postmenopausal animal model. In low-estrogen conditions, dietary genistein acted in a cumulative manner to stimulate cell growth, suggesting that consumption of genistein-rich products might not be completely safe for postmenopausal women diagnosed with estrogen-dependent breast cancer [159]. However, rodents have higher circulating concentrations of biologically active genistein compared to humans due to a different phase II metabolism of isoflavones. Thus, gaining insight into the effects of isoflavones, especially genistein, using a mouse model might cast doubt and conclusions might not be extrapolated to humans [160].

Behind the antiestrogenic effect, isoflavones can act through a plethora of cellular and molecular mechanisms to inhibit breast cancer cell growth. Genistein was shown to interfere in cell proliferation and survival by blocking important signaling pathways such as NF-κB pathway activation or PI3K/Akt/mTOR pathway. Moreover, genistein can trigger cell apoptosis, promote antioxidant defense and DNA repair, and inhibit the progress of tumor angiogenesis and metastasis. The aglycone can also interfere in other important ER-independent signal transduction pathways [157].

The non-cytotoxic effects of genistein and other isoflavones have been validated by the extensive clinical and epidemiologic data. Clinical trials consistently showed that isoflavone consumption does not adversely affect the markers of breast cancer risk. Furthermore, soy intake after breast cancer diagnosis significantly reduced the cancer recurrence and improved the overall survival [161].

A recent notification made by the American Cancer Society (ACS) reassures breast cancer survivors that soy foods are healthy and safe. Moreover, the health benefits of soy consumption appear to outweigh any potential risk and eating traditional soy foods such as tofu, tempeh, miso, and soymilk may even lower the risk of breast cancer, especially among Asian women. Beyond its isoflavone content, soy also represents an excellent protein source. However, patients are advised against taking soy supplements, which contain much higher isoflavone concentrations than food, until more research is done [162].

A similar position was adopted also by the American Institute for Cancer Research, which states that consumption of moderate amounts of soy foods does not increase a breast cancer survivor's risk of recurrence or death. The institute also explains that a moderate amount of soy consumption is represented by 1–2 standard servings (one serving averages about 7 g of protein and 25 mg isoflavones) daily of whole soy foods (tofu, soy milk, edamame, and soy nuts). According to studies, up to 3 servings/day—up to 100 mg/day of isoflavones—consumed in Asian populations long-term does not link to increased breast cancer risk [163].

4.2 Soy isoflavones and cardiovascular diseases

Clinical trials have shown that soy isoflavones can attenuate blood pressure, but this effect is more probable to occur in hypertensive or equol-producing individuals [164]. Also, a daily average consumption of 30 g soy protein was associated with a significant improvement in lipoprotein risk factors for coronary heart disease [165]. Similar to soy proteins, soy isoflavones were also shown to improve cardiovascular disease risk markers. Apparently, supplementation of soy protein with isoflavones (15 g soy protein with 66 mg isoflavone) for 6 months significantly improved the cardiovascular markers in women during the early menopause compared to soy protein alone (15 g soy protein) [166].

The molecular mechanism explaining the cardiovascular effects of soy isoflavones are multiple. First, isoflavones can mimic estrogen action and interact with estrogen receptors inducing receptor conformations similar to the action of selective estrogen receptor modulators (SERMs). Moreover, isoflavones can promote the activation of endothelial nitric oxide synthase in blood vessels through signaling pathways such as ERK1/2, PI3-Kinase/Akt, and cAMP. Apart from vasculature, isoflavones can also have a renal mechanism, increasing renal blood flow and sodium excretion. Finally, soy isoflavones can have humoral mechanisms, interacting with the renin-angiotensin-aldosterone axis [167].

The cardioprotective effects of soy have been demonstrated in multiple clinical trials, which have finally led to the FDA approval of soy consumption in order to lower the cardiovascular risk. According to 101.82 FDA health claim "Soy protein and risk of coronary heart disease (CHD)," the daily dietary intake level of soy protein that has been associated with reduced risk of coronary heart disease is 25 grams or more per day of soy protein. Moreover, when soy protein is included in a low saturated fat and cholesterol diet, it helps lower blood total and LDL cholesterol levels [168].

In 2017, the FDA proposed to revoke the healthy claim released in 1999 for soy protein, citing mixed results in more recent studies of the heart benefits of soy. However, a recent cumulative meta-analysis of the data selected by the FDA indicates continued significance of total cholesterol and low-density lipoprotein cholesterol reduction after soy consumption [169].

4.3 Soy isoflavones in diabetes mellitus

There are multiple animal and cell-culture studies demonstrating that soy isoflavones, and particularly genistein, exert anti-diabetic effects at physiologically relevant concentrations (<10 μM) [170]. However, a clear conclusion over the anti-diabetic properties of soy isoflavones has not been drawn.

In humans, data on soy isoflavone intake are relatively limited. Still, most studies have linked soy consumption to positive outcomes on glycemic control and insulin resistance. A meta-analysis of observational studies suggested an inverse association between soy food consumption and risk of type 2 diabetes, especially in women and

Asians [171]. This is in accordance with another study, which suggested that post-menopausal women who consumed a high soy diet had a lower fasting insulin, compared with those with no daily genistein consumption. Besides, women with high genistein intake had a significantly lower body mass index and waist circumference [172]. Recently, another study has drawn a similar conclusion, that dietary soy intake is inversely associated with risk of type 2 diabetes in Japanese women, but not in men [173].

As a molecular mechanism, most studies showed that genistein treatment increased β-cell proliferation in cell culture models and reduced apoptosis, protecting against β-cell mass destruction. The exact mechanisms appear to involve cAMP/PKA, NF-κB, and ERK-1/2 pathways signaling pathway and several studies suggested an effect on epigenetic regulation of gene expression. Furthermore, genistein has been shown to protect against oxidative stress and inflammation, and to enhance glucose homeostasis through stabilization of pancreatic β-cell function [170, 174].

Although many studies have investigated the benefit of soy isoflavone consumption of blood glucose, well-designed studies are needed to fully understand the underlying mechanisms and evaluate the exact effects of soy isoflavones on diabetes.

In April 2018, the American Diabetes Association (ADA) released a nutrition report with eating recommendations to help manage and prevent diabetes, and also to prevent complications such as heart disease. In this report, there are no amendments related to soy consumption for diabetic patients. The only specific remark is for patients with diabetic kidney disease and macroalbuminuria, who can change to a more soy-based source of protein in order to improve the cardiovascular disease risk factors but proteinuria is not altered [175].

4.4 Soy isoflavones and osteoporosis

The connection between soy consumption and bone health has emerged with epidemiologic studies, which found that Asian women have a lower hip fracture incidence in the elderly compared to Caucasian women. Later, it was confirmed that consumption of soybean and soy-based products, much higher among Asians, could potentially lower the bone loss rate and decrease the risk of fracture [176].

To date, the exact effects of dietary soy isoflavones on osteoporotic bone loss remain inconclusive, and results vary from study to study. Most studies, performed *in vitro* or using animal models, have found an inverse relation between the consumption of soy isoflavones and the percentage of bone loss. As an example, genistein was shown to reduce biochemical markers of bone metabolism, to prevent trabecular bone loss, and affect thyroid follicular cells in a male rat model of osteoporosis [177].

In humans, a post-hoc analysis of a multicenter randomized controlled trial suggested that genistein may be useful not only in postmenopausal osteopenia, but also in osteoporosis. Also, genistein has possible implications for the reduction of fracture risk in postmenopausal women with osteoporosis. These effects seem to be time-dependent and a long-term intake of genistein will produce ongoing effects on bone health [178].

The exact regulating model of soy isoflavones is still unclear, but mechanisms usually imply stimulation of bone formation and/or inhibition of bone resorption. Specifically, genistein was found to retard bone resorption by decreasing the viability of 1,25-dihyroxyvitamin D-induced osteoclasts. Other mechanisms implied enhanced bone formation by increasing serum osteocalcin concentration,

femoral insulin-like growth factor 1 mRNA transcription, and serum alkaline phosphatase activity [176].

However, clinical trials outcomes are still conflicting and more well-designed studies are warranted to delineate the underlying mechanisms, the efficacy, and safety of soy isoflavones in osteoporosis. Perhaps due to these current uncertainties, the National Center for Complementary and Integrative Health (NCCIH) declared that soy isoflavone combinations do not lower the rate of bone loss in Western women during or after menopause [179].

4.5 Soy isoflavones and menopausal symptoms

The use of soy-based foods or soy supplements in alleviating menopausal symptoms such as hot flashes, night sweats, and vaginal dryness has long been a controversial subject. A systematic review and meta-analysis published in 2016 has shown that individual phytoestrogen interventions such as dietary and supplemental soy isoflavones were associated with improvement in daily hot flashes and vaginal dryness score, but no significant reduction in night sweats. However, the study concludes that further rigorous studies are needed to determine the exact association of plant-based and natural therapies with menopausal health [180]. Also, a recent analysis concluded that frequent consumption of soy products (e.g., soy beans, tofu and tempeh), but not soy milk, may be associated with a reduced risk of subsequent vasomotor menopausal symptoms [181]. In contrast, a Cochrane systematic review determined that there is no conclusive evidence that phytoestrogen supplements effectively reduce the frequency or severity of hot flushes and night sweats in perimenopausal or postmenopausal women. Still, the study admits that genistein concentrates might pose beneficial effects, which should be further investigated [182].

The 2011 North American Menopause Society report on the role of soy isoflavones in menopausal health has concluded that initial treatment with soy-based isoflavones is reasonable for stressful vasomotor symptoms in postmenopausal women. The starting isoflavone dose should be 50 mg/day or higher, for at least 12 weeks. Supplements providing higher proportions of genistein or S(Y)-equol may provide more benefits. If a woman responds to isoflavone supplementation, treatment can continue with monitoring for side effects, but if a woman does not respond after 12 weeks, other treatment options should be discussed. The report also emphasizes on the urge of larger clinical studies aimed to investigate the exact role and mechanisms of isoflavones in postmenopausal women [183].

