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Probiotics and Prebiotics as Functional Foods: State of Art

Amin Mousavi Khaneghah[1]*

Department of Food Science, State University of Campinas (UNICAMP), Brazil

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***Corresponding author:** Amin Mousavi Khaneghah, Department of Food Science, State University of Campinas (UNICAMP), Rua Monteiro Lobato, 80. Caixa Postal: 6121, CEP: 13083-862 Campinas, Sao Paulo, Brazil, Email: mousavi@fea.unicamp.br

Abstract

Foods, beyond their nutritional value, are increasingly perceived in connection with disease prevention and physical and mental well-being. Functional foods are defined in basic terms as "foods that may provide health benefits beyond basic nutrition." An underlying premise is that functional foods are foods, not medicine. Although primarily a problem of developed societies, health problems associated with modern eating habits are having a profound impact on developing countries as well, where deleterious effects are likely to be more evident because of economic constraints. Functional foods and health eating habits are then responses to the global change in lifestyle. The market for functional foods has been steadily growing in the last decades, representing both an opportunity and a challenge to food producers willing to meet such demand and conquer consumer acceptance. Probiotics and prebiotics are outstanding components of functional foods whose types, compatibility and their use in food products have been discussed in this paper.

Introduction

Now-a-days, health concerns are popular among consumers. Demand for nourishing, healthy and valuable products are increasing, to not only improve nutritional profile but also maintain body weight. A variety of probiotication techniques and probioticated products are available in the market. Dairy and nondairy-based food products are available in word wide markets.

Probiotics

Probiotic bacteria can be defined as the live microbial cells which give health benefit to their host when administered in sufficient amount [1]. Currently, the most important benefits of the probiotics include their antibacterial effects, stabilization of the intestinal microbial balance, elimination or reduction of pathogens by toxic chemical production or competition, improvement of lactose intolerance and modification of the host immune system. A wide range of beneficial bacteria has been investigated for their health benefits i.e., *lactobacilli, Bifidobacteria* or *streptococci* [2].

Probiotic bacteria are now used in the treatment or inhibition of oral diseases, which are caused by the change in the biofilm composition of the bacteria. Regarding the human gastrointestinal tract most important probiotic bacteria are lactic acid bacteria [3]. LAB are Gram-positive, homo-fermentative and non-spore forming. Among the Lactic acid bacteria, lactobacilli strains are gaining popularity in the food industry. The most familiar Lactic acid bacteria include *L. plantarum, L. paracasi, L. rhamnosus* and *L. bulgaricus* [4].

Background

In 1992 Fuller defined probiotic as a supplement of live microorganism feed which beneficially affect by promoting its microbial balance [5]. They were used at that tie mostly as "intestinal microbiota" later studies revealed that other health benefits of probiotics like immune system improvement, different preventive effects related to urinary tract, oral infections, respiratory tract, alleviation of allergic reactions. Probiotics additionally defined as alive microorganism used as food supplement which affects the health of the host beneficially [6].

Identification of beneficial bacteria

Microorganisms have been used in various types of food products for thousands of years. These microbes have been used in the preparation of fermented foods. The fermented foods increase the digestibility of food components. *L. rhamnosusis* considered subspecies *L. casei* but later it is known that it is a separate species. These are rods that appear in chains. These are beneficial for the human digestive tract, improve the immune system and oral health [7].

Probiotics types

Probiotic bacteria are living cell supplements which have health-promoting effects on the host. Food and Agriculture Organization have been accepted *Lactobacillus strains* and many Gram-positive bacteria and different eukaryotic species like yeast as a probiotic organism for animal and human use. Probiotic bacteria are being used from prehistoric times and in the first report in which health the Nobel laureate Mectechnic off published improving effects proposed 100 years ago. The scientific basis of using probiotics has been revealed recently [8]. During the last decade, probiotic bacteria have been used in different infectious, inflammatory and allergic disorders. Most of the probiotics effect has been attributed due to the variation in the gut flora and stimulation of the immune by these friendly bacteria [9]. In infants to prevent a different allergic reaction, *L. caseistrains* are potential inducer. In human colon, the most common *Lactobacillus strain L. plantarum* has been widely used in critically ill patents. Establishing and maintaining the normal microflora of the intestine help to abrogate various inflammatory and infectious conditions and prevent different distal organs like brain and lungs from infections [10].

Probiotics can also be used to prevent the disorder which occurs in infancy and manifested in later life like allergic reactions and atopy, some immune disorders and urinary tract related infections. Most frequently used bacterial strains belonged to genera *Bifidobacteria*, and Lactobacillus. These bacterial strains broadly used for the preparation of ready to eat fermented food products [11]. However, for many years the bioavailability and physiochemical stability of the bacteria in non-refrigerated foodstuff was a challenge.

Identification of the strain is very important to gain positive effects on their host. For selection of the health, promoting bacterial strains there are different selection criteria. The strain selected by following these criteria should be safe and health promoting, viable and active [12]. These probiotics must show their activity in the gastrointestinal tract to beneficially effect the host metabolism. After the strain selection it must be evaluated for its health-supporting benefits [13].

Lactic acid bacteria

Fermentation through Lactic acid bacteria is ancient discovery. To increase the storage stability and nutritional value of the perishable foods like fruit, vegetables, meat, fish and cereals various bacterial and yeast species have been used for fermentation in different parts of the world [14]. Such types of bacterial cultures which have the ability to ferment the food play very important role in food preservation. In the developed world, these cultures are mostly used in the fermentation of dairy foods like yogurt, buttermilk and cheese [15]. In this century the use of the starter cultures has changed dairy industry. The bacteria, which produce organic acid such as lactic acid from lactose sugar of milk through fermentation process, are termed as Lactic acid bacteria. *Lacto bacteriaceae* name was applied to the family of the bacteria which produce organic acids like acetic acid, lactic acid, carbon dioxide and alcohol through fermentation of the sugar components of food products [16].

