Bioactive Ingredients in Functional Foods: Current Status and Future Trends

Fatemeh Hosseini, Mohammad Reza Sanjabi, Mehran Kazemi and Nasim Ghaemian

Abstract

Bioactive ingredients (BI) bestow various health-promoting outcomes on consumers, including treating or preventing diabetes, obesity, cancer, coronary heart diseases, and so on. Several BI have been found in nature, such as flavonoids, carotenoids, polyphenols, curcumin, phytosterols, probiotics, bioactive peptide, minerals, and nano-bio minerals, which can be incorporated into foodstuffs to improve their nutritional values. The foods containing BI are considered functional food. This review shed light on the health benefits of various BI for consumers. Due to the growing rate of population and surging demands for healthy foods in the future, it is pivotal to use affordable natural sources of BI to provide functional foods for a vast majority of people. Thus, in this review article, some potent by-products are addressed as alternative sources of BI.

Keywords: bioactive ingredients, functional food, nutrients

1. Introduction

Nutrients are classified into two major groups, including essential and nonessential nutrients. Essential nutrients can be synthesized by the human body, while nonessential nutrients cannot be made in the body. The former provides normal cellular function, and the latter is not required for life yet brings about health-promoting outcomes for consumers [1]. Our body is not capable of synthesizing nonessential nutrients. Thus, it is crucial to ingest these vital constituents in the form of dietary supplements or foods. In this regard, the foods containing these beneficial substances that are considered bioactive ingredients (BI), are called functional foods. Functional foods confer a broad spectrum of physiological benefits, including the prevention of cardiovascular disorders, obesity, diabetes, cancers, stress, Parkinson’s and Alzheimer’s diseases, neurological diseases, and pulmonary and respiratory disorder [2].

Generally, functional foods can be available in two forms. One of the forms is those that possess indigenous BI intrinsicality (such as fruits and vegetables), and the
other forms are ones that are fortified by BI extracted from available sources (such as snacks fortified by carotenoids) [3]. As the amount of indigenous BI in various sources might not be enough to provide health benefits, the purified BI are incorporated in foods to increase their nutritional values.

Phytochemicals are plant-produced substances that represent unique features, some of which could be utilized in maintaining human health and strengthening the body’s defense shield against diseases. Phytochemicals can be classified based on the chemical structure or their properties: flavonoids, carotenoids, polyphenols, curcumin, and phytosterols are the noteworthy groups [4].

WHO defines probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. They might be available in different forms of products, from fortified foods to supplements and drugs formulated with colony-forming units per gram (CFU/g) of probiotic strains of bacteria. Prebiotics, on the other hand, are a group of carbohydrates found in food, which the human body enzymes are incapable of digesting. Probiotic bacteria and some strains of gut microflora can break down these molecules and allow the body to gain nutritional benefits. The products that contain both probiotics and prebiotics are referred to as symbiotics [5]. Various functions in the body are carried out by interactions between proteins. Peptides are counted as the builder blocks of the proteins thus they also play a crucial part in the regulation of many functions in the body. Bioactive peptides have shown advantageous qualities such as immune system booster, cardioprotection, and neuroprotection [6]. For decades, the food industry strived to enrich the food with vitamins and minerals. Fortification of margarine with vitamin A, wheat with folic acid, cereals with vitamin B1, B2, and B3, and adding iodine to salt are among the processes that were done to produce more nutritious foods in the past. In recent years, encapsulating fat-soluble vitamins has been employed in order to increase their bioavailability [7].

Bio-mineralization is attributed to the formation of mineral complexes through living organisms, like bacteria. It can happen through two paths: (a) the metal complexes are formed due to electrostatic interactions between the negatively charged functional groups like COOH groups on the surface of the bacteria and positively charged ions, (b) the other path happens inside the bacteria and the microorganism synthesizes mineral biogenic, also called bio-mineral [8].

Among a myriad of BI, which play a substantial role in human health, we seek to address some of the most prominent ones, such as phytochemicals, prebiotics, probiotics, bioactive peptides, vitamins, minerals, and nano-bio mineral. Future trends: probiotics and bioactive.