5. Polysaccharides' contribution to health

Polysaccharides are natural polymers, found in various plants, algae, animals, and microorganisms. These polymers have exceptional properties and essential roles to sustain life. They are an important class of polymeric molecules composed of long chains of monosaccharide units bound together by glycosidic linkages [184]. General classification of polysaccharides is highly diverse; they are classified in different ways, based on their composition, function, and origin [185].

Therefore, an overview of the main polysaccharides, including their potential food and medical applications, is presented in **Table 2**. Depending on the single sugar moieties (glucose, galactose, fructose, mannose), polysaccharides are classified in two groups: (1) *homo-polysaccharides*, which contain only one kind of polymerized sugar unit like starch, xylan, galactan, and froctan, and (2) *hetero-polysaccharides,* containing two or more kinds of sugar units such as pectin [186].

Polysaccharides	Major sources	Applications in health care	Ref.
Starch (amylose/ amypectin)	Cereals, tubers, legumes	Starch esters—matrix former in capsules for medical application Maintaining human colonic function and preventing colonic disease	[189] [188] [186] [185]
Cellulose	Fungi, algae, fruit and vegetables	Oxidized cellulose and regenerated cellulose are widely used as excellent hemostatic materials in various surgical operations and postsurgical adhesion prevention layers Antitumor, immunostimulant, wound healing, and adhesion—prevention properties Drug delivery systems	[187] [198] [190]
Inulin	Chicory root, wheat, onion, garlic	Hypolipidemic effects, prebiotic properties which influence gut microbiota Reduces the plasma total cholesterol, LDL-cholesterol, triglycerides, and increases HDL-cholesterol concentrations Decreases adipose tissue pro-inflammatory cytokines	
Pectins	Citrus peel and apple pomace Spruce bark, mango waste	Gelling and thickening agents In the pharmaceutical industry, as an excipient due to its non-toxicity Specific drug delivery Immunomodulating activities In tissue engineering applications for bone cells culture	
Xylans	Beechwood Perennial plants, fruit, legumes, and nuts	Adsorption, separation, and drug release applications Wound dressing and antimicrobial agents Anticoagulant properties, anti-inflammatory and anticancer effects Immunomodulating activity	
Alginate	Brown seaweed	Cartilage regeneration agent Microencapsulation agent Drug delivery system, bionanoreactors, nanofiltration, and biosensors	
Chitin/chitosan	Shells of crabs and shrimp, cuticles of insects	Target drug delivery In oral administration for lowering serum cholesterol concentration and hypertension Orthopedic/periodontal materials, wound-dressing materials, tissue engineering Drug delivery systems Hemostatic action, anti-inflammatory effect, antitumoral antibacterial, and fungicidal properties Antibacterial coating	
(Galacto) glucomannans	Guar, locust, and carob beans (seeds), fungi and alga, spruce and *Aloe vera*	Thickeners and stabilizers agents Drug delivery system Anticoagulant and antithrombotic drugs Immunomodulating and radical-scavenging activities	
Xyloglucan	Tamarind seed and most land plant	Hypocholesterolemic and hypotriglyceridemic effects Antitumor activity Drug delivery system Gelling and thickening agents	

Table 2.
Main groups of polysaccharides, their origin and applications.

Polysaccharides, in many forms, play a central role in all living organisms for supply and storage of energy and/or structural integrity and protection of cells. Polysaccharide-based substances are increasingly used in health and cosmetic products manufacturing, food and feed production, and for obtaining cellulose-derived materials [187].

Recently, there has been an increased interest for polysaccharides use in various novel applications due to their biocompatibility, biodegradability, non-toxicity, and several specific therapeutic activities [188]. The relationships between polysaccharides, the effects of processing on their structures and interactions, and their behavior in the gastrointestinal tract are crucial for elucidating the relationships between diet and health [189]. Many foods contain a great number of polysaccharides that cannot be completely digested by the digestive system. These indigestible polysaccharides can be called dietary fibers [186]. The class of polysaccharides such as pectin, inulin, and gums are able to slow the food movement in the digestive tract and to slow the sugar absorption from food into blood. The specific action of polysaccharide at digestive tract is given by the fermentable process. Prebiotics are selectively fermented ingredients that result in specific changes of the gastrointestinal microbiota. They improve the mucosal barrier function of the intestine by reducing the expressions of pro-inflammatory cytokines [190].

Therefore, regular consumption of polysaccharides is suggested to beneficially enhance the gut physiology and the metabolic balance by influencing metabolic functions [185]. Intestinal microbiota degrades the polysaccharides to produce metabolites and many intestinal bacteria can use these polysaccharides as unique carbon sources during the fermentation process. An *in vitro* study, which simulated the human colonic fermentation and used two types of indigestible polysaccharides (apple pectin and inulin) as energy sources to three different human bowel microorganisms, showed that the low degree of polymerized inulin positively modulated the intestinal microbiota and improved the flora diversity [186]. However, pectin may also treat or prevent several diseases/disorders such as intestinal infections, atherosclerosis, cancer, and obesity. The oral administration of β-glucan reduced the intestinal inflammation levels and exerted a protective effect on other intestinal diseases and symptoms, especially celiac disease and constipation [191].

Incidence of inflammatory bowel disease has increased considerably in recent years. Therefore, the development of a new adjuvant therapy strategy that may involve natural sources such as dietary modifications is a challenging task [190]. Non-starch polysaccharides such as pectin, cellulose, hemicellulose, β-glucan, pentosane, and xylan are selected targets to reduce the incidence of inflammatory bowel disease, due to the resistance to hydrolyzation by endogenous digestive enzymes of human [192]. The inflammatory symptoms were decreased after the oral administrations of a guar gum or partially hydrolyzed guar gum mixture, a pectin-type polysaccharide; also, the bowel movement, stool consistency, the abdominal pain and diarrhea were improved [186, 193].

Current research shows that the immune-stimulating and immune-modulating functions [194, 195] of polysaccharides; these polysaccharides are called bioactive polysaccharides, they can also stimulate the immune system against cancer cells by increasing immunoglobulin. [186]. The apple-derived pectin is one of the polysaccharides that have been reported to ameliorate metabolic syndrome, and it reduces body weight and the excessive accumulation of fat. Also, the exopolysaccharides isolated from Kefir grains present the same effects as pectin. [186]. Studies have shown that moderate intakes of dietary fiber like polysaccharides can effectively lower risks for developing diabetes. [188]. Diabetes mellitus is a chronic metabolic disease characterized by dysfunctions of carbohydrate, lipid, and [A1] lipoprotein metabolism, which affects approximately 4% of population

worldwide and is expected to increase in next decades [186, 196]. Oral administration of β-D-glucans and other soluble non-starch polysaccharides, such as arabinoxylans, had anti-diabetic activities. The major effect of soluble non-starch polysaccharides in slowing glucose absorption is therefore of considerable benefit in terms of diabetes risk and management but also has implications for overall starch digestion [197]. In recent decades, new exploitation of polysaccharides and their derivatives focused on tissue engineering applications, such as biological signaling, cell adhesion, cell proliferation, cell differentiation, and cell responsive degradation. The obtained results showed that a variety of polysaccharides, such as alginate, chitin/chitosan, cellulose and starch and their derivatives, have been developed as biomaterials for tissue engineering applications. For example, chitin/chitosan possesses the requisite properties to act as a scaffold for tissue engineering, regarding their degradability, immunogenicity, and mechanical strength [188].

5.1 Effects of processing on polysaccharides structure and composition

In the initial processing stage, there are several factors that may trigger important modifications of polysaccharide properties. A careful attention paid to these factors is essential in establishing the polysaccharides use in food and biomedical applications. Mechanical fractionation has action on crystalline structure of starch. Dehulling and milling of cereal grains and peeling and chopping of potatoes cause physical damage to a proportion of starch granules. However, this type of starch damaged possesses a water absorption capacity 10 times greater than native starch and it is more prone to gelatinization with implications for end-use properties and digestion [189, 199].

Thermal degradation has an important action with respect to dynamic distribution of polysaccharides' molecular weight. High temperature accelerated the degradation of high-molecular weight polysaccharides to low-molecular weight oligosaccharides and monosaccharide. Thermal processes induce two different major reaction pathways, such as the Maillard reaction, which takes place in the presence of amino acids, and caramelization, that occurs when simple sugars are heated at high temperatures [200, 201]. The predominant products of thermal decomposition of pure starch in toasted bread are the dehydrated oligomers of glucose and individual molecules of dehydrated glucose, which are involved in the Maillard reaction [189]. However, in the case of starch, the thermal decomposition showed no significant relationship between microstructure (crystallinity, granule size) and the thermal degradation process [189, 202]. Heating to higher temperatures of β-glucan solutions induces depolymerization. Similarly, β-glucan in food products that are heat treated at higher temperatures (100) has been shown to become depolymerized as a result of the processing, which was also interpreted to influence their beneficial health effects [189, 203]. It should be noted that many dietary products containing polysaccharides are processed by thermal treatment, and the chemical structure of the carbohydrates is dramatically altered by heat treatment [203, 204]. The main effect of physical modification is to truncate the original polysaccharide backbone to get fragments with lower molecular weights and only cause some conformational changes.

Microwave exposure could degrade polysaccharide structure, and thus increase solubility and biological activity. Microwave heating is described as more homogeneous, selective, and efficient as compared to conventional heating, resulting in faster reactions with fewer or no side products. The polysaccharide degradation in a microwave oven is generated by the interaction between electromagnetic field and chemical constituents of polysaccharide, due to molecular vibration and intense friction [205].

New applications of microwave heating were used in the grafting modifications of polysaccharides, with the precise control of the graft polymer. Microwave irradiation can be a method for the development of valuable products with tailor made properties [206]. It has been shown that the properties of microwave-synthesized graft polysaccharides are normally superior to the derivatives synthesized conventionally, but it still requires very careful control of reaction parameters to obtain polysaccharides with suitable properties and grafting efficiency.

