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Rajeev Ravindran
Technological University Dublin

Samriti Sharma
Hannover Medical School

Amit Jaiswal
Technological University Dublin, amit.jaiswal@tudublin.ie

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Application of Enzymes in Processing of Functional Food and Nutraceuticals

Rajeev Ravindran[1], Samriti Sharma[2] and Amit K. Jaiswal[1*]

[1]School of Food Science and Environmental Health, College of Sciences and Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1, Republic of Ireland; [2]Institute of Medical Microbiology, Hannover Medical School, Germany

Corresponding author: Amit K. Jaiswal, PhD. School of Food Science and Environmental Health, College of Sciences and Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1, Republic of Ireland

ABSTRACT

Functional foods are nutriments consumed to cover the nutritional needs that cannot be met by a normal diet alone. Consumption of functional foods has proven to have physiological benefits such as reduced risk of certain chronic diseases, thus differentiating them from conventional foods. Several health benefits have been reported to be associated with the consumption of nutraceuticals and functional foods. Many natural sources have been successfully implemented in imparting additional health benefits and preventing the onset of chronic diseases related to aging, faulty genetics, or lifestyle. Functional foods can basically be categorised into different groups based on their nature and source of origin. The functional food industry utilises enzymes in large scale for synthesis of new and better products. Enzymes play different roles in the production of nutraceuticals and functional foods as well as the fortification of food. Enzymes are used over conventional methods due to cost effectiveness and higher yield in addition to being a cleaner and greener alternative. Nutraceutical and functional food products developed by the application of enzymes include non-digestible oligosaccharides, cereal and dairy based ingredients, prebiotic products, phytochemicals, nutraceutical lipids, bioactive peptides, special protein hydrolysates, and anti-oxidant peptides. However, there are certain limitations pertaining to the use of enzymes. Enzymes are proteins and therefore extremely sensitive to ambient conditions. Furthermore, they cannot be reused adding up the production cost. Nevertheless, this problem can be resolved by immobilising enzymes to compatible nanoparticles. Studies have shown that immobilised enzymes exhibit higher activities compared to their native counterparts in aqueous phase.

Key words: Extraction, Functional food ingredients, Nutraceuticals, Enzymes, Non-digestible oligosaccharides, Bioactive peptide

Introduction

Food has always played an essential role in the survival of life on earth. In the absence of research, some of the early civilisations in the world relied on plants and animals for food to derive energy necessary for everyday activities and also to meet a feeling of satisfaction and well-being. However, modernisation and advancement in research over the centuries have identified the importance of food beyond its role in survival and hunger satisfaction [1]. A healthy diet is now also being seen as a natural method to reduce the occurrence of chronic diseases. An aging population and increasing occurrence of lifestyle diseases in children and adults has called for additional nutritional complements that can be consumed along with food to meet inadequacy in daily nutrition. Increase in awareness about wellness is drawing more consumers towards seeking healthier dietary options and viewing nutrition as the key to a better life [2]. Increased health care cost has all the more drawn the interests of consumers, researchers and food manufacturers towards this concept of a healthy diet, resulting in the birth of two terms namely, functional foods and nutraceuticals.

Functional foods are the foods that can be consumed as the part of a normal diet which offers proven physiological benefits and has ability to reduce the risks of certain chronic diseases, thereby making them different from conventional foods. Food that is cooked with 'scientific intelligence', with or without the knowledge of how or why it is being used, is a "functional food". Functional foods provide the body with the required amounts of vitamins, minerals, protein, carbohydrates, etc., which is critical to achieve a state of healthy well-being. A portmanteau between 'nutrition' and 'pharmaceuticals' coined by Dr Stephen DeFelice in 1989, nutraceuticals on the contrary are the "concentrated form of a certain bioactive compound isolated from normal food offered in medicinal form" and when consumed, it imparts enhanced health benefits [3]. However, due to lack of universally accepted definitions often misleads consumers regarding the two terms.

In the food-drug spectrum that spans from health foods on one end and prescription drugs on the other, nutraceuticals find its place in the middle by incorporating functional foods, nutritional supplements and medicinal foods. Although, it has been difficult to differentiate between nutraceuticals and functional foods, several articles have tried to define both terms in different contexts. For instance, according to Sohaimy most functional foods possess certain antianemic properties, thus if a functional food is used to prevent or treat a disease or disorder other than anaemia, it is called a nutraceutical. Thus, functional food for one consumer may serve as a nutraceutical for the other [5]. Several guidelines have been laid down by the International Life Sciences Institute (ILSI), Europe and the European Parliament, which emphasises on the need for scientific proof to recognise the additional benefits on consumption of a particular functional food product [6]. From a consumer's perspective a major boundary line that separates functional foods from nutraceuticals is the difference in their physical forms; while nutraceuticals resemble pharmaceutical products (pills, granules, capsules, syrups and solutions, powders etc.), functional foods are just like normal food products in appearance [7].

Legislation on the regulation of ingredients in dietary health supplements became relevant in the late 1980's because of the shift in the mind-set of a generation that was youth-centric and believed in the preservation of health through lifestyle changes and better dietary choices. The

Japanese Government decided to address this problem by introducing the Foods for Specialised Health Uses Act (FOSHU) and thus became the first country to recognize functional foods as a separate category [8]. Since then, the country allows marketing of more than 200 functional foods under the present FOSHU legislation [5]. According to the U.S. Food and Drug Administration (FDA), over 50% of American citizens regularly consume vitamins, minerals, herbs and other supplements with intentions to improve their nutrition. The FDA uses the term 'dietary supplements' to define products with improved efficacy and increased health benefits. The US congress also came up with the Dietary Supplement Health and Education Act (DSHEA) of 1994 to amend the Federal Food, Drug and Cosmetic Act for establishing guidelines with respect to these dietary supplements. DSHEA defines dietary supplements and comprises of a set of guidelines which should be followed for the production of the same. DSHEA authorises dietary supplement manufacturers to produce and sell their product and to provide information on the health benefits on their labels with significantly reduced requirements, as opposed to conventional drugs. Supplements that were already available in the market prior to drafting of the act were presumed to be safe. Accordingly, DSHEA must prove that these supplements, or ingredients used in these supplements are harmful before they can prohibit the marketing of products that contain them [9]. The FDA is not accountable for new ingredients in these supplements and the manufacturers have been given the responsibility of making sure that the product is market safe. This is contrary to the rules laid down for release and marketing of a drug where a manufacturer has to perform safety studies complying to guidelines defined by the FDA and then submit the results before the FDA for review and subsequent approval.