2. Components of functional foods

Many definitions have been provided for functional foods by different organizations and since it is not legally accepted in any country except Japan, they are not unified. What is identical in all of them is that functional foods are food substances that have been modified in a way so that they have health-related benefits, and may subside the risk of some diseases. They may come from various sources: plants, fruits and vegetables, animals, probiotics, prebiotics, etc. Functional food components can be classified based on the product and the ingredients they contain.
2.1 Animal-based products

Meat is a key supply of amino acids, (e.g. cysteine, methionine, glycine, etc.) [9], and vitamins such as linolic acid, taurine, and creatine. By exerting some modifications on meat products, such as reducing the content of sodium chloride and the addition of nutrients like fish oil, natural extracts, and fiber from nuts, meat products can be a precious source of functional food. Moreover, chondroitin sulfate and glucosamine are ingredients that can be extracted from bovine skin and employed in the process of making functional beverages that promote joint health.

Egg also is one of the products that is being used largely in the market and its modification can diminish the risk of ischemic heart diseases. One of the strategies used to produce functional egg is enriching them by omega-3. For accomplishing this, there are two approaches, in the first one, linseed or flaxseed that contains large amounts of linolic acid would be added to the hen’s diet, as a result of which the linolic acid would be incorporated in the egg. However, the health-promoting effects of n-3 are mainly linked to the DHA (docosahexaenoic acid) and the conversion of linolic acid to DHA does not occur sufficiently in the human body. In this case, the second way of enhancing of n-3 level plays a significant role. It carries out via fortifying hen’s food with fish oil. Nonetheless, this strategy can lead to an undesirable fishy taste in the egg yolk, which is considered an undesirable characterization [10].

2.2 Dairy products

Proteins, vitamins, and minerals are the main functional components of dairy products along with hormones, cytokines, and immunoglobulins. The amino acid sequences of the peptides and proteins in the dairy-based products can promote health benefits by reducing cardiovascular diseases, e.g., hypertension, myocardial infarction, stroke, which are the leading causes of death globally. There are some products that have been modified by adding a number of functional Peptides with the sequences of VPP (valine-proline-proline) and IPP (isoleucine-proline-proline), with lowering blood pressure properties, beta-lactoglobulin, and glycomacropeptide (GMP) with the same quality along with increased protection against viral and bacterial infections [11].

2.3 Herbal products

For years ago, herbs have been used in various forms to treat several pathological conditions, some examples are polyphenols (quercetin, catechins, caffeic, and tannic acid), which can chelate iron consequently, this can mitigate neurodegenerative diseases like Alzheimer’s disease since the iron build-up is one of the causes of neuronal damage [12]. A study conducted by Chopra et al. showed that propolis can have protective effects against myocardial injuries due to its free radical scavenging effect [13]. Curcumin has shown neuroprotective properties by modifying the expression of inflammatory cytokines, reducing the oxidized protein, and increasing interleukin-1β. Curcumin also interacts with iron and copper ions and chelates these metals and suppresses oxidative damage, inflammation, and cognitive deficits in neurons [14]. Thus, the use of herbal extracts in different food products can have disease prevention and health-promoting advantages.
2.4 Fruits and vegetables

Fruits and vegetables contain a wide range of substances, which have health-promoting effects and can reduce the risk of various diseases with their antioxidant, anti-inflammatory, and cardioprotective properties. A variety of polyphenols, in which anthocyanins, flavanols, and catechins, phenolic acids like hydroxycinnamic acids, and tannins like proanthocyanins and ellagittannins are among the more important ones due to their disease preventive effects are substances that mainly exist in berry fruit [15]. Resveratrol, which is the phytochemical present in red grapes has shown lipid-modifying and antioxidant effects [16]. Lycopene is a carotenoid that majorly is known for existing in tomatoes and it is valuable as a functional food component Because it inhibits oxidative stress, hypertension, and atherosclerosis [17].

2.5 Marine products

Marine world has incredible biodiversity which includes marine plants, microorganisms, sponges, fish, etc. Therefore, the aquatic environment offers a vast array of functional compounds including omega 3 (ω3)-polyunsaturated fatty acids (PUFA), chitosan, chitosan oligosaccharides, glucosamine, carotenoids and xanthophylls, marine enzymes, and protein hydrolysates [18].

2.6 Probiotics and prebiotics

Probiotics are live microorganisms, commonly bacteria, that consuming an adequate amount of them can benefit the host. Prebiotics are oligosaccharides that the human body’s enzymes cannot digest, but are used by limited strains of bacteria, mainly microflora (intestinal bacteria), and promote their growth and again have advantages for the host’s health [19]. *Lactobacillus* and *Bifidobacteria* are the common strains of probiotics that have been investigated in research and used in functional foods [15, 20].