Microwave application has advantages of economical usage of time and power energy, and also, it is easy to operate [205–207]. Another type of physical treatment is application of ultra-high pressure widely used in food and medicine. Depolymerization is the main effect caused by the application of high pressure treatment on polysaccharides; it was shown that the effect of high pressure was found to be dependent on the structure and conformation of the polysaccharides and strongly on their structure: globular branched structures similar to gum arabic are nearly unaffected, while linear stiff polymers undergo depolymerization [208, 209].

Gum arabic was found not to be affected by the high pressure treatment, probably because of its branched and globular structures [208]; the same effect was also identified on cellulose [210]. Radiation processing of natural polymers has received much less attention over the years because most of the natural polymers undergo chain scission reaction when exposed to high-energy radiation and because of the difficulty in processing natural polymers in various forms and sizes [211]. There have been many reports about the effects of gamma irradiation on the degradation of polysaccharides, including the treatments of chitosan [211], cellulose [212], β-glucan [213], and so on. Gamma irradiation improved the solubility and decreased the viscosity of β-glucan by the radiolysis of the glycosidic bonds, and this effect was dependent upon the absorbed dose. Therefore, gamma irradiation could be used in commercial processes as an effective method to resolve the physical problems involved in the use of β-glucan with high viscosity and low solubility [213]. Regarding the effect of gamma irradiation on starch, the result showed increased water solubility and water absorption and, also, an increase of antioxidant activity [205].

Besides new technologies based on polysaccharides, irradiation can be used for the decontamination of food and food additives as well as for the sterilization of materials containing polysaccharides. The irradiation of polysaccharide-containing systems has already found or has potential to find use in plastics technology, in nanotechnology, in medicinal and pharmaceutical areas, in the food industry, and in the chemical and other technical industries [214].

6. Marine bioactive compounds: functional properties

Seafood products are considered inherently functional due to their many valuable compounds and bioactive molecules possessing health benefits [215, 216]. Bioactive components can be isolated from seafoods and seafood co-products and further added to various foods to enhance their functionality in terms of human health [217]. According to recent studies, biologically active protein and lipid compounds can be extracted from fish and other marine organisms like sponges, tunicates, sea hares and slugs, soft corals, bryozoans, as well as marine animals and seafood side streams.

The bioactives with strong health-promoting effects include vitamins, fish muscle proteins, marine peptides and depsipeptides, collagen and gelatin, fish oil, PUFAs, etc. [218–220]. Some of these bioactive components are of particular

pharmaceutical and nutraceutical interest due to claimed health benefits [221]. A big part of marine bioactive compounds has been isolated, characterized, and further modified for the development of analogs with improved activities [222–224].

Bioactive peptides extracted from marine organisms and seafood by-products have been reported to possess various activities, including antimicrobial, immunomodulatory, antithrombotic, antioxidant, mineral binding, hypocholesterolemic, and antihypertensive actions [225]. These bioactive compounds can also be used in diverse therapeutic applications for the prevention and/or treatment of chronic diseases, as well as modulation and improvement of physiological functions [224]. The following extraction methods have been mainly used to obtain lipid and protein bioactive ingredients from seafood sources: solvent extraction, heating/cooking, enzymatic hydrolysis, and microbial fermentation of marine proteins. However, heating and enzymatic hydrolysis are the most preferred methods in the food and pharmaceutical industries due to lack of residual organic solvents and/or toxic chemicals in the end products [224, 226].

Fish is a rich source of valuable protein and lipid components worldwide [227]. Moreover, fish muscle proteins possess the potential of providing bioactive peptides to the food, pharmaceutical, and nutraceutical industries [228]. Other marine sources for bioactive peptides include sponges, ascidians, tunicates, and mollusks. A number of these marine species have been studied in depth for presence of bioactive peptides and depsipeptides, including clinical assays, and an extensive group of bioactive peptides has been found [224].

The reported group of bioactive peptides includes compounds with antitumor activities such as Aurilide from tunicate *Dolabella auricularia* [229], Didemnin from tunicate *Tridemnum sp*. [230], Homophymines from sponge *Homophymia* sp. [231], Trunkamide A from ascidian *Lissoclinum* sp. [232], and Keenamide A from mollusk *Pleurobranchus forskalii* [233]. Antiproliferative bioactivities were found in Mollamide from ascidian *Didemnum molle* [234] and other bioactive peptides such as Geodiamolide H, Phakellistatins, and Jaspamide isolated from sponges of the genus *Geodia* sp. [235], *Phakellia carteri* [236], and *Jaspis* sp. [237], respectively. The most preferred method to extract bioactive peptides is enzymatic hydrolysis. Enzymatic hydrolysis results in several peptides with different bioactivities, which offers a huge potential to use them in pharmaceuticals and nutraceuticals.

The biological activity of small peptides present in protein hydrolysates depends on their molecular weight and amino acid sequences [238]. A fractionation step is generally applied to crude hydrolysates to separate individual peptides by using different techniques, such as gel permeation chromatography or reverse-phase high-performance liquid chromatography (RP-HPLC) [219, 239]. Bioactive peptides recovered by enzymatic hydrolysis are usually consisted of 2–20 amino acid residues, and their activities are influenced by their amino acid composition and sequence. A high number of hydrolyzed proteins extracted from seafood by-products have been assayed for various bioactivities, such as antioxidant, antiproliferative, antitubulin, and cytotoxic activities [225]. These biological activities can possess anticancer potential, providing the opportunity to use the recovered peptides in cancer therapy [224]. As mentioned above, seafood side streams and co-products resulting after fish processing are rich sources of valuable protein ingredients for further exploitation in the production of new products such as feed, functional foods, cosmetics, and nutraceuticals [240].

Various seafood rest raw materials such as heads, skin, cut-offs, frame, bone, and viscera can be utilized to isolate a number of bioactive protein ingredients [241]. Fish rest raw material resulting after filleting contains high amounts of high-value proteins containing all essential amino acids. Enzymatic hydrolysis can be used to obtain fish protein hydrolysates (FPH) for further isolation of bioactive

peptides. FPH have been shown to contain peptides with, for example, immunostimulating and blood pressure-lowering (ACE-inhibiting) properties, in addition to antiproliferative (antimicrobial), anticoagulant, and immunomodulatory effects. These peptides may be used in novel formulations of nutraceuticals and cosmeceuticals [242]. Bioactive peptides generally include 3–20 amino acid residues and their biological activities are based on their molecular weights and amino acid sequences.

Fish bones and skin are a good source of gelatin and collagen. Collagen finds wide applications in pharma/nutra/cosmeceutical, biomedical, tissue engineering, and film/coating industries either as collagen polypeptide/peptide or gelatin (denatured form of collagen consisting of low-molecular weight peptides and proteins) [243–245]. Gelatin is formed from collagen polypeptide chains by partial thermal hydrolysis. Thus, gelatin is a denatured form of native collagen. Gelatin has been reported to possess unique rheological properties including gel strength, thermal stability, and viscoelastic properties [246]. It is widely applied in the food industry as gelling agent to improve the texture, water-holding capacity, and stability for certain food products. Gelatin can be also used as carrier of active substances such as antimicrobials, antioxidants, flavors, and colors, for production of coatings, as well as microencapsulation of bioactive compounds in pharma-/nutraceuticals [247].

Nevertheless, collagen is characterized by greater mechanical strength, higher enthalpy and resistance to protease hydrolysis, as well as more dense structure with more rigid and firm fibril networks compared to gelatin [248]. Collagen can be successfully used both as a drug carrier and in the treatment of hypertension and pain associated with osteoarthritis, in tissue engineering and inhibition of angiogenic diseases, as well as in production of wound dressings and skin substitutes [249]. Marine collagen and gelatin have attracted a great interest for their unique properties and potential applications in pharma/nutraceuticals, cosmeceuticals, and food manufacturing. The extraction and characterization of collagen and gelatin has been reported from different fish species, including hake [250], ocellate puffer fish [251], Pacific [252], Baltic cod [253], logbarbel catfish [248], Jumbo squid [254], golden goatfish [254], red tilapia and barramundi [255], rainbow trout [256], albacore tuna [257], African catfish [258], Atlantic salmon [259], channel catfish [260], bluefin tuna [261], and others.

As mentioned before, using specific physical and chemical pre-treatments (heat, enzymes, etc.) followed by tailored extraction procedures, seafood rest raw materials might provide bioactive protein components (protein hydrolysates, bioactive peptides, collagen/gelatin, etc.). Some peptide fractions can be individually isolated from hydrolysates using tailored biotechnological processes for potential applications in various industries. Recovery and separation of low- and high-weight peptides from different protein fractions resulting from seafood co-products could provide valuable streams for exploitation in different sectors. The complete exploitation of seafood side streams is often compromised by their high susceptibility to microbiological spoilage and oxidation, as well as proliferation of pathogenic agents. In addition, despite a potential to recover bioactive proteins from seafood side streams, there is still a challenge to meet the growing consumer demands for sensory characteristics of the recovered protein ingredients and foods prepared thereof.

Moreover, a crucial step in the transformation of seafood co-products into new protein ingredients is the use of technological processes that permit the production of microbiologically and biochemically stable ingredients, while minimizing loss of bioactive, nutritional, and functional properties. Marine lipids play role of valuable components of cell membranes, while being carriers of fat-soluble vitamins and

energy providers. They also serve as an excellent source of polyunsaturated fatty acids (PUFAs) such eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are part of the omega-3 group. These fatty acids cannot be found in plant sources. Plant oils contain another type of omega-3 fatty acids called α-linolenic acid, which is a metabolic precursor of the omega-3 fatty acids found in fish and fish oils [262].

Although human body is able to convert dietary α-linolenic acid into eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids found in marine lipids, this conversion cannot be considered efficient for modern consumers proffering typical Western diet rich in saturated fat and omega-6 fatty acids [263]. Thus, consuming foods rich in α-linolenic acid, our tissues are exposed to very little action of EPA and DHA. Regardless the fact that some biological activity has been associated with the action of plant-derived omega-3 fatty acids, the main health benefits are attributed to the conversion of α-linolenic acid to EPA and DHA [262]. Omega-3 fatty acids are mainly found in the body of fatty fish (salmon, mackerel, herring, tuna, etc.), the liver of white lean fish (cod), and the blubber of marine mammals feeding on phytoplankton or other aquatic species containing these fatty acids [220]. Microalgae play role of the main generators and suppliers of omega-3 fatty acids to the whole marine ecosystem [264]. In addition, omega-rich algal oils can be obtained via fermentation processes or from kelp and seaweed [265] and are currently in huge demand due to a number of positive effects and industrial applications [266] in the food and feed industries, fisheries, aquaculture, agriculture, pharmacy, and cosmetics. The omega-3 fatty acids that are highly sought after by the nutraceutical and pharmaceutical industries are cis-5,8,11,14,17-eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA).