Lactic acid bacteria are mostly Gram-positive bacteria which are categorized on the basis of their metabolic, physiological and some of their morphological characteristics. They are lactic acid producing, catalase negative, ferment carbohydrate, acid tolerant, and non-spore producing [17]. They are further divided into four different genera *Lactobacillus, Pediococcus, Leuconostoc*, and Streptococcus. *Bifidobacteria* were also included into *Lactobacillus genus* and named as *Lactobacillus bifidus* [8].

Lactic acid bacteria classified into different genera mostly depending upon their glucose fermentation mode, their growth at various temperature and bile salts concentration and ability to bear acidic conditions. Some newly developed genera have also use the property of motility and fatty acid composition [18]. Now a day, health promoting foods increase the use of these bacterial strains. Lactic acid bacteria are mostly present in such habitats where nutrients are abundantly available such as vegetables, milk, and meat but some of the members of these genera are present in the oral cavity, vagina and intestine of mammals [17].

Lactobacillus rhamnosus

L. rhamnosus formally called lactobacillus casai is an aerotolerant, homofermentative. It is Gram-positive, motile, non-spore forming rod-shaped bacterium. It was originally isolated from the gut flora of healthy children [2]. Actually, it is a mesophilic organism but depending on the strain, it can grow at different temperature ranges. It can grow at a higher temperature above 40 °C and at a lower temperature of below 15 °C. It requires a lot of vitamins for its growth such as folic acid, riboflavin, niacin, pantothenic acid and calcium. The best pH for initial growth of *L. rhamnosus* is 4.5-6.4 and grows in the form of single rods or in the form of short chains. It converts hexoses into lactic acid, and due to phosphoketolase and aldolase, pentoses also formed [7]. This bacterium is very resistant to the technological processes e.g. freeze-drying and it has the ability to stick to epithelial cells of the intestine [19].

Bifidobacterium spp.

Bifidobacterium is an irregular branched or rod-shaped, gram-positive, non-motile, microaerophilic and facultative anaerobic microorganisms. They vary in size is around 0.5-1.3x1.5-8.0μm. It is naturally present in human and mammals oral cavity, intestinal tract, and vagina [5]. Many research studies have proven their probiotic properties and these microorganisms have been widely used in commercially available products, such as fermented milk (i.e. *L. acidophilus*), yogurt (i.e. *L. acidophilus*), as well as in capsules (i.e. *L. rhamnosus*, *B. longum*, and *B. bifidum*). In 1905 Tisser was first who started the use of *Bifidobacteria* as probiotics. There are some other *bifidobacteria* which are classified as probiotics that includes *B. thermophilum, B. bifidum, B. animals, B. adolescentis, B. longum* and *B. Bifidum* [20].

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Streptococcus spp.

They are small Gram-positive cocci that are commonly present in the form of short chain or pair. They can grow in the absence or presence of oxygen and known as facultative anaerobes [21]. In yogurt and sour milk firstly used probiotic was Streptococcus salivarius subspecies thermophiles. Other Streptococci that are classified as probiotics include, S. intermedius, S. cremoris, S. lactis, S. diatilactis.

Lactobacillus spp.

The main genera of probiotics are *Lactobacillus* and *Bifidobacterium. Lactobacillus spp*. vary in size (0.5-1.2x1.0-10.0μm), a rod shape, Gram-positive, non-spore forming, facultatively anaerobic bacterium and its primary fermented end product is lactic acid. Gram-positive bacteria cell wall is made up of peptidoglycan. The presence of peptides and glycogen in their cell wall make a thick layer. Anaerobic microorganisms grow in the absence of oxygen. Microaerophilic organism requires a lower concentration of oxygen than that found in air [22]. Lactobacillus bacteria are microaerophilic and most frequently used probiotics in foodstuff e.g. in yogurt fermentation process they are used as a starter culture, Lactobacillus species used as probiotics include, L. Reuters, Lactobacillus brevis, *L. delbreuckii, L. cellobious, L. germentum, L. lactis, L. casei, L. plantarum* and *L. acidophilus*.

Prebiotics

The concepts of improving the colonic health by maintaining the microbial flora are gaining much interest. Traditionally, it was achieved by the use of probiotics. Prebiotics are basically carbohydrates of short chain length [23]. And also those carbohydrates that escaped from the upper portion of the gastrointestinal tract support bacterial growth in the lower portion of the gastrointestinal tract. The prebiotic concept refers to the non-digestible components of foods, selectively breakdown by health-promoting intestinal bacteria [24].

Prebiotics motivate the activity of these useful bacteria in the colon. Different organization defined probiotics in many ways organizations depending on the parameters related to selectivity, site of action, grade of causality or association provided, and the requirement of fermentation or metabolism. Recently, with the aim to clarify the prebiotic concept it is defined as a food component that is non digestible by human intestinal secretions such as enzymes, bile salts, and pancreatic juice but breakdown by the bacteria of colon this prebiotics modify or improve the activity or composition of the gut microorganisms thus giving a beneficial physiological effect on the host [25]. The mechanisms through which prebiotics show their beneficial effects are: 1) selectively stimulation of the bacteria associated with health, mainly *Lactobacilli* and *Bifidobacteria* and 2) production of some short chain fatty acids particularly butyrate, which have antimicrobial activity through the reduction of intestinal pH and other immunological and physiological activities. Most of the studies on prebiotics have been focused on fructans like insulin-type fructans, galactooligosaccharides and fructooligosaccharides [26].

The location

Where they seem to exert their activity depend on the degree of polymerization (DP), acting in proximal areas of the colon those with low DP and in the distal areas with high DP. Several new sources of prebiotics from plants with high fructooligosaccharide content are under investigation like dextran, oligosaccharides, and glucomannan. The development of future prebiotics should take into account their capacity to alter the gut microbiota and short-chain fatty acid profiles: together with their ability to decrease systemic inflammation: increase safety, and reduce oxidative stress [27].