3. Bioactive compounds from vegetables and fruits by-product

According to WHO (world health organization), a healthy diet consists of 5 servings of different fruits and vegetables or 400 g of them on a daily basis. People’s diet varies in different countries, but in recent years many individuals consume more packaged food rather than fresh vegetables, which indicates the need for producing healthier food products. This can be achieved through producing bioactive compounds from vegetables and fruits by-products and adding them to conventional food [21].

Bioactive compounds are molecules that are produced in natural procedures, many of them are plant-based and can portray beneficial effects on human health. Commonly, fresh parts of fruits and vegetables are consumed, however, a huge part of phytochemicals exist in the seeds, shells, peels, bran, bagasse, and trimming, which are the by-products of the fruits and vegetables [22]. Tomato by-products (paste waste, skins, and seeds) can be used for the extraction of carotenoids, which regard as a lycopene source. Apple pomace contains phenolic compounds such as naringin and phlorizin. Phlorizin could be used in the synthesis of a new generation of antidiabetic drugs with the mechanism of inhibiting the sodium-glucose co-transporter 2.
Grapes skins and other by-products including seed, stem, skin, and pomace are also rich in polyphenols namely resveratrol. White grape seeds could be used to extract gallic acid, catechin, and epicatechin [22]. Avocado's seed and peel are rich sources of carbohydrates, they also contain, lipids, proteins, fibers, and minerals. These by-products contain terpenoids, alkaloids, saponins, and acetogenins [23].

4. Bioactive compounds from diverse plant, microbial, and marine sources

Plant-based bioactive compounds, which are called phytochemicals, are the secondary metabolites of the plant that normally are produced to protect the plant against insects and animals. In the human body, however, they can prevent and reduce the risk of some types of cancer or chronic diseases such as diabetes. Phytochemicals’ favorable properties have brought researchers’ interest towards them and resulted in isolating and identifying thousands of plant-based bioactive compounds. Vitamin C, folate, provitamin A, potassium, calcium, magnesium, flavonoids, phenolics acids, alkaloids, carotenoids, and fibers are among the outstanding ones that could be mentioned [24]. Here, some of the plants that are sources of phytochemicals are mentioned. The *Origanum Spp.* has different terpene derivatives and phenolic compounds. These compounds exist in the essential oil extracted from the plant and have shown antibacterial, antioxidant, and anti-inflammatory activities. The *Thymus spp.*, especially the flowers are rich in terpenes, terpene alcohols, phenolic derivatives, ketones, aldehydes, ethers, and esters. Although the chemical composition highly depends on the species. *Salvia Spp.* has terpenic, flavonoid, phenolic acid, and steroid structures and therefore can be utilized to extract anti-microbial and anti-oxidant phytoconstituents [25].

There are various microbes such as bacteria, fungi, and cyanobacteria that produce bioactive compounds through their metabolic pathways [26]. Cyanobacteria are microorganisms with characteristics similar to bacteria and fungi, they have 2000 species and are a great bioresource of pigments. During screening programs in search of bioactive compounds, cyanobacterial metabolites are with potential use in fluorescent probes have been found. Cyanobacteria consist of phycobiliproteins, molecules valuable as sensing elements in biosensors for light characterization [27].

Probiotics are a vast source of beneficial bioactive materials, among them *Lactobacillus spp.*, *Bifidobacterium spp.*, *Clostridium spp.*, and, Enterobacteriaceae are known for producing metabolites with health benefits such as amino acids, vitamins, and folicates [28].

There are two major groups of marine algae: (A) Microalgae that is divided into 4 types *Chlorophyta* (green algae); *Chrysophyta* (golden-brown, yellow algae, and diatoms); *Pyrrhophyta* (dinoflagellates); *Euglenophyta*. They produce a type of toxin called dinoflagellate and could be used in studying cellular processes. (B) Macroalgae that consists of seaweeds and marine vegetables, they are a resource for hydrocolloids and phycocolloids, these two are being used as gelling substances, emulsifying agents, and wound dressing [29].