Health benefits of nutraceuticals and functional foods

Several health benefits have been reported to be associated with the consumption of nutraceuticals and functional foods. Guarana is an Amazonian herb has strong platelet aggregation inhibition properties. Approved as a food additive in the U.S., Guarana has been used as a replacement for aspirin to avert the risk of thrombosis, which is a major factor that leads to stroke and coronary heart disease [10]. The example of Guarana is only one among many where natural sources have been tried, tested and succeeded in imparting additional health benefits or used in the prevention of onset chronic diseases related to ageing, faulty genetics, or lifestyle. Dietary fiber intake has immense health benefits ranging from lowering blood pressure and serum cholesterol levels and increasing insulin sensitivity levels to significantly lowering the risk of cardiovascular diseases, stroke, hypertension, and diabetes. The consumption of dietary fiber also averts the onset of gastrointestinal disorders [11].

Reactive oxygen species are a by-product of aerobic metabolism that accumulates in our body over a period of time. Aging, along with several other diseases such as arthritis, inflammation, ischaemia, Parkinson's disease, and atherosclerosis are directly linked to the detrimental effect of reactive oxygen species. The endogenous phenolics found in plants are phytochemicals capable of anti-oxidation and providing relief from oxidative stress [12].

Phytosterols are plant derived sterols that resemble cholesterol. They were initially thought to be rejected by the living systems, but studies later showed that phytosterols are metabolized to some extent. Experiments related to administering phytosterols to individuals at different concentrations resulted in the lowering of cholesterol levels. Phytosterols were primarily marketed as pharmacological agents before finding ways to incorporate them in margarine. Nowadays fruit juices, ice creams, and other foods are available that have been fortified with these phytosterols [13].

According to the UNICEF, malnutrition affects 161 million children worldwide. It is a problem that needs to be tackled not only in the under-developed and developing countries, but the developed world needs to pay an equal attention to this issue. It is also a serious problem among older adults [14]. The evolution of nutritional intervention to address global malnutrition has shifted over the years from controlling protein deficiency followed by controlling protein-energy deficiency to finally focusing on prevention and treatment of micronutrient deficiency. Micronutrient deficiency is considered the main cause of malnutrition in the world. This problem can be addressed and solved in an inexpensive and effective manner by supplementation and fortification of the available diet [15]. Vitamins and minerals, especially vitamin A, thiamine, iron, zinc, and iodine are very important micronutrients whose absence in the diet causes severe deficiency diseases [16-18].

Rice is the staple food for over 50% of the world's total population. Several studies have been conducted on the fortification of rice with zinc, iron and vitamin A. Vitamin A fortified rice supplemented with iron and riboflavin have been used in the treatment of night blindness that occur in pregnant women in Nepal [19]. Fortifying foods are considered the best long term and cost effective method to tackle iron deficiency. Commonly consumed food such as milk, cereal and salt can be fortified with iron, which can easily be distributed among populace [20]. Biofortification is the application of genetic engineering techniques to introduce micronutrients in staple foods such as rice and corn. The much fabled 'golden rice' is a result of the introduction of genes encoding the β-carotene biosynthesis pathway in rice endosperm. Ferretin is a protein that stores iron and is found in bacteria, plants and animals alike. Genes that code for ferritin can be obtained from different plant sources which are then transferred to *Oryzasativa* to improve the iron content in rice. Studies have shown that the expression of soy bean ferretin gene in transgenic rice leads to an increase in iron and zinc content the grains [21].

Types of Functional Foods & Nutraceuticals

In the past few decades, the concept of functional foods and nutraceuticals have gained increased attention due to their increased health benefits and ease of administration as part of daily diet that has revolutionised the human health and nutrition research, and subsequently, a range of products are available in the market. A functional food can be divided into following types [22]:

(a). Natural food,
(b). Food containing an additional functional component,

(c). Food or food product from which some component has been removed,
(d). Food with modified one or two components,
(e). Food with improved bioavailability,
(f). Combination of any of the above forms

However, functional foods can also be categorised based upon their source of origin as plant-derived foods or animal derived foods [23]. Foods derived from plants possess certain bioactive compounds that are known to provide health benefits in addition to normal nutrition. These bioactive compounds are mainly secondary metabolites which are synthesised as part of the natural metabolism in plants to withstand the external conditions. Despite of their low abundance, these compounds are being exploited for their role in nutrition. For instance, oats, tomatoes, garlic, etc. are known for lowering cholesterol, reducing the risks of prostate cancer, antibacterial, and antioxidant properties. On the contrary, animal derived products such as fish are a rich source of omega-3 fatty acids that helps reduce the chances of cardiovascular diseases. Dairy products derived from animals have long been known to us as reserves of calcium which promotes bone health.

Functional foods are also marketed as (a) whole foods where the food contains several ingredients of proven health effects, (b) enriched foods, where one or more functional ingredients are added to the food that were lost during food processing, (c) fortified foods contain one or more additional ingredients besides the naturally occurring ones, (d) enhanced foods, where foods are modified to contain additional nutrients [5]. Another concept introduced recently into the functional food market that promises the state of health being are the designer foods [24]. This class of foods includes designer eggs that are rich in omega-3-fatty acids and antioxidants that help in reducing the cholesterol content of eggs; designer broccoli fortified with selenium provide protection against certain cancers and foods fortified with different vitamins like vitamin C, D, etc. [25]. However, all these categories of foods contain one or more functional ingredients that are either present naturally or fortified or enriched into conventional foods to provide a natural cure for diseases like cardiovascular diseases, cancer, deficiency disorders, etc.