Oligosaccharides

Oligosaccharides consist of 2 to 20 monosaccharides which polymerize to form short chain non-digestible carbohydrates. Oligosaccharides which are most widely distributed in nature are fructooligosaccharides present in chicory, onion, garlic, wheat, and oat. For commercial scale production of oligosaccharides, they are produced by the hydrolysis of long chain polysaccharides such as starch. They can also be produced from lower molecular weight sugars by enzymatic reactions [28]. A variety of oligosaccharides has been tested for their prebiotics activity for different probiotics. Significant increase in the number of *Bifidobacteria* has been reported in the faces of animal models and also human clinical trials after the consumption of different fructo oligosaccharides [29]. They did not break down in the upper part of human digestive tract where a huge variety of enzymes are secreted, but these are utilized in the lower portion of the digestive tract by intestinal probiotics like *Bifidobacteria* [30].

Insulin

Insulin is the mixture of many fructan chains which are widely distributed in nature as a storage carbohydrate of plants and more than 36000 species of plants contains these carbohydrates such as onion, garlic, chicory roots, wheat and oat [31]. Fructan extracted from chicory root have a chain length of 2 to 60 fructan units with 10 degrees of polymerization. Maltodextrin is an important encapsulating material. As a dietary fiber, it performs the function of prebiotics by increasing the bioavailability of calcium. Insulin has many health related benefits which can be used in different fields [32].

As a functional ingredient of food, insulin can be used in food products as prebiotic and increase the growth of microbial flora of the gut which has the potential to decrease the risk of cancer. Insulin is also used in cosmetics and pharmaceuticals for ethanol and energy production. Insulin can be extracted from plants by using by the use of extraction-based methods [33]. The most important character of insulin is their solubility which is very low in the cold water and more soluble in hot water. So hot water

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extraction is one of the common methods of insulin extraction. Conventionally, extraction of insulin from chicory roots is done at 70-80 °C in a continuous extractor in almost 1.5-2hrs.

Fructooligosaccharides

Oligofructose and insulin both have a particular chemical composition that can't be a breakdown by the enzymes secreted in our digestive tract. Both of these carbohydrates are hydrolyzed in our body as dietary fibers by the probiotic bacteria of the colon [34]. And these useful dietary fibers have positives effects on the host such as facilitate the movement of food in our gastrointestinal tract, relief from constipation, increase the volume of stool and increase the acidity of fecal matter.

Insulin and oligofructose fit in the group of carbohydrates which is recognized as the non-digestible oligosaccharides [32]. Chemically inulin is composed of the mixture of polysaccharides and oligosaccharides which have chemical arrangement GFn where G is glucose; F for fructose and n is the fructose units which are attached to each other. The maximum number of fructose units present in insulin is 60 obtained from chicory roots. The linkage between these molecules is of special nature which makes them non-digestible for higher mammals [35].

Synbiotics

The symbiotic term is used for the product which contains both the probiotic bacteria and the prebiotics. Synbiotic should be specifically used to explain such products where prebiotic compounds support the growth of probiotic organisms. Synergism can be explained as the ingestion of probiotic bacteria such as Lactobacilli and the growth promotion of intestinal *Bifidobacteria* [8]. Synbiotic foods effect was tested by giving different concentration with control results revealed that Lactobacillus and *Bifidobacteria* population were considerably increased in fecal matter and coliform bacteria markedly decreased. Digestive enzymes activity significantly improved with the high and low dosage of prebiotics and probiotics because of both increases the microbial population and production of digestive enzymes [36].

Dairy products like drinks and yogurt have been largely produced on the concept of symbiotic. Because it shows the synergistic association between health supporting bacterial strains and they are substrate to promote the growth when they pass through the stomach and reach into the large intestine to establish predominance [32]. Insulin and fructooligosaccharides have the property to be fermented by intestinal bacteria. Recent investigation has revealed that to ferment prebiotics is a strain-specific property of *Bifidobacteria* due to their specific enzyme production which utilizes insulin and fructooligosaccharides as their substrate. While the other bacterial strains such as *B. longum* and B. animalis has the property to hydrolyze a number of oligosaccharide with a higher degree of polymerization which can't be digested by other bacterial strains. In this way, nature provides a diverse ecosystem in the intestine of human beings to ferment a variety of non-digestible carbohydrates by specific bacterial strains [37].

Safety aspects of probiotics

Generally, probiotic bacteria can be defined as the live microorganisms which on consumption confer health benefits on the host. There is an increasing interest in probiotics use due to their perceived health effects. *Lactobacillus, Lactococci*, and *Bifidobacteria* are generally regarded as safe [28]. Regarding the safety of probiotics, there are basically three theoretical concerns, the first occurrences of any disease like bacteremia, second toxin production which can affect the gastrointestinal tract and third their property to be resistance to antibiotics. There are very rare chances of any disease caused by the use of prebiotics like bacteremia otherwise most of the clinical trials show the safe and health promoting effects of probiotics. Lactobacillus have been using safely since, long time ago in different food products and in dairy products. Lactobacillus is naturally associated with human digestion tract and also found in some animals and plants [38]. Lactobacilli are mostly used in fermented milk products. People Lactic acid bacteria rarely cause infection mostly in people who are immune deficient and these are fall in the category of organisms which are generally regarded as safe. Whereas other probiotics like Bacillus, Enterococcus, and spore-forming bacteria are used as probiotics but not generally regarded as safe.

Compatibility of the probiotics

Probiotic bacteria are to be beneficial for the health of human beings. For the selection of a particular type of strain various factors are considered which are as follow [39]:

a. Probiotic strains should have the capability to exert health benefits e.g. increase the immunity.

b. It should be safe for use e.g. non-pathogenic.

c. The viability of the selected probiotic bacteria should be high so that it can show its effects.

d. Selected strains should be resistant to harsh conditions e.g. low pH.

e. Storage stability of the selected strains should be excellent.

f. It should be human origin so that it can stay in the gut tissues.