They are essential components of a healthy diet and are indispensable for the proper development and function of the nervous system, brain, and eyes, as well as serve as a preventative for cardiovascular diseases and inflammation [267, 268]. Their health benefits also include lowering of triacylglycerols and reducing the incidence of non-communicable diseases such as metabolic syndrome, type-2 diabetes, cancer, arrhythmias, as well as inflammatory diseases and immunomodulatory effects [220]. Other beneficial effects ascribed to PUFAs include antithrombotic, hypolipidemic, antiarrhythmic, and antihypertensive properties [269, 270]. The first studies regarding the health benefits of PUFAs have investigated the diet of the Eskimo population of Greenland, characterized by high intake of seafood rich in omega-3 fatty acids. The research performed by Bjerregaard et al. in these individuals has revealed a correlation between the high consumption of PUFA-rich foods and the low incidence of cardiovascular diseases [271]. These pioneer investigations became a starting point for further epidemiological and interventional studies on the cardioprotective role of omega-3 fatty acids [272]. PUFAs contribute to the formation of special biologically active compounds called eicosanoids, which include prostaglandins, leukotrienes, and thromboxanes [273]. Lipoxins, resolvins, and neuroprotectins with strong anti-inflammatory effects are also derived from EPA and DHA [274].

In addition, the products of PUFA metabolism participate in the maintenance of cell membrane architecture [275] and support homeostasis and vasoconstriction [276]. The cell membrane is exposed to various positive modifications under the influence of the long hydrocarbon chains and double bonds in EPA and DHA. These PUFAs enhance fluidity of the cell membrane [277] and change the distribution and size of lipid rafts in aortic endothelial cells [278]. A number of research investigations have also revealed that PUFAs can successfully prevent weight gain [279, 280]. Omega-3-rich marine lipids were shown to reduce the activity of some nuclear receptors, among which is peroxisome proliferator-activated receptor γ

(PPAR γ) regulating the transcription of several genes responsible for lipid metabolism. Thus, low PPAR γ activity leads to low fat deposits in the adipose tissue and the brain, decreasing the stimulus to consume fat-rich products [281, 282]. The main challenge associated with EPA and DHA is that these omega-3 fatty acids are highly polyunsaturated and readily undergo oxidation [283, 284]. Out of the biological context, DHA and EPL are highly sensitive to oxidation by molecular oxygen present in air due to their polyunsaturated nature. In the enzymatic path of PUFA oxidation, fatty acid oxygenases control the formation of fatty acid-peroxyl radicals. Thus, peroxyl radicals generated during the reaction of PUFAs with molecular oxygen upon biosynthesis of fatty acid peroxides as intermediates in autacoid formation are instantly reduced within the enzyme active site to form the corresponding peroxide. However, in the non-enzymatic path of PUFA oxidation, a temporarily generated peroxyl radical targets at abstracting a hydrogen atom from any of the nearby hydrogen-donating molecules. This molecule can be an antioxidant or a nearby-situated PUFA molecule if the concentration of reduced antioxidants is low enough to effectively scavenge peroxyl radicals formed [285]. At later stages of non-enzymatic peroxidation reactions, secondary lipid oxidation products are formed in the chain reactions involving fatty acid peroxides.

Generation of secondary lipid oxidation products depends on the initial formation and further consumption of fatty acid peroxides. Alkoxy radicals generated from the previously formed peroxides can be involved in reactions with conjugated dienes derived upon earlier hydrogen abstraction-promoted double bond rearrangements, thus fostering the formation of chain-shortened α, β-unsaturated aldehydes through cleavage of the fatty acid chain [285]. The entire description of the free radical-mediated oxidation reactions involving PUFAs is extremely complex and depends on many factors [286]. Thus, marine lipids exposed to oxygen are subjected to fast quality deterioration due to the free radical-mediated propagation of PUFA peroxidation. PUFA-rich marine oils are highly prone to oxidation under ambient conditions. Nevertheless, the oxidation rate can be significantly slowed down by adding and maintaining sufficiently high concentrations of antioxidants and limiting exposure to external factors such as air, heat, and light [287]. According to quality requirements established by GOED (Global Organization for EPA and DHA Omega-3 s), omega-3 PUFA-rich oils should comply with the following limits: 1) on primary oxidation: peroxide value (PV) less than 5 meq O_2/kg and 2) secondary oxidation: para-anisidine value (p-AV) less than 20, as well as a combined measurement of total oxidation comprising both the level of primary and secondary oxidation (TOTOX < 26) [288].

7. Conclusions

Today, food market is richer than ever and consumers have begun to pay more and more attention to what they consume. In this light, functional foods, also known as medicinal or pharmacological foods, have experienced a tremendous growth, as various health-related claims are dispatched on their label. One of the most appealing groups of functional foods is represented by probiotics. Although there are numerous studies that highlight good viability profiles with excellent sensorial properties of a wide range of probiotic functional foods, an obvious conclusion has not yet been drawn. Therefore, more *in vivo* studies are needed to establish a concrete relation between probiotic functional foods intake and prevention, amelioration, or treatment of specific disorders (e.g., colon cancer). In case of secondary metabolites, their pharmacological activities have been widely demonstrated in numerous researches. Apparently, these functional molecules can reduce

the incidence of developing chronic degenerative diseases, stroke, and cancer. A distinctive class of secondary metabolites, soy isoflavones provide a series of health benefits such as chemoprotective and chemotherapeutic effects, help reduce the menopause-related symptoms, prevent postmenopausal osteoporosis, or reduce the risk of coronary heart diseases.

Another important group of functional biomolecules is represented by polysaccharides. Several studies have found a positive interaction between polysaccharide-rich diet and metabolic health in the sense of reducing obesity, diabetes, and cardiovascular diseases, and also an inverse relationship between dietary fiber intake and body weight.

A similar positive impact on human health was observed after increasing the consumption of seafood and enrichment of food products with bioactive components extracted from fish, shellfish, seaweed, and seafood co-products. Therefore, for a healthy diet, it is extremely important to promote the consumption of seafood products, while reducing the consumption of high-sugar and high-fat foods.

All data presented herein aim to provide current, precise, and relevant information for nutritionists, education specialists, public health organizations, different organizations (prevention education programs), policy makers, and food industries. They represent key players that can influence consumers to make healthier food selections through labeling and nutrition information on food and beverages. Only in this way, customers will benefit from balanced and healthy diets that are essential to prevent diseases and illnesses.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 6

Nutritional Profile and Medicinal Properties of Pumpkin Fruit Pulp

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Abstract

Having high nutritional value and low cultivation costs, pumpkin fruit makes a great candidate to be used by the food industry as a functional ingredient. To prolong its shelf life and widen the array of its potential uses in food products, drying and powdering have been applied, producing pumpkin flour. Several studies have been done to optimize the drying method of pumpkin in order to preserve or reduce the loss of its nutritional constituents and color changes during drying and storage. As vacuum freeze drying produces great quality pumpkin powder and best preserves the β-carotene and phenolic contents of the fruit, it is considered an expensive technique that could be inconvenient to be used in developing countries or for cost-reduction purposes. Air drying is a cheaper technique but results in less nutrient preservation than vacuum drying. This highlights the role of pretreatments in order to reduce the loss of nutrients and produce better quality pumpkin flour. Hot water blanching followed by metabisulfite pretreatment results in the best carotenoid stability and preservation of phenolic compounds in the produced powder. Incorporation of pumpkin powder in wheat bread could increase its nutritional value by increasing the levels of dietary fiber, pro-vitamin A β-carotene, calcium, iron, and zinc and by decreasing the carbohydrate and caloric contents.

Keywords: pumpkin fruit, pumpkin flour, artisanal food

1. Introduction

Pumpkin belongs to the family *Cucurbitaceae*, genus *Cucurbita*. It is extensively grown throughout the tropical and subtropical countries, with the most common types worldwide being *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* (**Figure 1**) [1]. The giant type pumpkins tend to be *C. maxima* varieties ('Boston Marrow' and 'Mammoth'), and the miniature pumpkins tend to be *C. pepo* ('Jack-O-Lantern'). *C. moschata* is the most commonly cultivated species in Asia and United States [2]. The characteristic yellow-orange color of pumpkins is due to the presence of carotenoids that have major roles in nutrition as pro-vitamin A [3]. Pumpkins have an abundance of macro- and micro-nutrients and antioxidants that promote the human body immunity against cancer and other diseases; "*It has such nutritional potential unequal to any other single crop*" [4].

Pumpkins are high-yield fruits and their cultivation is inexpensive [5]. They are stable for 1–3 months after their harvest, but they become susceptible to microbial spoilage, moisture loss, and color changes after peeling. Thus, in order to prolong their shelf life, drying and powdering techniques have been applied. This also allows pumpkin to be used as an ingredient in manufacturing foods such as bakery

Figure 1.
(A) Cucurbita moschata Duchesne; (B) Cucurbita pepo (variety ovifera) [8].

products for quality addition [6], as the rich nutrient base of this vegetable increases the nutritional quality of baked products [7]. Pumpkin wheat composite bread has been found to have good nutritional value and sensory characteristics that could make it acceptable and well-appreciated to consumers [3].

2. Nutritional profile and medicinal properties of pumpkin fruit pulp

A growing interest in pumpkin fruit and its derived products has been taken by agriculture, pharmaceuticals, and food-processing due to its nutritional and health promoting values [9]. Many countries, such as India, China, Brazil and Argentina have been using different species of this fruit as a medicine. The Traditional Chinese Medicine considers pumpkin as being *immensely valuable for human health* [8]. The various health benefits of pumpkin nutritional components include anti-diabetic, anti-carcinogenic, antioxidant [10] and possible anti-fatigue effects [11].