Nutraceuticals on the other hand include food products that provide health or medicinal benefits and may be broadly classified based on their source (animal, plant, or microbial derived products), pharmacological condition or chemical constitution [26]. They can include isolated nutrients, dietary supplements, dietary fiber, herbal products, and processed foods [27]. Based upon the type of nutritional functions achieved, the nutraceutical market includes vitamins, minerals, fatty acid nutrients, herbal products, energy supplements and medicinal products with special health benefits. Out of all these, dietary supplements and herbal products contribute a major segment to the global nutraceutical industry [26]. Wide varieties of bioactive phytochemicals like carotenoids (lycopene, lutin, zeaxanthin, β-carotene), soluble and insoluble dietary fibers, polyphenolic compounds (phenolic acids, flavanones, resveratrol, curcumin), omega-3- fatty acids, plant sterols, isoprenoids, prebiotics and probiotics, minerals (iron, zinc, selenium, potassium, copper), sugar alcohols (xylitol, sorbitol), terpenes, etc. because of their their antioxidant properties and role in prevention of several degenerative and chronic diseases, form a significant source for nutraceutical products [28, 29].

Role of enzymes in processing of Functional Foods & Nutraceuticals

Enzymes are catalysts that are biological in nature with high substrate specificity. They are highly precise in the reaction they catalyse while being very specific and can function in mild to harsh reaction conditions. Enzymes are employed as a part of the biochemical operations involved in the biotransformation and stabilization of foods such as baked goods, fruit juices, dairy, wine, and brewed beverages. Traditionally, several enzymes have been used for the production of compounds such as xylose oligosaccharides and bioactive peptides [30, 31]. Recently, a lot of focus has been devoted to the application of enzymes in the extraction of bioactive compounds from waste such as lignocellulosic materials and marine waste [32]. Enzymes have wide applications in the food industry owing to the many advantages associated with it. It is quite difficult to categorize enzymes with respect to the different roles they play in the production of nutraceuticals and functional foods. Nonetheless, the roles of enzymes played in nutraceuticals and functional food production can be summarised as follows:

Production of functional food ingredients and nutraceuticals

The use of enzymes for the synthesis of bioactive compounds provide the manufacturer leverage over the choice of raw materials that can be used for production. Waste generated from the meat, dairy, fish, and agriculture industries can be valorised using different enzymes for the production of dietary supplements and functional food ingredients. Enzymatic hydrolysis of different foods rich in proteins is an approach followed in the industry for the production of bioactive peptides. Milk and fish waste have been experimented to synthesise peptides that possess functional properties and a range of proteolytic enzymes derived from plant, animal, and microbial sources are used for this purpose. Trypsin, papain, neutrase and α-chymotrypsin are some enzymes that have been used to produce bioactive peptides from fish waste. Integrating a three enzyme sequential digestion system with ultrafiltration mechanism results in a multi-step recycling membrane reactor, which is ideal for the extraction of bioactive peptides from marine substrates [33].

Glucosamine supplements are taken by people who suffer from arthritis to ease joint pain. Commercially glucosamine can be produced by the hydrolysis of chitin using concentrated acids. However, it results in a lower yield and higher downstream processing costs. Alternatively, d-glucosamine and *N*-acetyl-d-glucosamine can be produced by the application of two enzymes *viz.* exo-chitinase and endo-chitinase. These enzymes can be used to synthesise therapeutically viable glucosamine from crab and shrimp waste [34]. Enzymes are widely used for oligosaccharide synthesis from different sources. Enzymatic hydrolysis of sugar beet pulp using commercial pectinolytic enzymes Rohapect DA6L and Macer8 FJ have resulted in the formation of rich fractions of hemicellulose [35]. Other enzymes that are used for oligosaccharide production include β-galactosidases, glucansucrases, and fructansucrases [36]. A schematic representation of nutraceutical production from different forms of food processing waste has been shown in Figure 1.

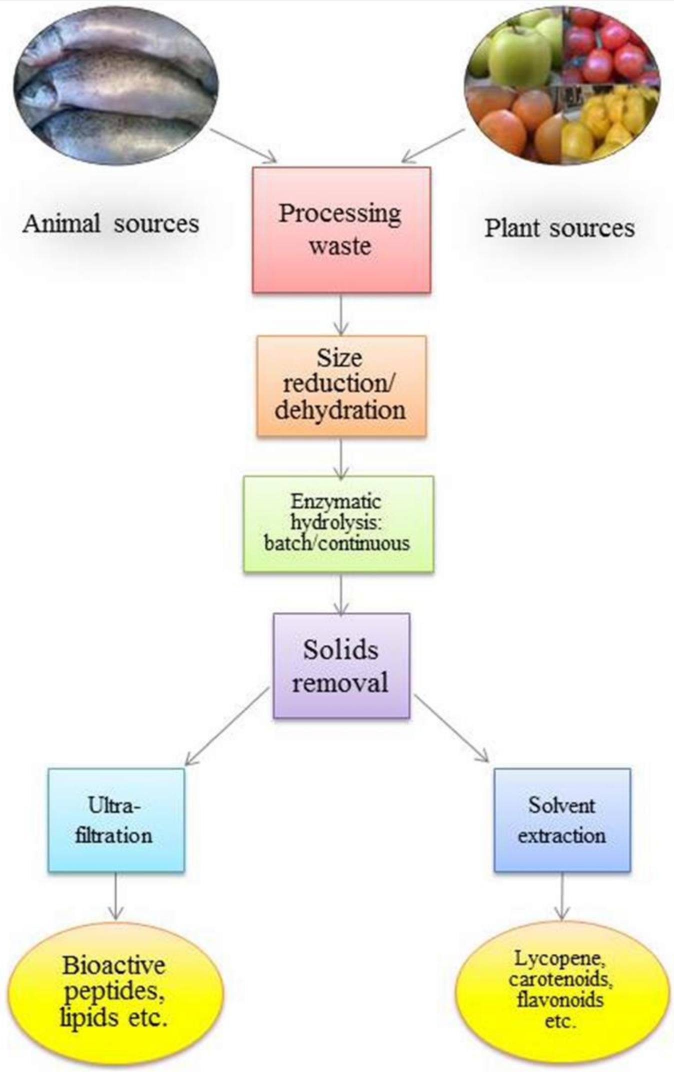

Figure 1. Schematic representation of production of nutraceuticals from food processing waste