Probiotics in the intestine

Probiotics are considered as food supplements as well as feed who complementary affect the health of the host. It is important to know the health benefits of particular strains which they exert on the health of the host. Probiotics include a large number of microorganisms like bacteria and yeast. The most important probiotic bacteria are Lactic acid bacteria and some yeast can also be considered as probiotics as they represent favorable effects on the health of their host [20].

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These bacteria have value able and very useful role in the maintaining the health of the host. These bacteria are considered very vital for the human gastrointestinal tract. They possess the following characteristics; they are Gram-positive and prefer to survive as anaerobic. Some studies showed that they can also survive in the aerobic environment. Different bacterial strains have different health-related importance for their host.

So each species of probiotic bacteria promote and support the health of their host by producing different types of acids and some strains function by the production of bacteriocins. The literature cited has shown that 106 to 107Cfu/g/day usually recommended. It has been investigated that a healthy human adult contains about 107 bacteria per gram of saliva in their oral cavity. Their number decreases in the stomach due to increasing in acidity up to 102 to 103 cells per gram of gastric secretions [40].

Bacteria present in the intestine are three types; 1) beneficial bacteria 2) harmful bacteria 3) neutral bacteria. The microflora of the human intestine includes the bacteria from the genera Lactobacillus, Terobacteria, *Bifidobacteria*, and Streptococci. Some of the harmful bacteria include Escherichia coli and clostridia. These harmful bacteria produce toxins like phenols, ammonia, and amines [24]. These bacteria also compete with beneficial bacteria by replacing them and killing them. *Lactobacillus rhamnosus* is one of the utmost important bacteria which have the ability to reduce the toxin which was produced harmful bacteria. They also detoxify the metabolites produce by these pathogenic bacteria. To alter the metabolic processes is also an important probiotic characteristic [41]. It includes the steps like repressing the actions which produce toxic metabolites, motivating response that detoxifies toxic substances, inspiring enzymes of mammals for digestion of composite nutrients and by preparing vitamins and additional necessary nutrients not taken through diet [42].

Probiotics based food products

There are lots of health benefits of probiotics consumption. It has been shown that probiotics provide nutritional effects, such as producing water soluble vitamins in the host colon, increasing the bioavailability of iron, production of short chain fatty acids and improving lactose digestion. Besides Lactobacillus and *Bifidobacteria*, probiotics also include some species of yeast as probiotic yeast like *Saccharomyces boulardi* and *Saccharomyces cerevisiae* [43].

It is been observed that these probiotic strains have been mainly used in pharmaceutical applications such as premature infant formula. Some clinical trials data indicate that probiotics have the potential to prevent and treat different diarrheas. Besides treating diarrhea cases like Rotavirus diarrhea, traveler's diarrhea and antibiotic-related diarrhea probiotics have the potential to treat irritable bowel syndrome disease [44]. The main symptoms of the disease are flatulence, abdominal pain, diarrhea and sometimes constipation. A mixture of the *B. breve* and *L. rhamnosus* has been shown significantly decrease irritable bowel syndrome.

Probiotics also have the potential to prevent postoperative infections. *B. breve, L. plantarum*, and L. caseihave been used for the treatment of patients who were recovering from surgery and probiotics significantly decrease the chances of bacterial infection. Many studies indicated that probiotics have the ability to decrease allergic reactions. For example *L. rhamnosus, L. reuteri*, and *B. longum* significantly decreased atopic eczema [45]. Moreover, is has been proposed that Lactobacillus may decrease the level of serum cholesterol by breaking down and retarding its re-absorption. Short chain fatty acid can also be produced by Lactobacillus which suppresses the synthesis of hepatic cholesterol and its distribution in liver and serum. Probiotics have been indicated to have potential to bind with carcinogens chemicals and further decrease the occurrence of colon cancer [46].

Probiotics dairy based products

Fermented products containing probiotics are gaining popularity all over the world such as yogurt and some fermented beverages. Due to their functional properties and viable bacteria fermented milk products give numerous benefits to their host. These products minimize the problem of lactose intolerance. Yogurt is the fermented product which is developed by using *Lactobacillus bulgaricus, Lactobacillus delbrueckii*, and *Streptococcus thermophiles*. Yogurt has attributed therapeutic effects like digestion improvement, immune system enhancement, a decrease in serum cholesterol and anti-carcinogenic property. Besides of their health benefits which are due to the presence of live microorganisms the other factors like appearance and color are important for consumer acceptability [47].

Ice cream

Ice cream containing probiotics was prepared by properly mixing fermented milk which contains probiotics and ice cream mix followed by freezing and evaluated for culture survival, chemical properties, and sensory characteristics during storage at -26 °C for 12 weeks. Analysis of ice cream mixes showed higher acidity, viscosity, specific gravity and decreased freezing rate in the case of mixes containing probiotic bacteria [7]. The viable counts in logs CFU/g decreased up to 2.23 for *L. acidophilus*, 1.68 for *B. bifidum*, 1.54 for L. reuteri, 1.23 for *L. gasseri* and 1.77 for L. rhamnosus, log CFU/g, during frozen storage of 12 weeks. All ice cream samples obtained a high score in the organoleptic evaluation. L. reuteri containing ice cream was arbitrated to be attained higher score for probiotic flavor and more sour taste.

Ice cream with probiotics was prepared by fermenting the standard ice cream with *B. bifidum* and *L. acidophilus* cultures and undergo freezing in a batch freezer. The viability of the *L. acidophilus* and *B. bifidum*as well as B-galactosidase activity was checked during 17 weeks of frozen storage at -2 °C. After freezing of the mixture, bacterial count for *B. bifidum* was 2.5x108CFU/mL and for *L. acidophilus* it was 1.5x108CFU/mL. The probiotic

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ice cream was prepared at pH 6.0, 5.5, and 5.0, which evaluated by 88 judges. The ice cream was preferred with pH 5.5, based on overall acceptance. It is recommended that symbiotic ice cream is a preferable vehicle for delivering health beneficial microorganisms for consumers and remain viable during storage period [13].