The composition of fresh pumpkin is shown in **Table 1**. Additional physical and chemical characteristics of ripe pumpkin fruit are shown in **Table 2**. It must be noted though that differences in the chemical components are found between different species of pumpkin, and among cultivars grown in different regions [5]. Pumpkin fruit is composed of pulp and seeds. Pumpkin pulp contains polysaccharides, pigments, amino acids, active proteins, and minerals. Pumpkin seeds are high in lipids and proteins, and they are a good source of many elements such as potassium, phosphorus and magnesium [8]. This chapter aims at characterizing the main nutritional components of pumpkin fruit pulp and its medicinal properties.

Composition of fresh pumpkin (%)	
Moisture	92.24
Fat	0.15
Protein	0.98
Ash	0.76
Crude fiber	0.56
Carbohydrate	5.31

Table 1.
Proximate composition of fresh pumpkin [9].

Weight, g	3730.0 ± 67.71
Length, cm	32.6 ± 2.32
Diameter, cm	69.1 ± 2.05
Color	YGY
Pulp recovery, %	76.7 ± 0.006
Pulp:Skin:Seed	23:6:1
Firmness, lb./in^2	21.3 ± 0.11
Seed oil recovery, %	35.7 ± 0.003
Moisture, %	6.2 ± 0.07
Total soluble solids, °B	9.2 ± 0.06
Total sugars, %	3.9 ± 0.01
Reducing sugars, %	2.1 ± 0.02
Titratable acidity, %	0.07 ± 0.003
pH	4.5 ± 0.003
β-carotene, mg/100 g	11.2 ± 0.007
Ascorbic acid, mg/100 g	14.5 ± 0.03
Pectin, %	1.2 ± 0.01
Fiber, %	0.66 ± 0.003
Ash, %	0.52 ± 0.003
Minerals, mg/100 g edible portion	
Ca	10
P	30
Fe	0.44
Mg	38
Na	5.6
K	139
Cu	0.05
Mn	0.05
Zn	0.26
S	16
Cl	4

(n = 4), YGY: Yellow to golden yellow.

Table 2.
Physical and chemical characteristics of ripe pumpkin [2].

2.1 Pumpkin pulp polysaccharides

Many studies have been done on the anti-diabetic effect of pumpkin polysaccharides. They have been shown to decrease blood glucose and lipid levels in diabetic rats. *C. moschata* polysaccharides, which include soluble and insoluble dietary fiber, had a clear effect on reduction of serum glucose in diabetic rats. Clinical trials have also demonstrated significant reduction of post-prandial serum glucose and fasting glucose in non insulin dependent diabetes mellitus (NIDDM) subjects, after oral administration of pumpkin polysaccharides liquid and granules; and they have also shown that a daily supplement of 30 g pumpkin powder can significantly reduce blood glucose

concentrations in NIDDM patients [8]. A Protein-bound polysaccharide isolated from water-soluble substances of pumpkin fruits, was also proven to improve tolerance to glucose by reducing blood glucose levels and increasing the levels of serum insulin in alloxan diabetic rats [5].

Pectin, a complex polysaccharide, is an important structural component of the cell wall of plants. It is mainly found in the peels of pumpkin, but the pressed pulp also contains a promising amount of it. Pumpkin pectin has been reported to have remarkable effects on lowering cholesterol levels in blood plasma and reducing triacylglycerols in liver, and thus reducing fatty acids in blood. It also simultaneously decreases the rate of fat assimilation and causes quick dissimilation of fat. In addition to their hypoglycemic and hypolipidemic activities, pumpkin polysaccharides antitumor effects were investigated and observed [8].

2.2 Pumpkin pulp pigments

Pumpkin pulp pigments are widely used as additives in food products, in medicine and in cosmetics. Pumpkin pigments include carotenoids, lutein and zeaxanthin. The carotenoids are responsible for the characteristic yellow-orange color of pumpkins [8]. In fact, the yellow color of pumpkin at its young stage develops to orange in its ripened stage due to a dramatic increase by 11 fold in the carotenoid content of the fruit [12].

The high carotenoid content is one of the reasons why pumpkin is such a nutritionally valuable fruit [13]. Carotenoids are considered a major source of vitamin A which is necessary for embryonic development, growth, and normal eyesight. Pumpkin is an excellent source of pro-vitamin A carotenoids. The major carotenoid in pumpkin is β-carotene, followed by small amounts of α-carotene, lutein and lycopene [8]. β-carotene content of pumpkin varies from 1.6 to 45.6 mg/100 g [14]. Indian cultivars have 132 to 527 mg/100 g of β-carotene content [1]. Research has indicated that pumpkin could be a primary vegetable to satisfy children's needs for carotenoids [8]. Moreover, β-carotene can protect against certain cancers and is considered a *powerful ally against degeneration aspect of aging*. Analysis of β-carotene content of pumpkin fruit has also been done for its possible use in combating eye diseases [2].

2.3 Pumpkin pulp minerals, amino acids, and active proteins

The human body acquires its needed minerals from the daily diet. Minerals have key roles in several body functions. Pumpkin is considered as an eminent source of many minerals important for human health [8]. Pumpkin pulp is rich in K, Fe, Mn, Mg, P, vitamin C, vitamin E and phytosterols [2]. The pulp of *C. moschata* contains high amounts of calcium (205.45 µg/g) and potassium (1840.30 µg/g) and a low amount of sodium (28.70 µg/g), making it a suitable food for the prevention of osteoporosis and hypertension. Chrome is another mineral that is found in pumpkin in an amount higher than any other vegetable. Chrome is part of glucose tolerance factor which is essential for the activity of insulin and improves tolerance of blood glucose. Cobalt is also an essential microelement present in pumpkin. It is essential for islet cells of the pancreas and improves the body's metabolic capacity and participates in the synthetic action of vitamin B12 [8].

Protein content of pumpkin is less than 2.0% of dry matter weight. Yet, there are some essential amino acids present in pumpkin pulp. *C. moschata*, for example, contains 0.609% valine, 0.700% leucine, and 0.508% lysine, which are relatively

high amounts. Several studies of purified pumpkin extracts including proteins and polysaccharides have shown anticancer activity against melanoma, ehrlich ascites and leukemia. In addition, enzyme preparations of pumpkin have been found to possess antitumor potential [8].

3. Pumpkin flour

3.1 Characteristics of pumpkin flour

Processing of fruits or vegetables can transform these perishable foods into more stable foods that can be advantageous to both consumers and food industries. Pumpkins are consumed in various ways, whether fresh, canned, frozen, or dried. Preservation of pumpkin by drying is an important way to prevent postharvest losses. Though they keep longer than other fruits and vegetables, they can only do so if the fruits are free from any bruise. However, this is sometimes not possible because of insect bites or bruises acquired by harvesting, or by transportation after harvest [4].

Pumpkin fruit can be processed into flour which has a longer shelf-life, highly desirable -sweet- flavor, and deep yellow-orange color [2]. The rich nutrient potential of dried pumpkin makes utilization of pumpkin flour or pumpkin flour based products a good source of vitamin A from the β-carotene content, protein [4] and dietary fiber [15].

Analyses on the composition of pumpkin (*Cucurbita moschata* Decne) flour, for example (**Table 3**), show that it contains high levels of carbohydrates, starch, dietary fiber, protein, total ash, and low levels of lipids and crude fiber. Authors proposed that it is an ideal food for diabetes patients, cardiovascular disease patients, and elderly [9]. Moreover, the functional properties of the flour such as water solubility and absorption indices and the pasting properties suggest that it may have suitable applications in the food industry such as a thickener in soup, gravy, and as an ingredient in bakery products such as bread, cake and fried noodles [9].

3.2 Effect of different convective drying methods on selected characteristics of pumpkin fruit flour

Production of powders from vegetables and fruits have been done by various drying techniques such as hot air-drying, freeze drying, spray drying, vacuum

Parameter	
Moisture content (%)	3.73 ± 0.01
Fat (%)	3.60 ± 0.12
Crude fiber (%)	3.65 ± 0.14
Protein (%)	7.81 ± 0.18
Ash (%)	5.29 ± 0.01
Carbohydrate (%)	79.57 ± 0.01
Dietary fiber (g/100 g)	12.1 ± 0.00
Starch (%)	48.30 ± 0.54
Vitamin A (µg/100 g)	262 ± 0.32

Table 3.
Proximate composition of pumpkin powder [9].

drying, and microwave vacuum drying. Spray and freeze drying have been reported to produce a good quality product, but are too expensive. On the other hand, hot air-drying *could result in a quality product that is characterized by uniform, hygienic, and attractive color of dried fruit and vegetable powder* in a condition that it is not done in a rapid manner which might result in an inferior product quality [6].

A study performed by Kiharason et al. [4] aimed to determine the effect of three drying methods of pumpkin fruit slices on the nutrient integrity of certain components of pumpkin: open solar (OSD), oven electric (OED), and enhanced solar (ESD) drying methods. The drying methods were applied, then dry fruit slices were milled and analyzed to determine their nutritive value.

3.2.1 Drying of pumpkin slices

After washing, peeling and deseeding mature fruits, the fruit pulps were sliced and then cut at 2.55 cm length by 0.5 cm width. Next, they were blanched by dipping fast in boiling water for 1 min, cooled with running tap water for 1 min as well, and then wiped with absorbent paper. Afterwards, they were subjected to drying while weighing every 3 h until constant weight was recorded. The dried pumpkin slices obtained were ground, sieved and analyzed to determine their nutritive value [4].

3.2.2 The effect of the three drying methods on the moisture content and nutritive value of pumpkin powder

3.2.2.1 Moisture content

In ESD and OED (temperature set at 50°C), different shelves at which pumpkin slices were put had great variations in terms of drying time, whereas the drying time in OSD, where tables used to dry pumpkin slices were at the same height, did not have much variation. Generally, OED took the shortest time and ESD took the longest time to dry the pumpkin slices (**Table 4**). As for the moisture content (MC), milled pumpkin slices that were dried by the OED exhibited the highest MC while ESD had the lowest (**Table 6**) [4].