Extraction and recovery of functional food ingredients and nutraceuticals

Enzyme assisted extraction methods are finding wide popularity because of the environment friendly approach and applicability for the extraction of a variety of pigments, flavours, and medicinal compounds. Recent studies on enzyme assisted extraction techniques has revealed a higher yield and recovery of the compound, faster extraction process, reduction in the use of solvent and reduced energy requirements. Enzymes have successfully been used for the extraction of bioactive compounds such as catechins from tea, lecithin from egg yolk, soybean oil, sunflower oil, and vegetable oil [37, 38]. Lipases such as phospholipase A_1 and phosphlipase

C are the major enzymes involved in the extraction of lecithin and associated bioactive compounds [39, 40]. Enzymes, particularly help in facilitating the extraction of compounds with biological activities by improving their accessibility to solvents. For example, in the case of production of bioactive compounds from seaweed, enzymes are used for the degradation of cell wall polysaccharide which is present in high concentrations. The successful application of enzymes for cell wall polysaccharide hydrolysis has spread their use over extraction of polyphenols from citrus peel, grape skin, apple skin, black current, and *Gingko biloba* leaves [41].

Concentration of functional food ingredients and nutraceuticals

Enzymes are also used for the concentration of bioactives like polyphenols, lipids and antioxidants in particular. Recent advances in enzymatic treatment of wheat bran and rye revealed that α-amylase can be used to concentrate bioactive compounds with increased antioxidant potential [42]. The concentration of PUFAs is usually achieved by the application of lipases. Lipase obtained from *Yarrowia lipolytica* was immobilised on functionalised Fe_3O_4 magnetic nanoparticles (MNPs) in reverse micelles system and was used for the enrichment of polyunsaturated fatty acids [43]. Phytosterols and phytostanols are nutraceutical lipids derived from plants. A few examples of phytosterols include sitosterol, sitostanol, campesterol, campestanol (and their respective esters), stigmasterol, brassicasterol [44]. Lipozyme RM IM, Lipozyme TL IM, Lipozyme NS-4004 TLL, and Lipozyme 435 produced by Novozymes A/S have been successfully used to recover phytosterols from plant oil deodoriser distillates mixture [45]. Enzyme extracts obtained from tea leaves were used in a study to obtain superior vanillin from green vanilla pods. Enzyme assay of the extract revealed amylase activity and results indicated that a higher vanillin content can be obtained using tea leaf extracts than the commercially used Viscozyme [46].

Fortification of food

The application of enzymes in different food products impart specialised characters that increase their nutritive value and sensory qualities. Enzymes are extensively used in the baking industry, dairy, juice industry, wine and brewery, and for cereal production. The treatment of cereals with enzymes results in the increase in the amount of soluble dietary fiber, water-soluble antioxidant activity and phenolic compound bioavailability [47]. Pectinases are enzymes are used to stabilise the cloudiness in fruit juices and clarify them. The application of these enzymes in fruit juice processing results in the formation of polyphenolic compounds [48]. Protein fortification of fruit juices is achieved by the addition of soy protein hydrolysates which is initially treated with proteases, such as trypsin and alcalase, which renders the digests soluble in water based solutions [49]. In wine production, pectinases find applicability in improving stability, colour extraction and increasing the anthocyanin content in red wine [50].

Benefits of using enzymes over conventional methods

Chemical methods for the synthesis of bioactive compounds such as oligosaccharides have been much researched on and established. However, these methods cannot guarantee region specific modifications and the amount of steps increase as the size of the oligosaccharide increases. The resulting yield is low and scaling up does not remain a pragmatic option. Additionally, achieving stereospecific anomers (α and β) is often a difficult task. Using enzymes, all of these targets can be achieved as enzymes are region-selective in the reactions they catalyse, and the application of a different enzyme can result in the same molecule with a different optical activity [51]. The extraction and production of bioactive compounds for their application in nutraceuticals and functional foods require methods that are cost intensive and provides better yield. Together with conventional thermal processing method, a range of novel methods such as high hydrostatic pressure, pulse electric field, and ultrasonication are some of the methods that are currently employed in the industry for the production of functional foods [52]. However, these processing methods have several drawbacks by being expensive, tedious to operate, hazardous, requiring stringent process control operations, and the occasional incidence of toxic components. For example, marigold flowers have traditionally been an important source of carotenoids. Conventional extraction techniques accounts for almost 50% loss of carotenoids depending upon the conditions and solvent used. However, 97% carotenoid recovery yield was achieved using an alternative extraction method involving enzyme-assisted extraction simultaneously [53]. Microbial synthesis and enzyme-assisted production of functional food is an age old concept whose extensive applications have recently come into the limelight with the advancement of bioprocess technology [54]. The use of enzymes in the food industry also provides a cleaner and greener alternative as opposed to conventional chemical reaction processes employed for nutraceutical production. The ability of enzymes to cleave biomolecules of specific sites result in better yields of the desirable compounds as compared to physical and chemical processing methods.