The survivability of free and microencapsulated probiotic strains *L. acidophilus* (LA-5) and *L. casei* (NCDC-298) in four type ice creams was studied. The viability of two probiotic was checked at -23 °C for the storage period of 180 days. In the free-state in prepared ice cream mixture, the viable cell count of *L. acidophilus* (LA-5) was 5.1±0.2×109CFU/mL at day one and viability decreased to 4.1±0.3×106CFU/mL after 180 days of storage. The survivability of *Lactobacillus casei* (NCDC-298) at day one was 4.3±0.2×109 CFU/mL and viability decreased to 1.9±0.2×107CFU/mL after storage of 180 days. The survivability of both probiotics was increased up to 30 percent after encapsulation along with calcium alginate and whey protein concentrate beads, during the same period of storage. Microencapsulation can enhance the survival rate of probiotics in ice cream for a long period of shelf-life and sensory properties were not affected.

The viability of encapsulated bacteria has at least 6 months and in a frozen state at least 18 months, when held under normal atmospheric humidity (RH 50%) at room temperature. Probiotics are widely used food ingredients as supplements that provide health benefits to consumer's health. Precise inspection and epidemiological studies make possible strains identification and their link with consumer's health. The symbiotic ice cream was prepared by addition of *L. acidophilus* to enhance the nutritional and textural properties. Incorporation of insulin in ice cream mix significantly ($P<0.01$) reduced the melting rate, improved the growth of Lactobacillus acidophilus by increasing count from 0.61 to 0.77 log counts in end product at -18 to -23 °C in ice cream contained therapeutic minimum probiotic level of 106cells/mL for 15 days of storage [48].

The study was conducted to assess the stability of two proven bacterial strains viz., *L. casei* (NCDC-298) and *B. animalisin* ice cream having four treatments, free and encapsulated *Lactobacillus casei* and B. animalis by using microencapsulation technique. Probiotic viability was monitored at -23 °C up to 180 days of storage. The viable cell count of B. animalis) and *L. casei* (NCDC-298) in a free state in ice cream prepared mixture was 4.6±0.2×109CFU/mL and 5.3±0.2×109CFU/mL at day one and count was reduced to 2.1±0.1×107CFU/mL and 4.5±0.2×106 CFU/mL respectively. A procedure of microencapsulation can meaningfully improve the survival percentage of probiotic bacteria above 30 percent during the same period of storage, with no significant effect on the sensory properties [49].

Fermented frozen desserts and ice cream fortified with live probiotic bacteria *L. acidophilus*, P. jensenii and B. animalis are gaining popularity among the dairy products. This study also checked the physicochemical and sensory characteristics while storing ice cream in various packaging materials. It is concluded that by storing ice cream at -20 °C these probiotics can remain viable up to 52 weeks above the minimum therapeutic level. Survival characteristics of *B. lactis* and *L. casei* encapsulated in alginate were 30% higher in frozen ice cream, these can undergo mechanical stresses of production process due to this cell walls of free bacteria can be damaged by the formation of ice crystals [50].

Free and encapsulated probiotics are utilized in dairy products commonly but the main problem of concern is the lactose intolerance and allergic problems that arise after the consumption of dairy products. Many people around the world are negatively affected by allergy. It was found that traditional prospects and economic concerns restrict the usage of dairy products in different nations like China, Japan, and some African countries [51]. It promoted the concept to reduce the use of dairy products. The major concerns using dairy products are lactose intolerance, allergic problems and cholesterol content; all these problems paved the way for the development of non-dairy products containing probiotics.

2.22. Probiotic non-dairy based products

Research work on vegetables showed that these are the best ones for providing substrates for probiotics because they contain nutrients, dietary fibers, antioxidants and also do not contain allergens. Adding probiotic cultures to different beverages is a novel concept and provides a research opportunity for food professionals. Wheat is a widely available and commonly used cereal in Pakistan because due to its nutritional profile containing vitamins and different minerals (iron, potassium, calcium, phosphorus, and sodium).

Useful nutrients are present in vegetables like antioxidants, dietary fiber, minerals, and vitamins while missing the dairy allergens and making them suitable for probiotic cultures. It is reported that the Vegetables and fruits contain a wide variety of antioxidant components such as phytochemicals [52]. The phenolic compounds present in phytochemicals are essential to decrease the risk of degenerative diseases by inhibition of macromolecules oxidation and by the reduction of oxidative stress due to these properties these compounds are beneficial for human health. The non-dairy based functional beverages with probiotics are developed due to certain reasons because they are cholesterol free and serve as a healthy alternative for dairy probiotics.

Cereal based beverages and fruit juices consider as a good source of minerals and vitamins also give refreshing effect to the consumer. Beverages are liked by the people of all age groups irrespective of the dairy product which are avoided by a segment of the population due to some potent allergens like lactose and casein intolerance. Because of the above mention reasons beverages are considered as good carrier for transferring of probiotics, as they are a good source of nutrients [16].

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Non-dairy probiotic beverages can also help as a healthy substitute for dairy probiotics and also favor consumption by lactose intolerant consumers. The technologies work to alter the structural composition of beverages prepared from cereals. These changes could convert them into ideal products for health and contain a lot of useful compound like minerals, different vitamins, and antioxidants. The milk based products are available in the market nowadays but, due to the consumer demand for cholesterol free products the probiotics containing non-dairy beverages have encouraged scientists and researchers to explore new matrices such as fruits and vegetables based drinks [53]. Cereals based beverages contain higher contents of sugars which also support the growth of probiotics. A large number of benefits are related to the use of foods containing probiotics. Probiotics positively influence the intestinal microflora, improve the functionality of intestine, reduce the pH of the intestine, act as antimicrobial agents, detoxify the microbial metabolites, reduce the cholesterol level, produce vitamin B (folic acid) and improve the immune system regulating the host cells. Because of a range of benefits provided by probiotics, there is an increase in the incorporation of probiotics in different food product especially juices beverages [54].