5. Recent advances in production of bioactive compounds

Fermentation by probiotic bacteria is an approach used to the preservation of food for a long time. Fermented commodity contains bioactive compounds owing
to the activity of probiotics in foodstuffs. The bioactive substances produced by probiotics in functional foods prompt beneficial outcomes for consumers [30]. In this regard, *Enterococcus sps*, *streptococcus sps*, *Bacillus sps* have the ability to synthesize amino acids, such as tryptophan and tyrosine, which are claimed to be advantageous to the function of the male and female reproductive system. Not only that, some probiotics are capable of yielding vitamin B groups. For instance, *Lactococcus lactis*, *Bifidobacterium sps.*, *Lactococcus fermentum* CECT 5716, *Lactobacillus reuteri* JCM1112 and could take part in the production of vitamin B2 (responsible for energy metabolism), B6 (responsible for amino acid metabolism), B9 (responsible for energy metabolism), and B12 (responsible for helping red blood cell formation and making DNA), respectively. For producing bioactive in protein-based foods (such as meat products, dairy products, soy milk, etc.), lactic acid bacteria are considered nontrivial. Besides, fungal fermentation of foods is employed to produce bioactive peptides in both plant and animal sources. In this regard, proteolyze activity of *Aspergillus oryzae* and *Aspergillus flavipes* in goat milk brought about the generation of peptides with antimicrobial/antioxidant nature [30]. Another conventional method used for the generation of bioactive peptides is the enzymatic hydrolysis of protein, by which one or more peptidases are added to a reactor containing deionized water and concentrated protein and controlling the temperature and pH throughout the process [31]. In addition to these conventional techniques, there are also innovative technologies, such as ultrasound-assisted and subcritical water extraction, which have been operated to produce bioactive peptides. Ultrasound-assisted extraction is a non-thermal and green technology that works based upon mechanical waves with frequencies of higher than 20 kHz, which traverses through a medium. Ultrasound waves give rise to strong forces at a microscopic scale in form of vibrations with large amplitudes in the medium, which then cause physical or chemical changes in foods [30]. This technique can be performed along with enzymatic hydrolysis in order to accelerate the process. Liang et al. [32] utilized low-frequency ultrasound with powers between 45 and 65 W/L on the enzymolysis of corn protein, which resulted in the production of short-chain peptides with a molecular weight of 200~1000 Da (11.84%) and 1000~3000 Da (21.29%) at optimal condition. In another study with a similar approach, Guerra-Almonacid et al. [33] used ultrasonic pre-treatment with a frequency of 80 kHz and an amplitude of 100% for 10 min prior to enzymatic hydrolysis of the plant protein. They reported that this procedure produced hydrolysates that possessed molecular weight ranging from 8 to 20 kDa. Subcritical water is liquid water at temperatures from 100 to 374°C under pressure less than 22 MPa (below the critical point of water). Once the dissociation constant (K_w) of subcritical water escalates as a function of temperature, subcritical water behaves as an acid or base catalyst [30]. Espinoza et al. [34] stated that the optimal condition regarding hydrolysis of whey protein isolate using subcritical water was at 300°C for 40 min, in which the free amino acids generation, especially lysin, was the highest.

6. Food technology and its impact on functional food development

Several strategies and technologies have been adopted for fortifying foodstuffs and increasing their nutritional value. Recently, vacuum impregnation as an emerging technology has captivated a lot of attention in food science and technology. This technique is an operation whereby a liquid medium containing bioactive compounds enters the solid porous food in favor of internal gas through capillary pressure [35]. In
this regard, numerous studies conducted to enhance the nutritional quality of foods via this method, such as enriching ready-to-eat sweet potatoes with polyphenols [36], enriching potato tuber with ascorbic acid [37], fortifying potato snacks with calcium, vitamin C, and E [38], and incorporating Lactobacillus casei into apple cylinders [39].

High-pressure processing is a widely applied technology by which 10–1000 MP is exerted on foods at mild temperatures. This technique influences non-covalent bonds, including hydrogen, ionic, hydrophobic bonds. As this approach excludes heat treatment, it is tailored for the sterilization of foods containing thermo-sensitive BI [40]. Aguayo et al. [41] evaluated bioactive stability under two processes, including the high-pressure homogenization (HPH) treatments (80 and 120 MPa) versus thermal treatment (80 °C, atmospheric pressure). They reported that the high-pressure process was a better alternative for the retention of heat-sensitive compounds such as vitamin C, vitamin A, and unsaturated fatty acids (10-hydroxy-2-decenoic acid). Not only might high-pressure processing averting loss of BI, in some cases, it could improve the nutritional value. In this regard, Saricaoglu et al. [42] claimed that high pressure homogenized rosehip nectars showed more antioxidant capacity after treatment owing to an increase in total carotenoid content.