Selection criteria of enzymes

Enzymes that are used for food processing may be of plant, animal or microbial origin. When sold, it doesn't only contain the desired enzyme activity, but also other metabolites of the production strain along with preservatives and stabilizers of food grade quality. Enzymes that are used in the food industry are supposed to comply with certain safety guidelines before they can be commercially exploited. As enzymes are specific in the chemical reactions they catalyse, there is little risk of formation of undesirable by-products. However, according to safety regulations for enzymes obtained from production strains, the following issues are considered:

- Pathogenicity and toxicity of the production strain
- Allergies and irritations
- Interaction of enzymes from other food components
- Products of enzymatic reactions, carcinogenic and mutation effects
- Effect of food enzymes on consumption

The primary focus when assessing a production strain is its toxigenic potential. Toxigenic potential is defined as the ability of a microorganism to produce chemicals (toxins) that can cause food poisoning. Bacterial and fungal strains have been known to produce toxins which on ingestion can cause sickness and even death. Meanwhile, pathogenic potential is the capability of a microbe to infect and cause disease. Pathogenic potential is not of major concern since the enzyme preparations rarely contain any viable microorganisms [55, 56].

In a commercial perspective, there are certain criteria that should be followed for the selection of enzyme for the food industry. Enzymes are capable of catalysis at lower temperatures and pH, making them an obvious choice over their corresponding chemical catalysts. Since so many enzymes fall in the GRAS category, making the right selection is a rather tedious process. Fortunately, enzyme catalysed reactions are so specific that the number of by-products produced to a bare minimum, eliminating the need to remove them at most times [57]. Another aspect that should be evaluated while enzyme selection is the over-all situations of the food or the beverage like pH, temperature, moisture content, reaction time necessary and other processing conditions. The performance of the enzyme in real time also needs to be assessed, as laboratory tests reveal very little in terms of changes in enzyme activity with minor operational fluctuations [58, 59]. The source of the enzyme also determines its operational efficiency. It is also necessary to study the side reactions of the enzymes as well. While fungal enzymes tend to be more active in acidic pH bacterial, yeast and animal sources show a higher activity in alkaline conditions. Furthermore, fungal proteases used in the baking industry also exhibit amylase activity [60, 61].

Application of enzymes in food industry

Foods from plant, animal and microbial sources available to us possess all essential compounds that could help in achieving the state of healthy well-being. However, the major disadvantage lies in the concentration of these ingredients in the foods of the daily diet. Also, the changing lifestyle and increasing health care cost have provided food for the thought for all major industries involved in food sector, to provide easy and natural alternatives to attain good health. Functional food is one major sector that is deriving attention. Food industries are making every possible effort to produce such foods that provide additional functionalities to our present diet. Currently, supermarkets are flooded with foods like prebiotics and probiotic products, functional meats, foods enriched with special oligosaccharides, dietary supplements, etc. that contain one or more ingredients that help control and deal with the problems of cardiovascular diseases, cancer, diseases of the gastrointestinal tracts, etc. These functional ingredients include flavonoids, catechins, lycopene, polyphenolic compounds, probiotic and prebiotic ingredients, glucosinolates, conjugated linoleic acids, plant sterols etc. that are used to enhance or modify probiotic and prebiotic foods, dietary fibres, bakery products, meat products, oils and dairy products like cheeses, etc. In the next section, some cases were discussed where enzymes are applied to produce functional food components and nutraceuticals. A summary of the role of enzymes in different functional food and nutraceutical industries have been mentioned in Table 1.

Non-digestible oligosaccharides: a functional ingredient in prebiotic products

Non-digestible oligosaccharides (NDOs) are low molecular weight carbohydrates of intermediate nature between simple sugars and polysaccharides that are used as functional ingredients in food products due to their important physiochemical and physiological properties [62]. Being non-digestible inside the human digestive tract, these oligosaccharides help stimulate the growth of healthy bacterial flora including *bifidobacterium* and *lactobacillus* inside the gut which eliminates harmful bacteria like *Escherichia coli* and *Clostridium spp.* [63, 64]. Increase in the population of host-friendly bacteria helps increase the availability of minerals, promotes absorption of ions like iron, magnesium, calcium and fermentation by these bacteria inside the gut helps to produce organic compounds that reduce the effects of lipids and cholesterol, thereby reducing the gastrointestinal infections which enhances the digestive health and improves the immune system, helping in weight loss [62, 65]. Non-digestible oligosaccdarides can be produced by either extraction from their natural sources like milk, honey, fruits and vegetables or by enzymatic or chemical synthesis from disaccharides. Examples of non-digestible oligosaccharides include fructooligosacchardies, galactooligosaccharides, lactosucrose, isomaltose, xylooligosaccharides, cyclodextrins, raffinose oligosaccharides, soybean oligosaccharides. All the NDOs except lactulose, raffinose, and soybean oligosaccharides, are industrially produced by enzymatic hydrolysis. Enzymes are used to produce two possible set of reactions of (a) transgalactosylsation of simple sugars or (b) controlled hydrolysis of polysaccharides to produce NDOs [62]. These enzymatic reactions can be tailored to produce individual or different oligosaccharides.

Fructose-oligosaccharides are one such group of non-digestible oligosaccharides that are used as functional ingredients in prebiotic food products and dietary fibers. These oligosaccharides are commercially produced either by transfructolysation of disaccharide sucrose by the enzyme β-fructofuranosidase or controlled enzymatic hydrolysis of inulin [62]. Other examples of non-digestible oligosaccharides include galactooligosaccharides, commercially produced from lactose by the action of β-galactosidase and isomaltose, a dissacharide produced by enzymatic rearrangement of glycosidic bonds in sucrose.

3.1.1 Cereal derived ingredients

Cereals offer a great source of functional foods and functional food ingredients like β-glucan, resistant starch and oligosaccharides. Cereals serve as fermentable substrates for the growth of probiotic microorganisms in the gastro intestinal tract. Also, as a source of oligosaccharides, they are used to extract prebiotic ingredients that could be used in the production of prebiotic products [67]. β-glucans and arabinoxylans are two very important dietary components that are extracted from cereals like oats, wheat, barley, etc. 1-3, 1-4 β-D-glucans commonly known as β-glucans consist of D-glucopyranose residues linked by β 1-4 glycosidic bonds. As dietary ingredients, they are used to fortify barley flour and enrich wheat flour to improve the consistency of the dough [69]. Consumption of a diet rich in β-glucans helps increase the peristalsis of the intestines and promote growth of healthy microflora. B-glucans can be extracted from wheat, barley, oats, edible mushrooms, waste brewer’s yeast, etc. by ultrasonic treatment [70, 71]. However, the final product is often contaminated with starch and several proteins. Thus enzymes like α-amylase and protease and amyloglucosidase are used to remove these impurities from β-glucans. In certain cases, enzymes like papin, α-amylase and protease are used to release β-glucans into the reaction medium [71, 72].