Drying in a solar drier occurs in a closed environment whereas open sun drying happens in the open without any barrier, leading to quicker drying. A high evaporation rate during drying leads to a high possibility of nutrient losses. In addition, open sun drying has the poorest protection against insects, dust, microbes, and is inconvenient due to certain weather conditions such as rain where samples become subject to spoilage. Both oven drying and open solar drying showed a moisture content above the acceptable safe level, which is 14%. Moisture levels of 14% and above make the food susceptible to attack by microbes and promote fungal growth, while lower

Drying method	Average drying time (hours)*	Average moisture content (%)
Enhanced solar drying	13.27[a]	12.82
Open solar drying	9.50[b]	14.91
Open drying	7.25[c]	15.15

**Means followed by the same letter within a column are not significantly different at P = 0.05.*

Table 4.
The mean drying time of pumpkin fruit slices and the average moisture content obtained by three drying methods [4].

levels slow down the growth of microbes and prolong the shelf-life. These results make enhanced solar drying a better method for longer preservation of pumpkin flour [4].

3.2.2.2 Analysis of nutritive value

Pumpkin flour obtained by oven drying OED retained the highest amount of β-carotene, followed by ESD (**Table 5**). The fast rate of drying caused less nutrients to be lost, and samples at the core were still intact by the time constant weight was achieved. OSD had the least amount of β-carotene, most likely since unprotected exposure to the sun's UV rays caused photo-degradation of the carotenoids. Protein content showed no significant differences between the three drying methods but it showed a significant difference between the flours and the fresh fruits for almost 800% increase in the flour [4].

As for mineral, drying generally was found to reduce their levels compared to fresh fruits. Zinc was significantly lower in enhanced solar drying than oven and open sun drying, yet the fresh fruit exhibited the highest amount. Calcium levels were reduced greatly after drying showing 200% loss from fresh fruit. Whereas for iron, calcium and energy levels, no significant difference was noted between all treatments. It is therefore concluded, in this study, that enhanced solar drying is the best method for drying pumpkin and obtaining better quality pumpkin flour [4].

Treatment	β-carotene (µg/g)*	Protein (%)	Zinc (ppm)	Iron (ppm)	Calcium (ppm)	Energy (kcal/g)
Fresh fruit	16.6150^{c}	2.6175^{b}	44.075^{c}	94.5000^{a}	1116.82^{a}	4.26575^{a}
Oven dried	74.8425^{a}	13.7850^{a}	24.948^{a}	66.3225^{a}	830.23^{a}	3.84675^{a}
Enhanced solar	62.9875^{ab}	16.4875^{a}	9.058^{b}	49.5400^{a}	539.08^{a}	3.76350^{a}
Open sun	27.1750^{bc}	16.4900^{a}	20.995^{ba}	94.7975^{a}	525.43^{a}	3.62875^{a}
F-value	8.497	58.832	17.616	1.595	1.705	2.376
P-value	0.003	0.000	0.000	0.242	0.219	0.121

**Means followed by the same letter within a column are not significantly different at P = 0.05.*
ppm parts-per-million, 10^{-6}.

Table 5.
Nutrient levels of fresh pumpkin fruit and pumpkin flour obtained from three drying methods [4].

Temperature (°C)	MC_{db} of fresh pumpkin	MC_{db} of dried pumpkin powder	a_w of fresh pumpkin	a_w of dried pumpkin powder
50	82.10	10.21^{a}	0.98	0.65^{a}
60	82.58	7.46^{b}	0.95	0.42^{b}
70	84.09	5.47^{c}	0.97 ns	0.30^{c}

Data are expressed as mean values. Mean values with different superscripts in the same column differ significantly at P ≤ 0.05. The symbol ns means that the mean values are not significantly different.
MC_{db} Moisture Content – dry basis matter.
a_w Water Activity.

Table 6.
Mean values for moisture content and water activity of dried pumpkin powders prepared by hot air-drying at different temperatures [6].

3.3 Hot air-drying of pumpkin: the effects of using different temperatures on physico-chemical characteristics of pumpkin flour

3.3.1 Drying of pumpkin slices by three different temperatures

A study performed by Roongruangsri and Bronlund [6] examined the effect of three hot-air drying temperatures (50, 60, and 70°C) on physico-chemical properties and sorption characteristics of pumpkin powder after the drying process. *Cucurbita maxima* Duch., also called buttercup pumpkin, was cleaned, peeled, and deseeded. The pulp was cut into slabs with a 5 mm thickness, 40 mm length and 20 mm width. Pumpkin slices were then blanched by immersing in hot water at 95°C for 5 minutes, then cooled to room temperature. Hot-air drying was then performed in a cross-flow cabinet hot-air tray dryer at three different temperatures of 50, 60 and 70°C. Afterwards, samples were weighed to calculate the moisture content (MC_{db}), and then ground in a blender and sieved.

3.3.2 The effect of drying temperatures on the characteristics of pumpkin powder

3.3.2.1 Moisture content and water activity

Results of MC_{db} and water activity analyses showed that dried pumpkin powder produced at 70°C exhibited the lowest MC_{db} and water activity levels compared to those produced at drying temperatures 50 and 60°C, as shown in **Table 6**. The low MC_{db} and water activity levels of pumpkin powders produced at 60 and 70°C suggest a better keeping quality than those produced at 50°C, since the occurrence of most unfavorable changes of food during storage is less when water activity drops below 0.4 [6].

3.3.2.2 Color of pumpkin powder

Color of food is one of the important quality parameters since it may indicate changes in food quality due to processing, storage or other conditions. As mentioned earlier, the yellowish color of dried pumpkin powder is due to the carotenoid pigments naturally found in the pumpkin fruit. Powders produced at drying temperatures of 50 and 60°C showed lighter color retention than those produced at 70°C. Pumpkin powder produced at 50°C had the lightest color compared to that produced at 60 and 70°C, indicating that increase of drying temperature causes increase in the darkening of the color [6].

3.3.2.3 Carotenoid content

Dried pumpkin powder produced at 70°C showed the highest percentage decrease in carotenoid content (56%) compared to the decrease in those produced at 50 and 60°C (18% and 33% respectively). Decrease in total carotenoid content may be attributed to the degradation of β-carotene and other carotenoids due to auto-oxidation, since the highly unsaturated chemical structure of carotenoids makes them very sensitive to thermal degradation and oxidation [6].

3.3.2.4 Powder properties

Table 7 shows the effects of drying temperatures on bulk density, solubility, water adsorption and oil adsorption capacities of the pumpkin powder. These

Temperature (°C)	Bulk density (g/ml)	Water solubility (%)	Water adsorption (g water/g dry sample)	Oil adsorption capacity (g oil/g dry sample)
50	0.62[c]	54[a]	3.50[a]	4.42[a]
60	0.86[b]	50[b]	3.00[b]	3.97[b]
70	0.91[a]	43[c]	2.33[c]	3.87[b]

Data are expressed as mean values.
[a, b, c]Mean values with different superscripts in the same column differ significantly at $P \leq 0.05$.

Table 7.
Physical characteristics of dried pumpkin powders obtained by hot air-drying at different temperatures [6].

properties affect the functional characteristics of the powder and are critical parameters for controlling quality; fruit and vegetable powders that have high water adsorption and oil adsorption capacities can convey water-retention and fat-binding properties that are important in bakery products [6].

The results implied that higher drying temperatures have an effect of decreasing water solubility and water and oil adsorption capacities of pumpkin powder: the dried pumpkin powder produced at 50 and 60°C had a water solubility above 50%, and higher water and oil adsorption capacities compared to that obtained at 70°C. These results indicate that dried pumpkin powders produced at 50 and 60°C have more potential for baking purposes than those produced at 70°C [6].

3.4 Freeze-dried pumpkin powder

Freeze drying is a dehydration process employing two steps: freezing the food material, and sublimation of ice from the frozen material. Freeze drying is generally recommended for drying foods that have heat sensitive components such as tocopherols, carotenoids, and phenolics. It is considered a great method for drying foods of high quality where color, flavor, texture, nutrient content, taste, chemical composition and biological activity of the fresh sample only undergo minimal changes [16].

In a study performed by Dirim and Caliskan [16], it was observed that the chemical compositions such as vitamin C and total phenolics contents of dried pumpkin powder obtained by freeze-drying were not significantly different from that of fresh pumpkin. Freeze drying only reduced the total phenolic content by 3% in this study, but in the study performed by Aydin and Gocmen [17], pumpkin powder that was produced by hot-air oven scored higher than that produced by

Samples	Pretreatment
T1	Control
T2	Dipping in 0.1% Citric Acid (CA) for 15 minutes
T3	Hot water blanching at 95°C for 3 minutes
T4	Steam blanching for 5 minutes
T5	Blanching at 95°C in 1%NaCl for 3 minutes
T6	Dipping in 0.2% potassium metabisulfite (KMS) for 45 minutes
T7	Hot water blanching for 2 minutes followed by dipping in potassium metabisulfite (KMS) for 45 minutes

Table 8.
Different types of pretreatments [1].