It is investigated that to check bacterial viability and survival rate eight different bacterial strains in the free and microencapsulated form in apple and orange juice. The viability of these bacteria was assessed up to six weeks when they were incorporated into apple and orange juices. It was noticed that encapsulated probiotics survived six weeks in fruit juices during storage while within five weeks the viability of free probiotic organisms was lost [55].

In fruits juices, microencapsulated probiotic organisms showed better survival as compared to free probiotics organisms. During storage, it showed that the pH and Brix concentration was decreased in probiotic fruit juices. The survival of probiotic bacteria may increase in buffering fruits juices while it causes the reduction in the shelf life of the products. Concentrations of malic acid were lower in microencapsulated probiotics containing fruit juices then free probiotic fruit juices. It was recommended that the food products may be stabled by using immobilized cells [16].

Maintain probiotics viability through GIT tract

Probiotics carry great health benefits and that's why there is direct need to improve their survival. The viability is of supreme importance to transfer the bacteria at their target place where they will perform their function. Several factors are responsible for decreasing the viability of the probiotics. Some of the important factors are pH, acidity, dissolved oxygen contents as they are anaerobic, storage conditions, hydrogen peroxide production during fermentation of product, strains type and concentration of citric acid [25]. These factors must be considered during manufacturing of probiotics containing products. To enhance the survival of the probiotics in food products we have to provide them such techniques which prevent them for adverse environmental conditions and increase their survival in food products. At the time of ingestion of food, probiotics must be present in sufficient amount to give health benefit on the consumer. One of such technology which enhances probiotics survival and viability in the food products is microencapsulation technology [56].

Effect of carrier food matrices on the survival of probiotics in the gastrointestinal tract and their adhesion ability have been studied which show that it has a significant influence on the viability of probiotics in the extremely acidic environment (pH 2.0) and also in bile salts solution 0.3%. Exposure of probiotics to lower acidic conditions results in a great reduction in viability as compared to pH levels Between 3 to 4. As compare to the plain and stirred yogurts, ice cream improves the bile salts and acid tolerance of the probiotic bacteria. Similarly the *in-vitro* adhesion ability of the probiotics influenced by carrier food matrices [57].

Different food products are produced by distinctive methods. The differences in the food production processes can affect the functioning and viability of the probiotics in the product [26]. During storage of the food products survivability of the probiotics is affected and also in adverse conditions like bile presence and high acidic conditions effects strain functionality. To deliver the probiotic strains into gastrointestinal tracts different dairy products like ice cream and other frozen desserts can serve as a carrier. The total solid level in dairy products like fat and milk solid provide protection to the probiotics from adverse environmental conditions [33].

Probiotics containing food products

The connection between the foods we consumed and our health is very obvious. Functional foods are those foods that provide additional health benefits along with their nutritional value. Functional foods have great potential to encourage health in such marvelous ways that cannot be expected from traditional nutritional food science [27]. Functional foods have been evaluated to be very effective towards personal health. Functional foods with a lot of health benefits are inspiring research and development in the field of food technology.

Although all foods are functional as they offer aroma, taste and nutritional benefits to the consumer. But the term functional food is used for those foods that provide supplementary benefits such as physiological benefits along with their nutritional benefits. By the use such ingredients, a new era of developing functional foods originated [18]. The concept of functional foods was promoted in japan when Japanese calculated the relationship among physiological systems modulation, fortification, sensory satisfaction, and nutrition. They concluded that functional foods are the need of the time.

Functional foods provide a number of health benefits by improving the stomach and colon health and functioning by providing prebiotics, probiotics as functional ingredients. These

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are designed to treat the existing health related problems as high blood pressure and cholesterol level in patients due to their gluten and lactose-free property. Along with their nutritional and health benefits, functional foods are also providing economic opportunities and market gain. With increasing trends of functional foods particularly probiotic based non-dairy drinks the main challenge is to attain maximum viability of probiotics in the food commodity during its storage and passage through intestinal tract [12].

In addition to taste and nutrition of the food products, the consumers are now interested in their health benefits. In this regard, the nutrition science is updating the consumer with the latest knowledge about the food products. Functional foods are defined from different aspects simply these are the food that not only gives nutrient but a variety of health benefits. The consumption of these foods increases the functioning of different body parts [15]. If a consumer takes foods containing the antioxidant, these will prevent from oxidation process decreasing the chances of cancer. Foods containing the high fiber contents and antioxidants fall in the category of functional foods. And functional ingredients may be tea, fruits, and vegetables. The ingredients of food should be active so that they can play role in preventing the human from different diseases. Some important functions in the human body can be improved by consuming the functional foods like physical performance, mode and behavior. Some gastrointestinal function like the immune system, food transit time, colonic microflora, antioxidant, and redox reaction are integral for normal functioning of cells and tissues. These reactions required a certain amount of vitamins and minerals. These functions can be improved by the consumption of fresh fruits and vegetables which are helpful in controlling the toxicity and carcinogenicity caused by different harmful chemicals [11].

Food producer and health professional are also taking these issues very seriously. They concentrate on prebiotic and probiotics foods having functional effects. A synbiotic product is the combination of functional ingredients with their specific health benefits. So cumulatively probiotic based functional food provides numerous health benefits under one umbrella. Different products containing probiotics and prebiotics are available in the market, for example, probiotic yogurt, probiotic juices and symbiotic beverages [9]. The shelf life of the food can be enhanced by controlling the pathogens that cause spoilage of the food. Another principle can also be applied to improve the shelf life of the food products [7].

Microencapsulation

Probiotics are mostly taken orally. The most commonly used probiotics strains are *Bifidobacteria* and Lactobacillus. However, some other species can also be used with them to achieve same objectives. Some yeast also used as probiotics such as *Saccharomyces cerevisiae*. Some of the probiotic microorganisms are incorporated into food products converting them into functional foods. Such kind of ailments can be defined as modified foods or food which provides health benefits with satisfying nutritional requirement [53].