In cereals, enzymes are also used to improve the bioavailability of micronutrients. In case of plant based foods, like wheat bran, cereals, pea, etc., phosphate is stored in the form of phytate in conjugation with certain salts of divalent cations like zinc. This restricts the availability of free zinc resulting in the deficiency of zinc. Deficiencies caused by these micronutrients result in poor growth, decreased immune function, and increased susceptibility to several infections and fetal abnormalities in pregnant women. Thus, bioavailability of these micronutrients can be improved by fermentation that causes the hydrolysis of this phytate to lower inositol phosphates and the release of low molecular weight organic acids that enhances iron and zinc absorption. Fermentation also improves protein quality and vitamin B content in these foods.

3.1.2 Dairy-based ingredients

Milk is known to us a complete diet due to the presence of all essential nutrients and protein required for the human body. Considering this importance of milk and milk based products in our daily lives, functional food industry largely relies on dairy-based foods and ingredients. A wide range of products are available in supermarkets whose functionality has been enhanced by the addition of prebiotic and probiotic ingredients. As the name suggests, prebiotic products contain ingredients that are indigestible inside the human body and their intake enhances the growth of healthy microflora inside the gastrointestinal tract. Probiotic products on the contrary contain live microbial cultures present in a concentration that is harmless to the body but their intake increases the colonisation and growth of microorganisms like *Bifidibacterium* that in turn inhibits the growth of infections pathogens. They also help regulate the immune system against pathogenic microorganisms and regulate the release of vitamin B into the body [73]. These prebiotic ingredients can be extracted from cereals, meat, and dairy products.

Prebiotic ingredients can be carbohydrate based ingredients like oligosaccharides, lactosucrose, lactulose, lactitol or peptides that can be added to dairy products like milk based beverages, yogurts, desserts, etc. [74, 75]. Galacto-oligosaccaride for instance is used as a low calorie sweetener in fermented milk products. This ingredient is synthesised from β-galactosidase derived from microorganisms like *Arthrobacter sp, K. lactis, L. reuteri, Thermus sp.*, etc. [76]. With advancements in enzyme engineering technologies, the enzymes used range from crude enzymes to recombinant enzymes. One of the major sources of lactose used for the production of Galacto-oligosaccaride is the waste generated from cheese processing known as cheese whey. Cheese whey is ultrasonicated and treated with enzymes to produce whey permeates that later serve as a source to produce lactose derivatives like lactulose, lactitol, lactobionic acids, etc. [77]. These carbohydrate ingredients are added to baby formulas, yogurts, desserts, buttermilk, etc.

Milk being the source of proteins is also used to extract peptides that could be used to produce functional foods. These peptides are inactive inside the protein but can be isolated by enzymatic hydrolysis. These peptides exhibit properties of metal chelation, radical scavenging and inhibition of lipid peroxidases [75]. Such peptides known for their antioxidant properties have been isolated from milk, casein, whey proteins using trypsin, chymotrypsin, caseinate, etc. [75].

3.1.3 Phytochemicals

Fruits and vegetables are a reservoir of specialized compounds called phytochemicals that are non-nutritive and can strengthen the immunity and prevent the occurrence of diseases. For this reason, phytochemicals are important in the nutraceutical industry. Cold pressing, super critical fluid extraction, and solvent extraction are some industrially important methods used for the extraction of phytochemicals from different plant parts. Maceration enzymes play a key role in the skin softening of fruits such as grapes and citrus varieties that are used in fruit juice and wine production. Additionally, these enzymes also play a vital role in the extraction of different anthocyanin compounds. The application of macerating enzymes in process resulted in the increase in release of anthocyanins by 8% to 15% or more [78] in a study using Novozym®, a commercial lipase for obtaining acylated derivatives of polyphenols from jaboticaba peels [79].

Flavonoids are a group of compounds that impart color to flowers. They are also found in tomatoes and citrus fruits. They are of particular interest in the medical world due to their several therapeutic properties. Flavonoids have been known to exhibit anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity and antiallergic activity, and antioxidant activity [80]. Flavonoids contain rhamnose in their structure. The presence of rhamnose in flavonoids prevents the effective absorption. Treating flavonoid rich food with rhamnoside enzyme effectively removes the rhamnose from the rest of the molecule by enhancing its intestinal absorption [81, 82].

Carotenoids are the orange yellow pigment that gives colour to carrots and tomatoes among plants, and crustaceans among animals. Carotenoids have been identified to have anti-oxidative capabilities in addition to reducing the risk of cancer and macular degeneration. The enzymatic extraction of carotene protein can be achieved by the combined activity of pepsin, trypsin and papain [83]. Commercial enzymes that are commonly employed for caroteniod production from different sources include Viscozyme, Pectinex, neutrase, corolase, and HT-proteolytic [84]. A 2.5 fold increase was reported while using pancreatin for the extraction of lycopene from tomato skin [85]. Polysaccharide degrading enzymes like cellulase and hemicellulase along with pectinases have been used extracting several bioactive compounds such as volatile oils, lycopene, and capsaicin [86, 87]. Flax seed and hulls were used in a study to extract secoisolarciresinol, a lignin using cellulase [88].