Composition	Pumpkin flour (0 days)						
	T1	T2	T3	T4	T5	T6	T7
Moisture (%)	6.40 ± 0.005	7.38 ± 0.01	12.78 ± 0.02	12.62 ± 0.01	13.8 ± 0.05	11.44 ± 0.01	10.99 ± 0.01
Protein (%)	8.51 ± 0.01	7.25 ± 0.01	5.17 ± 0.02	6.16 ± 0.01	6.68 ± 0.01	9.54 ± 0.01	5.45 ± 0.02
Ash (%)	6.52 ± 0.02	5.70 ± 0.15	4.02 ± 0.01	6.61 ± 0.02	6.59 ± 0.03	6.04 ± 0.01	6.54 ± 0.01
Crude fiber (%)	6.58 ± 0.02	6.41 ± 0.03	6.9 ± 0.2	7.04 ± 0.02	7.5 ± 0.05	8.36 ± 0.01	12.011 ± 0.02
Minerals (mg/100 g)							
Phosphorus	241.977 ± 0.02	317.545 ± 0.02	177.449 ± 0.06	167.514 ± 0.01	28.35 ± 0.05	269.451 ± 0.02	142.988 ± 0.04
Iron	22.54 ± 0.05	16.01 ± 0.03	5.078 ± 0.00	10.075 ± 0.01	11.629 ± 0.00	18.61 ± 0.04	21.794 ± 0.01
Total carotene (mg/100 g)	2.816 ± 0.01	5.492 ± 0.03	9.196 ± 0.01	10.35 ± 0.01	2.17 ± 0.01	7.581 ± 0.00	17.769 ± 0.00
Starch (%)	30.16 ± 0.05	40.77 ± 0.01	19.8 ± 0.01	23.7 ± 0.05	22.68 ± 0.02	30.22 ± 0.03	32.14 ± 0.04
SO_2 (mg/kg)	—	—	—	—	—	—	1279.14 ± 0.03

Table 9.
Effect of pretreatments on the proximate parameters of pumpkin flour [1].

freeze drying in terms of phenolic contents and antioxidant activity. The latter study, however, showed that freeze drying reduced browning, preserved redness, resulted in a lighter color, higher water holding capacity, oil binding capacity, emulsion stability, and the highest total dietary fiber compared to oven produced powders. Color values obtained by Aydin and Gocmen [17] and Mujaffar et al. [18] supported the overall results that freeze drying was able to preserve a closer color of powder to that of fresh pumpkin, producing pumpkin powder of high quality color. Moreover, freeze drying was reported to produce higher yields of powder [18] and less carotenoid degradation [16] than that of hot-air oven drying.

Although freeze drying preserved the deep-orange color of fresh pumpkin and produced better physico-chemical properties of pumpkin flour, the cost of freeze drying application is very high [17], making oven drying a more suitable technique in developing countries or for cost-reduction purposes.

3.5 The effect of pre-treatments on selected properties of pumpkin flour

Fruits and vegetables are often pretreated in order to extend their shelf life, preserve their color and flavor, decrease the loss of nutrients and reduce activity of enzymes. In the production of dried products, pretreatments can lead to improvement of product quality and help in the inhibition of enzymatic browning (Kripanand et al., 2016). Since conventional air drying can adversely affect the color, flavor and nutritional value of the dried products, pretreatments prior to air drying are considered one of the most important factors that affect the quality of the final powder product produced by drying [17].

In order to optimize different pretreatments to obtain good quality pumpkin powder and β-carotene retention during storage, Kripanand et al. [1] performed a study employing six different pretreatments for the production of pumpkin flour from fresh *Cucurbita maxima*. The different types of pretreatments are presented in **Table 8**, where the control sample (T1) represents no pretreatment.

Results of this study (**Table 9**) showed that pretreated flour samples retained a higher moisture content compared to the control sample. Blanching was found to significantly affect the protein content, where cold pre-treated samples (T1, T2 and T3) had higher protein values compared to hot pre-treated samples. Blanching was also found to reduce starch, ash, fiber, phosphorus and iron quantities due to leaching out during the blanching process [1].

As for the carotenoid content, it was observed that chemical pretreatments lead to improvement in the amount of total carotene in pumpkin flour. But the use of blanching and sulfiting together (T7) showed a most favorable effect on total carotenoid stability T7 pretreatment also attained the highest score for color and overall acceptability, followed by T6. In addition, less browning was observed in all T7 samples during storage indicating that metabisulfite reduced the formation of browning compounds during storage [1].

4. Pumpkin wheat composite bread

4.1 Nutritive value of pumpkin wheat composite flour

Consumers are becoming more aware of healthy eating and high quality foods that contain additional health benefits. Yet, the modern consumers rely on the food industry to provide such high quality food products as they purchase more processed foods and ready meals [7]. Development of healthy products with the incorporation of fruits and vegetables represent one strategy for the production

	Wheat Flour	Pumpkin Flour
Moisture	11.1%	4.8%
Protein	12.4%	11.6%
Fat	1.4%	2.4%*
Dietary fiber	10.1%	28.3%
Crude fiber	1.2%	16.9%
Ash	0.63%	6.7%
Calcium	17.0 mg/100 g	121.7 mg/100 g
Iron	5.3 mg/100 g	7.1 mg/100 g
Zinc	2.8 mg/100 g	3.1 mg/100 g
β-carotene	—	1.8 mg/100 g

**This study does not mention whether pumpkin seeds were removed or not before drying and powdering, which might explain the higher fat content in pumpkin flour compared to wheat flour if the seeds were kept.*

Table 10.
Proximate composition of wheat flour and pumpkin flour [19].

Level (%PF)	Protein (g/mg)	β-carotene (µg/g)	Calcium (mg/g)	Iron (mg/g)	Zinc (mg/g)	Energy (kcal/g)
1 (0%)	0.1108^{b*}	1.433^{b}	0.2736^{b}	0.0216	0.0344^{b}	2.6792^{a}
2 (5%)	0.1284^{ab}	3.583^{ab}	0.2850^{b}	0.0739^{c}	0.0407^{ab}	2.4494^{b}
3 (20%)	0.1298^{ab}	3.768^{ab}	0.4549^{ab}	0.0164^{bc}	0.0512^{ab}	2.3141^{bc}
4 (50%)	0.1350^{a}	5.125^{a}	0.8063^{ab}	0.1175^{ab}	0.0551^{ab}	2.2147^{bc}
5 (95%)	0.1378^{a}	5.128^{a}	1.0113^{a}	0.1495^{a}	0.0631^{a}	2.1104^{c}
RDI (adult)	34–71 g/d	600–1300 µg/d**	1000–1300 mg/d	8–18 mg/d	8–13 mg/d	2403–3067 kcal/d
RDI (child)	13–19 g/d	300–400 µg/d**	500–800 mg/d	7–10 mg/d	3–5 mg/d	1046–1742 kcal/d

**Means followed by the same letter within a column are not significantly different at P = 0.05. PF = pumpkin flour. g/d = grams per day. mg/d = milligrams per day. Kcal/d = kilocalories per day.*
***Applies to retinol: 1 µg retinol = 12 µg β-carotene, hence RDI values should be multiplied by 12 to relate to table values.*
RDI Reference Daily Intake

Table 11.
Mean of nutrient content in pumpkin bread at five blending levels [7].

of these 'functional foods' [19]. Use of functional ingredients in bakery products for the aim of nutrient enrichment is increasingly becoming important in bakery industries [7]. Pumpkin flour has been used to supplement cereal flours in bakery products, soups, sauces, instant noodles and spices [3].

Pumpkin wheat composite flour improves the texture, nutritional value, and color of different bakery products and thus, it is likely to produce bread with improved nutritional value and good sensory characteristics by using pumpkin wheat composite flour [20]. **Table 10** compares the proximate composition of wheat flour and pumpkin flour. Pumpkin flour was shown to have higher amounts of calcium, iron, zinc, β-carotene, ash and total dietary fiber. This indicates that pumpkin flour could be used to supplement wheat flour with these nutrients for the production of higher quality bread [19].

Table 11 shows the contents of various nutrients in wheat bread supplemented with different levels of pumpkin flour. Incorporation of pumpkin flour resulted

Composition %	Control	5%	10%	15%
Moisture	32.02 ± 0.54^{bc}	32.63 ± 0.50^{c}	34.25 ± 0.08^{ab}	35.32 ± 0.06^{a}
Fat	2.59 ± 0.01^{a}	2.55 ± 0.01^{a}	2.48 ± 0.01^{b}	2.44 ± 0.01^{b}
Protein	15.72 ± 0.04^{a}	15.17 ± 0.09^{b}	14.71 ± 0.02^{c}	14.47 ± 0.06^{c}
Ash	1.83 ± 0.07^{d}	2.09 ± 0.01^{c}	2.26 ± 0.02^{b}	2.43 ± 0.03^{a}
Crude fiber	1.56 ± 0.02^{d}	2.46 ± 0.03^{c}	2.62 ± 0.01^{b}	2.90 ± 0.04^{a}
Carbohydrate	46.28 ± 0.14^{a}	45.10 ± 0.21^{b}	43.68 ± 0.05^{c}	42.44 ± 0.05^{d}
Calorie (kcal/100 g)	271.31^{a}	264.03^{b}	255.88^{c}	249.60^{d}

$^{a, b, c}$ Means in a row with similar superscripts are not significantly different at α = 0.05.
Values are the Means ± SD and n = 3 for each group.

Table 12.
Proximate composition of bread for different levels of pumpkin flour [3].

in a uniform trend of increase in protein, β-carotene, calcium, iron and zinc, and uniform decrease in energy content with increasing levels of pumpkin flour. Reduction of calories with increasing pumpkin flour levels is attributed to increased fiber content and lower carbohydrate content in the composite flour, which is a good approach in the direction of health promotion [7].

4.2 Physico-chemical properties of pumpkin wheat composite bread

The effects of adding different levels of pumpkin flour on the physico-chemical properties of bread have been studied. Substitution of higher levels of pumpkin powder in bread have been shown to decrease the fat content of the bread. This might be attributed to the lower content of fat in pumpkin flour compared to wheat flour. The same effect was observed for the carbohydrate content, as increasing the level of pumpkin flour resulted in decreased total carbohydrate content of the bread [3]. Protein content has been also shown to decrease with increased incorporation of pumpkin flour (**Table 12**) [3, 20], which opposes the results obtained by Kiharason et al. [7] (**Table 11**) that shows increased protein content with increased pumpkin flour content. This might be attributed -as mentioned in chapter I- to the different nutritional profile of different species and cultivars of pumpkin, or to the pumpkin powder preparation methods in which seeds were removed or kept. Pumpkin seeds are rich in protein and lipids [2], and thus keeping them as part of the pulp in the flour preparation process would increase the amount of these constituents in the produced powder. Ash, total fiber and reducing sugar levels increased with increasing substitution of pumpkin flour in bread [3, 21]. Increasing the level of pumpkin flour also resulted in increase of the moisture content of the composite bread which could be explained by the higher water absorption capacity of the composite flour compared to wheat flour [3].