To give beneficial effects to host, probiotics should be able to alive and proliferate in the host. That's why, probiotic bacteria should be active and stable in the food products, survive in the channel through stomach and intestine inadequate number. However, the viability of probiotics has been affected by several factors including hydrogen peroxide, pH, oxygen and storage temperature [54]. To improve the resistance of the sensitive bacterial cells against harsh conditions different approaches has been suggested such as selection of strains that are resistant to acid and bile salts, use of oxygen-impermeable containers, micronutrients assimilation such as amino acids, peptides, and microencapsulation. Microencapsulation is the most efficient method and is under special attention and examination [55].

Microencapsulation can be defined as the technique through which probiotics are packed in the encapsulating material to decrease the cell death in such a manner that result in the proper release of microorganism in the gut. Some of the benefits of microencapsulation are as follow: Microencapsulation protects the probiotics from bacteriophage [58]. Protect them from damaging factors improving their survival in freezing, freeze-drying, and storage. Converting probiotics into powder form through microencapsulation make their use easier and also help in their homogeneous distribution in the product [59]. Encapsulation process may be divided into 3 steps. In the first step, the addition of bioactive compound is done which may be liquid or solid. The second step includes the dispersion of liquid matrices.

The last step deals with stabilization by a chemical and physical process like solidification, evaporation, and polymerization. After encapsulation of the bacterial cell, spherical or oval shaped structured are obtained which are known as beads or micro beads. Each micro bead consists of hydrocolloids which are also called capsule coated with the bacterial cells [38]. Hydrocolloids may be alginate or other modified starch. Each bead may contain one or several cells. When a single bead contains several cells then the liquid between the cells from encapsulation medium fills the spaces of the micro-bead. As a result of which superficial or deep cracks may occur in the beads when these cracks spread, they lead to pore formation in the capsule which considerably reduces the encapsulation efficiency.

Probiotic encapsulation technology

Encapsulation using probiotic is known as Probiotic Encapsulation Technology (PET) using many microorganisms which are immobilized in the globular structure of the membrane, so moderating the release of cells. Sometimes double layer is formed to protect the microorganism of interest. Biomolecules are materials used for encapsulation of the microbes. The material used as biomolecule should provide maximum protection to the active material and hold it active to have desired properties during its processing and storage. There are

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different types of the biomolecule is reported in literature like polysaccharides (chitosan, xanthan, dextrin, and carrageenan), milk and milk proteins. Sodium alginate is mostly used due to its low cost and easy availability [58]. Twelve encapsulated and non-encapsulated strains of Lactic acid bacteria and *Bifidobacteria* were tested for their resistance to the environment just like a human digestive tract. Results clearly revealed that encapsulated bacteria remain protected from the unreceptive environment and digestive tract preventing the loss of probiotic bacteria. The viability of the encapsulated bacteria increased at pH 2.0 attaining the mean value 58.9% as compared to the bacteria without encapsulation [60].

Encapsulation has many applications in the food industry that involves enhancing shelf life controlling oxidative reaction, pretense flavors, odors, and colors, giving a sustained release, etc. It is also being used for the protection of Probiotic bacteria from adverse environment Encapsulation is used for micro packaging of solids, liquids and living microbes in capsules, their contents are released in controlled amount under the influences of certain conditions [61]. A solid or liquid core is surrounded by spherical, semipermeable membrane in a microcapsule having a diameter up to 1mm.

Encapsulating materials

Important food ingredients that are encapsulated include enzymes, flavors, flavor enhancer, sweeteners, antioxidants, food preservatives, acidulates, amino acids, colorants, edible oil, essential oils, fats, leavening agents, vitamins, minerals, salts and microorganisms. Application of encapsulation is not only limited to the food industry but it has also a wide application in various other fields like biotechnology, pharmaceutical, and chemical industries.

In the food sector encapsulation can be utilized to stabilize the central material, to give a scheme for the start of reaction, provide a control release (both time and rate of release), to disguise flavor, colors and odors, to enhance shelf life or protect components against nutritional loss and to decrease volatility [62]. Different types of coating material used for encapsulation of probiotics. Some of which are proteins (albumin, hemoglobin, and casein), celluloses, gums (sodium alginate, agar, and gum Arabic), lipids (wax, hardened oils, and fats), and carbohydrates (starch, dextrin, and sucrose).

Maltodextrin

Maltodextrin is used as coating material due to its unique properties like water solubility, low sugar content, and low viscosity. Maltodextrin is a hydrolyzed product of starch and most commonly used in food industries for coating the materials. It has been utilized for encapsulation of hydrophilic and hydrophobic components e.g. oil, natural pigments, flavors etc. its stability depends on upon the formation of the amorphous glassy matrix during microencapsulation. Moreover, the hydrogen bonds between maltodextrin hold up the solid network of glassy material. Bioactive compounds are encapsulated in this matrix and consequently protected from the external environment [63].

Gum arabic

The main source of gum Arabic is acacia tree which is a good encapsulation material. Gum Arabic possesses different properties like low viscosity and high water solubility. It also behaves as oil in water emulsion it is a good source of dietary fibers. It can maintain the original taste of the food products. It can be added in large quantities in any products as it doesn't affect the organoleptic properties of the products and well resistant to the acidic and other processing conditions [35].

Gelatin

Literature indicates that gelatin is the most commonly used matrix for the encapsulation of the probiotics due to their non-toxic, high molecular mass, inexpensive and valuable characteristics. Main sources of gelatin are bones, skins, and hides. Gelatin can absorb water when put in the cold water and can absorb 5-10 times of its volume of water. After the absorption of water, it swells and has good film forming properties. It has good characteristics for the encapsulation; it can form the good protective layer. It increases the survival in many processing conditions [64].