3.1.4 Production of nutraceutical lipids

Triglycerides are the main form of fat in our body and thus an important part of our diet. They are the end product of fat metabolism and some form of triglycerides are synthesised within the body. The nutritional value of triglycerols is determined not just by the length of the polymeric chain but also by the position of the fatty acids on glycerol. Medium chain fatty acids contain more nutritional value, as compared to long chain fatty acids, and are of more ease for digestion by pancreatic lipase and gastric lipase. The digestion of medium chain fatty acids release 8.3 kcal as compared to 9.0 kcal that result from long chain fatty acid degradation. Therefore, the synthesis of triglycerols with long chain fatty acids at sn-2 position and medium chain fatty acids at sn-1 and sn-3 positions is of nutritional and industrial importance. Specific acylation of molecular positions in triglycerols using chemical methods does not always result in favourable results. Therefore, lipases have come of great help in the inter esterification of the respective positions [89]. Lipozyme® RM IM is a commercial lipase that is used for the inter esterification for the production of low calorie structured lipids [90]. Studies have shown that the consumption of bioactive lipids is associated with the prevention or delay of cardiovascular disease, osteoporosis and immune disorders [91].

Long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) have been widely used for the production of nutraceuticals and pharmaceuticals. They are particularly interesting in nutritional perspective due to the various health benefits associated with cardiovascular health, auto immune diseases, and neurological conditions. Lipases play a vital role in the processing and concentration of LC n-3 PUFAs from compounds of natural origin. Lipases are water soluble enzymes that hydrolyse ester bonds in water insoluble substrates like long chain fatty acids [92]. Another application of lipases includes the production of diacyl glycerol (DAG) and eicosapentaenoic acid (EPA) [93]. Studies have revealed that consumption of DAG, EPA and conjugated linoleic acid (CLA) result in the reduction of belly fat. DAG and CLA have been used to fight obesity. However, natural sources contain these two compounds in very less amounts. A patented method was developed which involves 1, 3-specific lipid catalysed esterification of free fatty acids obtained from hydrolysis of triglycerides to obtain high-purity DAG [94].

Polyunsaturated fatty acids are an important component of the cell membrane and are responsible for the maintenance of homeostatic cellular environment. Fish and fish oil supplements contain high concentrations of polyunsaturated fatty acids. Cheaper investment costs and lesser energy expense have promoted the use of enzymes in the extraction of PUFA. Omega-3-fatty acids have been extracted from salmon oil with a recovery of 77% using proteases. Alcalase, Neutrase, Protomex, and Flavourzyme are commercial proteases that are used for the extraction of PUFA from different fish varieties (tuna, salmon, etc.). Alcalase have been reported to be the best yield providing protease with regards to extraction of omega-3-fatty acids [95].

3.1.5 Bioactive peptides

Bioactive peptides that are derived from enzymatic hydrolysis of food sources have demonstrated great potential in the prevention of cardiovascular disease, inflammation, and cancer. Milk is a major source of bioactive peptides in our diet. Bioactive peptides may be formed in the intestine with the intake of milk due to the digestion of proteins. Bioactive peptides are also formed by microbial action in milk such as *Lactobacillus sp.* or by the application of proteases during processing [96]. Milk derived proteins are also a source of antioxidants. Although, they are inactive as part of the natural parent protein, enzymatic hydrolysis gives rise to peptide molecules that exhibit anti-oxidant properties. Radical scavenging, metal ion chelation and inhibition of lipid peroxidation are some of the properties showed by milk-derived peptides on release [97]. Most commonly used enzymes for the production of bioactive peptides from milk include pepsin, trypsin and chymotrypsin, which may be of bacterial or animal origin [98].

Fish can be an important source of bioactive peptide compounds besides polyunsaturated fatty acids, polysaccharides, vitamins, minerals and anti-oxidants. Bioactive peptides can be derived from fish by three methods: microbial fermentation, solvent extraction or enzymatic hydrolysis. The third method eliminates the need for organic solvents and the formation of toxic by-products. Fish protein degradation products of pepsin, trypsin, alcalase, neutrase, papain, and α-chymotrypsin have been reported to exhibit anti-oxidative and antimicrobial activity [99].

3.1.6 Special protein hydrolysates

Protein hydrolysates are defined as a mixture of aminoacids, oligopeptides and polypeptides produced by the partial hydrolysis. They are used for the preparation of dietary supplements for medical conditions that are caused by extreme protein deficiency, inability to digest proteins, allergic reactions on protein consumption, starvation, etc. Protein hydrolysates reinforced with cystiene has been used for the treatment of liver diseases. They have clinical applications and are used in treatment of phenyl ketonuria, Crohn's disease ulcerative colitis [100]. The hydrolysis of proteins involves the breakage of peptide bonds to release smaller molecules. Chemical hydrolysis of protein molecules result in the production of L-form amino acids which can be toxic in nature such as lysino-alanine. Production of protein hydrolysates using enzymes eliminates the formation of toxins and safeguards the nutritional properties [101]. Protein hydrolysates are prepared by the enzymatic treatment of mincemeat, defatted mince, soy protein, whey protein, and other protein rich sources [102]. Flavourzyme Type A, Protamex, Neutrase, and Alcalase the major enzymes used in the production of protein hydrolysates from different sources [103]. Protein hydrolysates are used to prepare carbohydrate protein supplements that help athletes for post exercise muscle glycogen recovery [104]. Protein hydrolysates produced by enzymatic treatment of fish and shrimp waste has been reported to have 54.67–55.93% and 39.27–38.32% of essential amino acids and flavour amino acids making it a valuable savoury agent [105].

3.1.7 Anti-oxidant peptides

Enzymes have been employed to isolate substances with antioxidant properties. Peptides produced by the hydrolysis of plant derived sources such as soy, rapeseed, wheat, sunflower, and barley have been known to possess antioxidant properties. Chymotrypsin and Flavouzyme mediated digestion of plant proteins release peptides with antioxidant potential. However, uncontrolled digestion using papain can have a detrimental effect on the antioxidant property of the molecule. Soy protein isolates possess high antioxidant capabilities. Although it does not affect lipid oxidation, it reduces the formation of conjugated diene, a secondary metabolite formed during lipid oxidation. Low molecular weight soy protein hydrolysate molecules can be extracted by ultrafiltration methods. These molecular fractions have been reported to exhibit maximum antioxidant activity [106]. Bioactive peptides are produced by enzyme-mediated hydrolysis in batch reactors. The application of hydrolytic enzymes has shown great promise in extracting secondary metabolites from plants as well as preserving the bioactive properties of the peptides [107].