In the study conducted by See et al. [3], incorporation of 5% pumpkin flour resulted in the highest loaf volume and specific volume compared to the other samples giving more significant softness in bread. The weight of the loaf significantly increased as increasing levels of pumpkin flour were incorporated, which was attributed to the increased water absorption capacity of pumpkin flour. Opposite results were obtained by Kundu et al. [19] where supplementation of increased levels of pumpkin flour lead to decreased water absorption (**Table 13**). The result was related to the dilution of gluten.

Dough development time, defined as *the time to the nearest half minute from the first addition of the water to the development of the maximum consistency of the dough*

Parameter	Flour supplemented with 5% pumpkin powder	Flour supplemented with 10% pumpkin powder	Flour supplemented with 15% pumpkin powder
Water absorption (%)	67.0 ± 0.0	65.0 ± 0.0	62.5 ± 0.16
Dough development time (min.)	2.5 ± 0.0	2.7 ± 0.0	4.1 ± 0.08
Dough stability	2.0 ± 0.83	3.0 ± 0.08	3.5 ± 0.08
Mixing tolerance index (BU)	70.0 ± 1.6	60.0 ± 1.6	50 ± 1.6
Time to break down (min.)	5.1 ± 0.0	6.0 ± 0.08	7.5 ± 1.6
Farinographic quality number	51.4 ± 0.0	60 ± 0.83	75 ± 1.6

Table 13.
Effect of incorporation of various levels of pumpkin powder on farinographic characteristics of wheat flour [19].

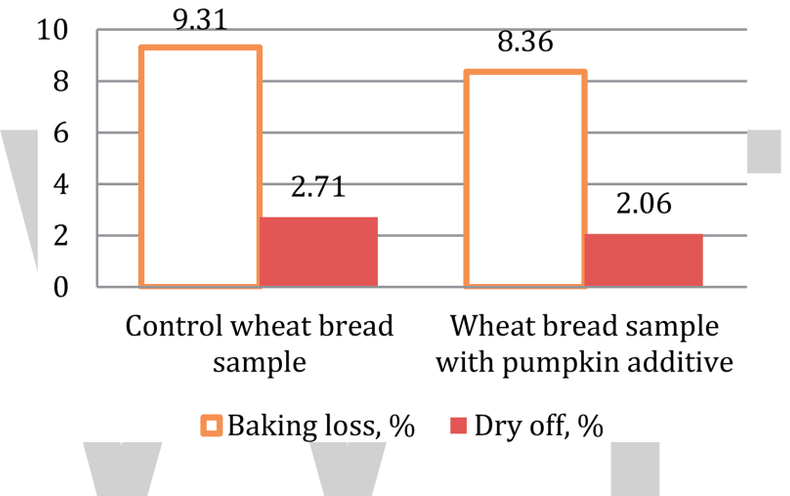

Figure 2.
The changes in the value of dry off and baking loss in bread samples [21].

increased with the addition of pumpkin flour, which was related to the difference in the physiochemical properties between the constituents of pumpkin flour and wheat flour. Dough consistency was also maintained almost at the same level after increased levels of pumpkin flour indicating that the dough was stable and had more resistance against mechanical mixing. Increased concentration of pumpkin flour also lead to decrease in mixing tolerance index indicating stronger flour, since the lower the mixing tolerance index, the stronger the flour. Extensibility and resistance to extensibility were also shown to significantly increase with increased incorporation of pumpkin flour, resulting in rubber-like properties [19].

Rakcejeva et al. [21] studied the effect of incorporating 10% pumpkin flour in wheat bread on the bread baking loss which *forms the biggest loss in technological processes*. Results showed an insignificant decrease by 0.95% compared to 100% wheat flour bread (**Figure 2**). Thus, technological bread weight loss during the addition of 10% pumpkin flour is considered insignificant. These results show that pumpkin powder supplemented bread can be used for making good quality bread.

4.3 Sensory evaluation of pumpkin wheat composite bread

Conducting tests that determine consumer acceptance, liking, preference and opinions is among the key activities that relay important information for consumer

Parameter	Control	5%	10%	15%
Crust color	6.00 ± 1.67[a]	6.07 ± 0.88[a]	5.67 ± 0.81[a]	5.33 ± 0.90[a]
Crumb color	6.13 ± 0.99[ab]	7.67 ± 0.49[c]	6.67 ± 0.49[b]	5.73 ± 0.70[a]
Moistness	5.60 ± 0.51[ab]	6.07 ± 0.80[a]	5.33 ± 0.49[bc]	5.00 ± 0.38[c]
Softness	5.93 ± 0.80[ab]	6.47 ± 0.83[a]	5.53 ± 0.64[bc]	5.20 ± 0.41[c]
Aftertaste	5.73 ± 0.59[a]	6.13 ± 0.52[a]	5.20 ± 0.41[b]	4.87 ± 0.35[b]
Overall acceptability	6.60 ± 0.74[ab]	6.93 ± 0.59[a]	6.13 ± 0.35[bc]	5.73 ± 0.46[c]

[a, b, c] Means in a row with similar superscripts are not significant different at α = 0.05.
Values are the Means ± SD and n = 15 for each group.

Table 14.
Mean value of sensory attributes of bread incorporated with different levels of pumpkin flour [3].

Treatments	Volume of bread	Crust color	Symmetry of form	Evenness of bake	Character of crust
T0	7.00[a]	7.00[a]	2.80[a]	2.90[a]	2.90[a]
T1	8.00[b]	7.00[a]	2.80[a]	2.80[a]	2.80[a]
T2	7.10b[c]	6.90[b]	2.60[a]	2.60[a]	2.60[a]
T3	6.90[c]	6.62[a]	2.20[a]	2.20[a]	2.20[a]

T0 = control (0% pumpkin flour), T1 = 5% pumpkin flour, T2 = 10% pumpkin flour, T3 = 15% pumpkin flour.
[a, b, c] Means in a row with similar superscripts are not significantly different.

Table 15.
External characteristics of bread [20].

Treatments	Grain of bread	Crumb color	Aroma of bread	Taste of bread	Mastication of bread	Texture of bread
T0	7.50[a]	8.10[a]	8.00[a]	12.60[a]	8.00[a]	12.20[a]
T1	7.50[a]	8.00[a]	7.70[a]	12.60[a]	7.60[a]	12.20[a]
T2	7.40a[b]	7.60[a]	7.50[ab]	11.00[b]	7.20[a]	11.90[a]
T3	6.80[b]	6.90[b]	6.90[b]	10.80[b]	6.40[b]	10.80[b]

T0 = control (0% pumpkin flour), T1 = 5% pumpkin flour, T2 = 10% pumpkin flour, T3 = 15% pumpkin flour.
[a, b, c] Means in a row with similar superscripts are not significantly different.

Table 16.
Internal characteristics of bread [20].

goods companies. The results of these tests help companies make product decisions concerning marketing, development of new products, reformulation of existing products, etc.. Sensory evaluation performed to assess pumpkin wheat composite bread showed the highest acceptability and preference for 5% pumpkin flour supplemented bread in the studies conducted by See et al. [3] and Pasha et al. [20]. **Table 14** shows the data of the sensory evaluation obtained by See et al. [3]. The data indicated that consumers preferred the crust color, moistness, softness and aftertaste of the 5% pumpkin flour bread and the control sample that were not significantly different. Similar results were obtained by Pasha et al. [20] where external and internal characteristics (**Tables 15** and **16**) of control bread and 5% pumpkin flour supplemented bread were only significantly different by volume of bread (higher for 5% pumpkin flour bread).

In the study performed by Rakcejeva et al. [21], the highest assessment after expert sensory evaluation was scored for 10% pumpkin flour bread, and elevated levels of pumpkin flour (over 10%) became unacceptable due to worse porosity, stickier bread soft part and unpleasantly sweet taste of bread. A higher degree of liking was also scored for 10% pumpkin flour bread over control bread: bread sample with pumpkin additive was shown to be tastier than the control bread sample.

5. Conclusion and discussion

The nutritional value of pumpkin fruit is high and exquisite, which calls for its exploitation by the food industry as a functional food. Studies have found antioxidant, anti-diabetic, anti-carcinogenic and anti-fatigue effects of pumpkin pulp nutritional components. Being a perishable fruit, means for prolonging its shelf life had to be employed. Drying is one of the methods that prolong the shelf life of food products by reducing the moisture content to inhibit the growth of microbes and thus prevent spoilage of the food material.

To preserve the nutritional value of the dried pumpkin, several drying methods were studied in an attempt to reduce the degradation of nutritive components during drying and during storage. Vacuum freeze drying was shown to be a great method to preserve the β-carotene and phenolic acid contents of dried pumpkin but is an expensive drying technique. Convective drying methods are common methods to dry food materials and are cheaper but could result in a greater loss of nutrients. To reduce this loss, the appropriate drying conditions such as drying temperature and pretreatments had to be optimized. It was found that a drying temperature of 60°C resulted in good quality pumpkin powder with acceptable water activity, β-carotene content retention, color quality and good potential for baking purposes. Drying temperatures of 50 and 70°C lead to unacceptable water activity level and greater degradation of β-carotene, respectively. Metabisulfite pretreatment of pumpkin slices preceded by hot water blanching was found to have the most favorable effect on total carotenoid stability, color, phenolic content and overall acceptability compared to several other pretreatments in the production of hot air dried pumpkin powder.

Production of pumpkin powder from dried pumpkin slices allows its supplementation into baking products –among others- to enhance their nutritional value. Development of pumpkin wheat composite bread was studied using different levels of pumpkin flour. Increasing the level of pumpkin flour incorporation into wheat flour led to increased contents of total fiber, β-carotene, calcium, iron and zinc, and it led to decrease in carbohydrate and caloric contents which is a good approach for health promotion. Incorporation of 5 and 10% pumpkin flour were found to have good dough and bread physical characteristics and the best sensory evaluation of pumpkin wheat composite bread.

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