Bioactive ingredients are usually susceptible to detrimental conditions such as low pH, and gastrointestinal conditions. Hence, encapsulation is a practical approach whereby bioactive compounds are protected from various deteriorative conditions by entrapping them in various non-toxic materials [43]. The most common materials exploited for encapsulation in food science are proteins [44], polysaccharides [45, 46], lipids [47], hydrogels [48], and metal-organic frameworks [49, 50] or a proper mixture of them for controlled release. A wide array of novel technologies has been utilized for the encapsulation of bioactive, some of the recent and intriguing ones are spray chilling [51], electrospinning [52], supercritical fluid [53], and microfluidic [54, 55]. The size of carriers is divided mainly into nano- or microcarriers [56]. The former has been used for most of the bioactive compounds [47], and the latter is suited for both bioactive and probiotics [52].

### 7. Future trends

Bioactive peptides (BP) are short sequences of amino acids, which in most cases are composed of 2–20 hydrophobic amino acids in the peptide chain. Nonetheless, longer sequences of BP have been reported, such as Linasin, which is a soy-derived peptide wherein 43 amino acid residues exist [30]. BP can be utilized in foodstuffs to produce functional foods due to the numerous health-promoting outcomes that they bestow on consumers [57], such as imparting antihypertension, anti-thrombotic, anti-cancer, antimicrobial, antioxidant, and immunomodulatory to the human body. Several natural sources of BP are exploitable, including soybean, cereals germ, potato, nuts, dairy products, egg, and meat proteins [58]. Moreover, marine microorganisms, for instance, microalgae, recently captivated increasing attention as a source of BP [59]. BP does not display biological activities unless they become activated through enzymatic, chemical, and microbial hydrolysis [58]. For the production of BP, enzymatic hydrolysis and fermentation are preferable to the chemical approaches [31]. The BP production via an enzymatic procedure possesses the advantage of complete control over the process [57]. However, in comparison to the enzymatic manner, fermentation is considered a more cost-effective strategy to obtain BP [31]. Moreover, novel technologies employed to produce BP in foods are
subcritical water extraction, ohmic heating, pulsed electric fields, and high hydrostatic pressure [30]. Since all peptides are not regarded as BP, it is pivotal to identify the BP among various other peptides. In this regard, bioinformatics and peptidomics are promising approaches proposed to discover BP effectively.

Peptidomics is the quantitative and qualitative analysis of a vast array of peptides in biological samples throughout protein hydrolysis, in which High-performance liquid chromatography (HPLC) and mass spectrometry (MS) are performed to discover or identify peptides, even in complex matrices [60–62]. Fermented foods contain probiotics that possess proteolytic activity. These probiotics can be able to release BP enzymatically in foods containing proteins as a result of fermentation. In this regard, Lactobacillus bacteria is the epitome of BP production in fermented dairy products [60]. Gu et al. [63] identified a novel BP (NENLLRFF) using UPLC-MS/MS, which was produced by multi-species probiotics in yogurt. Those authors claimed that the newly found BP might have angiotensin-I converting enzyme inhibitory, antioxidative, hypotensive, and stimulating properties. Peptidomics has advantages, such as it can accelerate peptide identification and does not require complete peptide separation [64]. Nevertheless, the main restriction associated with peptidomics is the difficulty or impossibility with regard to the identification of very short peptides (<5 amino acids), large-sized polypeptides, and disulfide cross-linked hetero-oligomers [60]. Employing appropriate tools to analyze and interpret the obtained peptidomics data is crucial, and bioinformatics, as a practical approach, provides a great opportunity for in silico analysis of biological data via various software (such as GalaxyPepDock) and database (which some of them are represented in Table 1).

### Table 1. Bioinformatic database.

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**8. Conclusion**

Bioactive ingredients (BI) play a substantial role in health, and consuming foods possessing these beneficial substances, which are called functional foods, can
therefore enhance human well-being. BI are abundant in various natural sources, including animal-based products, marine products, fruits, vegetables, and herbs. Probiotics can increase the nutritional value of these commodities via their enzymatic activity, which may lead to producing a vast array of BI in foods, such as vitamins, bioactive peptides, and folates. In addition to fermentation, there are other novel technologies that have been employed for the generation of BI in foods, like subcritical water extraction, ohmic heating, pulsed electric fields, and high hydrostatic pressure. In this regard, peptidomics and bioinformatics are two robust approaches to identify and discover the bioactive peptides formed in functional foods.

**Conflict of interest**

The authors declare no conflict of interest.

**Author details**

Fatemeh Hosseini¹*, Mohammad Reza Sanjabi², Mehran Kazemi³ and Nasim Ghaemian⁴

1 Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran

2 Agriculture Research Institute, Iranian Research Organization for Science and Technology (IROST), Iran

3 Faculty of Agriculture, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran

4 Department of Pharmaceutics, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Address all correspondence to: zinolife2014@gmail.com

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