It is considered as protein gum. This makes the thermo reversible gel and is used for the encapsulating the probiotic. This gum can be used alone for the encapsulation or it can also be used in the combination for encapsulation or for other different purposes. Its great advantage is that it is amphoteric [65]. That is the reason that it can be used with ionic polysaccharides that include the Gellan gum. These hydrocolloids are mixed at a pH greater than 6. From an industrial point of view, it is good polysaccharide which has extensive applications in different industries.

Carrageenan

Carrageenan is rarely used as a bio-molecule for encapsulation purpose. The gelation caused by the carrageenan helps to keep bacteria live. It is the natural polymer that requires the temperature between 40-50 °C and cooled to room temperature to get the desired properties. Microencapsulation of microbes is efficiently done by incorporating different stabilizing agents like potassium ions. The stability of these micro beads is very poor due to their brittleness [35]. As a bio-molecule carrageenan shows its suitability to encapsulation when used with locust bean. Raw milk is also used to enhance binding properties of carrageenan because it forms bonding with milk protein [66].

Pectin

Pectin is the component of cell wall which performs a biological function in the plant. It has good defensive power against the microorganisms. It maintains the physical as well as sensory properties of the fruits. It does not affect the processing characteristics of fruits. It is a good dietary fiber and has a lot of health benefits [38]. It is not digested but has well physiological

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and nutritional effect on human health. Enzymes that are produced by humans are unable to digest this fiber. It has been reported that it is good for lowering cholesterol in human and rats.

Chitosan

Chitosan is made up of polymers of glucosamine which are polymerized by cross-link formation in the existence of anions and poly anions. It is a linear polysaccharide [64]. It is not a good capsule material but we can use it as only as a protective material for the different ingredients. However, encapsulation of probiotics with a mixture of chitosan with alginate gives protection in the gastrointestinal tract.

Starch

Starch is the polysaccharide which is made up by polymerization of glucose units through a linkage known as glucosidal bonds. Starch regarding its composition is mainly made of amylase and amylopectin. The linkage is an alpha-1-4 glucosidic bond. Resistant starch is a type of starch that is digested by an enzyme known as pancreatic enzymes it is named as it occurs in the intestine known as amylase. Resistant starch fermented in the colon [62]. It is a very useful plant polysaccharide. Bacterial survival is higher in this encapsulation as compared to other application. It is considered as an ideal for the bacteria particularly the probiotic bacteria. This can increase the survival and make the successful release of the bacteria. Due to its different attributes, it is extensively used in the food and pharmaceuticals industries [61].

Emulsion technique

In this technique, all the glassware are sterilized in the oven at 171 °C for 30 minutes. All solutions that are prepared for encapsulation are also sterilized in the autoclave. Recommended encapsulating materials can be used for the encapsulation of the bacteria. Mostly calcium or sodium alginate is used in the food industry. Concentration may very it depend on the purpose of the encapsulation [25]. A mixture of 2% alginate is prepared. Then 0.1% culture and 3% sodium alginate or gum arabic can be added. After that the mixture is dropped into corn oil that contains Tween 80 (0.02%). The purpose of the addition of tween in the mixture is to increase the emulsification. This mixture is stirred strongly. It makes the mixture emulsified and to large extent as creamy. Then a solution of 0.1M calcium chloride is added hurriedly sideways the side of the beaker [45].

This causes the separation of an oil-water emulsion. This mixture is permitted to stand for thirty minutes so that the sodium alginate beads to separate and settle at end of the layer. After their settlement at the bottom the oil layer is drained [41]. The beads are collected by the low-speed centrifugation (350×15min) after that 0.9 % saline containing glycerol is used to wash the beads. These beads are stored at 4 °C for future use. This method is extensively used in the food industry as well as in other industries [57]. The beads that are obtained in this method are smaller in size as compared to the extrusion method. This technique is costly than the extrusion.

Spray drying

It is most suitable due to different factors spray drying is a widespread method for encapsulation in the food industry. It is very economical. It has the high stability of the most encapsulating material. It has rapid solubility power of the desired capsules and easy availability of the processing equipment. There are some disadvantages that are associated with this method. It includes the coating or encapsulated material must have high viscosity this makes the choice of the material limited. Another disadvantage is that heat drying may cause the oxidation of some active ingredients. This method is not applicable for the foods having intermediate moisture level. This technique is very useful for encapsulating flavor, starches, vitamins, pigments, proteins, oils, polysaccharides and leaving agents [47]. The most commonly used encapsulating or the coating materials in the spray drying method are dextrins, gum arabic, gelatin, soy proteins, and starches.

It is extensively used in the food industry. It is economical, flexible and produces good quality particles. It involves the following steps Emulsion or suspension formation of encapsulating materials and then the evaporation of moisture of the emulsion. It has different advantages and disadvantages. Advantages include high dispensability, beneficial for heat sensitive ingredients, the formation of large amount of capsules [67]. Disadvantages include it can cause the loss of low boiling compounds, oxidation of oil and it is only feasible for the water-soluble shell.

Extrusion technique

This is very simple technique. It is mostly used on laboratory level for the production of the beads. This technique is very economical. Some disadvantages that are associated with this are the slow production of beads and beads are larger in size. These larger beads create grittiness in the product. That is not a desirable character for the product. A hydrocolloid solution is prepared by adding 3 % sodium alginate in water [55]. In this solution, 0.1% probiotic bacteria are added. This suspension is extruded through a syringe. The syringe is used to form droplets. These droplets are dropped into a hardening solution that is prepared by adding the calcium chloride (0.5M). Droplets are allowed to stand for thirty minutes in the hardening solution [54]. Beads are collected by centrifugation and are stored at 4 °C for future use. Encapsulates are considered as a perfect example of extrusion technique, having high efficacy [68-114].

Conclusion

The role of Probiotics and Prebiotics as Functional Foods was reviewed in the current study. Employing the biotherapeutic mechanisms such as probiotic to improve of human health as well as controlling of linked food pathogens can be recommended as a promising and safe approach of bacteria in food product.

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Approaching emerging technologies can be point of interest on enhancing the performance of proposed functional foods.

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