Table 1. List of commercial enzymes that are used in functional food industry

Principle Enzyme	Enzyme activity	Industrial Application	Reference
α-amylase	Cleaves α-1,4 linkages found in starch to yield glucose and maltose	Cereal, Beverages, Sugar, Bakery	[108]
Cellulase	Hydrolyses 1, 4-beta-D-glycosidic linkages in cellulose to release glucose	Bakery, Sugar, Prebiotics	[109]
Mannanase	Catalyzes the hydrolysis of terminal, non-reducing β-D-mannose residues in beta-D-mannosides	Bakery, Sugar, Prebiotics	[109]
Xylanase	Degrades the linear polysaccharide β-1,4-xylan into xylose	Prebiotics, Probiotics, Bakery	[110]
Lactase	Converts the disaccharide lactose in to component glucose and galactose	Dairy, Beverage	[55, 111, 112]
Transglutaminase	Catalyzes the cross linking peptide bond formation between two different protein molecules	Dairy, Bakery, Meat, Improved the shelf life of vegetables	[113-115]
β-amylase	Hydrolyses β-1, 4 glycosidic linkages in starch from non-reducing ends giving rise to maltose.	Bakery, Beverage	[116]
Pectinase	Breaks down pectin, a polysaccharide in plant cell wall	Food and Beverage	[111]
Lipase	Catalyzes the hydrolysis of fats	Food, Beverage, Prebiotics, Pharmaceutical	[92]
Rhamnosidase	Hydrolyses the terminal rhamnose residues in rhamnosides	Food and Beverage, Prebiotics	[81, 82]
Protease	Proteolysis, protein catabolism, hydrolysis of peptide bonds	Dairy, meat, nutraceutical, prebiotics	[96, 117]

Limitation of using enzymes

Enzymes are highly sensitive to the ambient physical and chemical conditions, making them susceptible to denaturation. Slight changes in pH or temperature can cause a decreased activity. Enzyme activity can be inhibited by the presence of other compounds or an increased concentration of the substrate. This calls for stringent process control measures. The cost of enzymes can be high since its isolation and purification is a cost intensive process. Therefore, robust enzymes in reusable forms will be of great advantage. Genetic engineering and recombinant DNA technology have paved way to creating thermostable enzymes that can exhibit optimum activity in acidic as well as alkaline conditions. Biocatalytic membrane bioreactors have been used for commercial nutraceutical production for the past few decades. Membrane reactors score over physical processing methods that are generally employed in the manufacture of bioactive compounds owing to several advantages *viz.* elimination of heat in separation and concentration process, smaller, flexible equipment which is easier to scale up, low operating costs, minimal energy consumption, high quality products and co-products. Biocatalyst membranes are prepared by incorporating enzymes or cells in a well-defined physical and geometrical region such as the lumen or shell zone and immobilizing them on the surface of the membrane or within the membrane matrix. Enzyme immobilization onto a matrix does not result in any reduction of activity and imparts significant macromolecular stability [118]. Immobilization on nanoparticles such as modified iron oxide magnetic nanoparticles have shown to increase enzyme's activity and stability [119].

SUMMARY

- Functional foods are the foods that can be consumed as part of a normal diet and provide with demonstrated physiological benefits and avert the risks of certain chronic diseases, thereby making them different from conventional foods.
- Nutraceuticals are the concentrated form of a certain bioactive compound isolated from normal food offered in medicinal form, that is not food-like in nature, which when consumed imparts enhanced health benefits.
- Laws furnished for the manufacture of dietary supplements hold the FDA accountable for new ingredients in these supplements and the manufacturers have been given the responsibility of making sure that the product is market safe. This is contrary to the rules laid down for the release and marketing of a drug where the rules are more stringent.
- Consumption of functional foods and nutraceuticals has immense health benefits such as lowering the risk of cardiovascular diseases, stroke, hypertension, and diabetes. Global malnutrition can be addressed and solved by supplementation and fortification of the available diet with important micronutrients.
- Enzymes play important roles in the production, extraction, concentration and fortification of food and applied throughout the food industry. Enzyme assisted processes also result in a higher yield of product compared to conventional methods.
- Enzymes are sensitive to pH and temperature changes and may exhibit decreased activity in the increased substrate concentration or any other compound. However, the application of genetic engineering has resulted in robust enzymes that show optimum activity even in adverse conditions. Furthermore, biocatalytic membrane reactors and immobilisation of enzymes on nanoparticles has opened doors to the reusable enzyme technology.

TEST QUESTIONS

1. Nutraceuticals and functional foods are different in the fact that:
   a. They provide demonstrated physiological benefits and reduce the risk of chronic diseases
   b. They provide essentials nutrients and minerals to the body that cannot be achieved by following a normal diet
   c. Nutraceuticals are offered in medicinal form while functional foods appear to be like normal food
   d. They can be consumed as part of a normal diet

2. Japan was the first country to implement laws on the production and consumption of nutraceuticals and functional foods. What is its equivalent in the United States and when did it come into being?
   a. Food and Specialised Health Uses Act (FOSHU) of 1994
   b. Dietary Supplement Nutrition and Education Act (DSNEA) of 1984
   c. Dietary Specialised Health and Education Act (DSHEA) of 1992
   d. Dietary Supplement Health and Education Act (DSHEA) of 1994

3. Enzymes are valuable assets for the production of functional foods and nutraceuticals because:
   a. They are highly specific in the reactions they catalyse
   b. They are proteinaceous in nature
   c. They act upon a wide range of substrates
   d. They are inexpensive and can be obtained easily

4. One of the following can be quoted as a selection criterion for enzymes used in food processing
   a. Enzyme reaction rate
   b. Co-products formed
   c. Availability of enzyme
   d. Origin of the enzyme

5. The following functional food ingredients are produced by employing enzymes except:
   a. Protein Hydrolysate
   b. Prebiotics
   c. Probiotics
   d. Prolactin

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