Chapter 9

Functional Dairy Probiotic Food Development: Trends, Concepts, and Products

Aziz Homayouni, Maedeh Alizadeh, Hossein Alikhah and Vahid Zijah

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/48797

1. Introduction

In recent years, scientific investigators have moved from primary role of food as the source of energy and nutrients to action of biologically active food components on human health. On the other hand, consumer interest about the active role of food in well-being and life prolongation has been increased. In this way, a novel term -functional food- was introduced which refers to preventional and/or curing effects of food beyond its nutritional value. There is a wide rage of functional foods that were developed recently and many of them are being produced in all over the world including probiotic, prebiotic and symbiotic foods as well as foods enriched with antioxidants, isoflavones, phytosterols, anthocyanins and fat-reduced, sugar-reduced or salt-reduced foods. Among these foods, probiotic functional food has exerted positive effects on the overall health. We can divide it in both probiotic dairy foods and probiotic non-dairy foods. The market of probiotic dairy foods is increasing annually. An increased demand for dairy probiotic products comes from health promotion effects of probiotic bacteria which are originally initiated from milk products, bioactive compounds of fermented dairy products and prevention of lactose intolerance. Therefore, development of these products is a key research priority for food design and a challenge for both industry and science sectors.

Literatures about probiotic application in pediatrics have some characteristics including numerous, randomized, controlled clinical trials or meta-analyses but the substantial heterogeneity of these works greatly complicates the interpretation of the results and thus makes it difficult to draw univocal and general conclusions. Despite these complications, it is possible to draw some conclusions about the clinical effectiveness of probiotics by examining the most significant literature on each pathology. In particular, there is strong evidence indicating that probiotics have preventive and therapeutic effect on pathologies such as acute diarrhea, antibiotic-associated diarrhea, NEC, and allergic pathology. It was

reported that administration of L.GG to 50 infants, for a period of 6 weeks, did not improve abdominal pain but did reduce the incidence of abdominal tension compared to the placebo (Bausserman and Michail, 2005) But in other works it was clearly demonstrated that L. acidophilus did improve the symptoms in about half of the patients with IBS, that the blend of VLS#3 probiotics decreased abdominal swelling, while the combined use of L. plantarum and B. breve reduced pain intensity (Halpern, et al., 1996; Kim, et al., 2003; Saggioro, 2004). L. acidophilus and B. infantis for 4 weeks were administered alone or in combination with antibiotics ciprofloxacin for the first week to three different groups with IBS: diarrhea, constipation, and alternating diarrhea and constipation. Both therapeutic approaches have improved the quality of life and reduced symptoms in all three groups (Faber, 2000). In conclusion, although the use of some types of probiotics on IBS appears promising, additional studies are needed. Food supplementation with pre- and probiotics may reduce the prevalence for the infant in high-risk families developing an atopic eczema during the first 2 years of life. Those pregnant women should be advised to take probiotics (L. GG) in late pregnancy and the first 6 months postnatally during nursing. If breast-feeding is not possible, pro- or prebiotics can be supplemented to the infant. There are no known adverse reactions and it might prevent atopic eczema, especially in neonates after cesarean delivery. Therapeutic use of probiotics to improve atopic eczema is only supportive in infants 18 months and with IgE sensitization.

Recent experimental studies have shown that certain gut bacteria, in particular species of Lactobacillus and Bifidobacterium, may exert beneficial effects in the oral cavity by inhibiting Streptococci and Candida sp. Probiotic lactic acid bacteria can produce different antimicrobial components such as organic acids, hydrogen peroxide, carbon peroxide, diacetyl, low molecular weight antimicrobial substances, bacteriocins, and adhesion inhibitors, which also affect oral microflora. However, data is still sparse on the probiotic action in the oral cavity. More information is needed on the colonization of probiotics in the mouth and their possible effect on and within oral biofilms. There is every reason to believe that the putative probiotic mechanisms of action are the same in the mouth as they are in other parts of the gastrointestinal tract. Because of the increasing global problem with antimicrobial drug resistance, the concept of probiotic therapy is interesting and pertinent, and merits further research in the fields of oral medicine and dentistry (Meurman, 2005).

The number of microbial cells in the human gut is 10 times more than the number of cells in the adult body (Mountzouris and Gibson, 2003). So, the change of microbial balance in human intestine can impress the host health. The ratio between the beneficial microbes (probiotics) and harmful microbes would have an important effect on host health. One way to keeping up the probiotic cells in the gut, is to entering probiotics into the intestine through the regular consumption of food containing these bacteria. Among the functional foods, the dairy probiotic products, especially ice cream and cheese are good vehicle to transfer probiotics to the human intestinal tract (Homayouni, 2008a; Homayouni et al., 2012). Dairy products have an important role in human health and form the main part of the food pyramid. The therapeutical and health care characteristic of fermented dairy products has been used over long years. Another way to keeping up the probiotic cells in the gut is to

entering prebiotics into the intestine through the regular consumption of foods containing these components. It is clear that versus probiotics the amounts of prebiotics do not changes during the passage from upper intestinal tract (Homayouni, 2008a).

The main role of food is providing enough nutrients to meet metabolic requirements in human body, while giving the consumer a satisfaction feeling and well-being (Homayouni, 2008a). Beyond meeting nutrition needs, food may have different physiological functions and may play detrimental or beneficial roles in some diseases (Koletzko et al., 1998). Functional foods were developed in order to promote a well-being state, improving health, and reducing the diseases risk. "Functional food" means; special foods which have preventional and/or curing effects beyond its nutritional (Homayouni, 2008a). There is a wide rage of functional foods that were developed recently and many of them are being produced in all over the world including probiotic, prebiotic and symbiotic foods as well as foods enriched with antioxidants, isoflavons, phytosterols, anthosyanins and fat-reduced, sugar-reduced or salt-reduced foods. Among these foods, probiotic functional foods are the first choice to exert positive effects on the human health. Probiotic functional foods were divided into dairy probiotic foods and non-dairy probiotic foods. Some of dairy probiotic foods including probiotic ice cream, frozen fermented dairy deserts, probiotic cheese, bio-yoghurt, drinking yoghurt, kefir, Freeze-dried yoghurt and spray dried milk powder have been employed as possible delivery vehicles for probiotic bacteria (Haynes and Playne, 2002; Homayouni et al., 2008b; Homayouni et al., 2012; Ejtahed et al., 2011; Ejtahed et al., 2012; Mirzaei et al., 2012 Kailasapathy and Rybka, 1997; Ravula and Shah, 1998; Stanton et al., 2001). Probiotics are distinct as live micro-organisms which, when administered in sufficient amounts present a health benefit on the host (Food and Agriculture Organization of United Nations; World Health Organization - FAO/WHO, 2002; Homayouni, 2009). In recent years probiotic bacteria have increasingly been incorporated into dairy foods as dietary adjuncts. *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacterial cells that were used in the production of fermented and non-fermented dairy products.

Consumption of probiotic bacteria via dairy food products is an ideal way to re-establish the intestinal micro-floral balance. It must conform to certain requirements for a dairy food product to be considered as a valuable alternative for delivery of probiotic bacteria in one hand and for variety of probiotic cultures to use as a dietary adjunct and to exert a positive influence in the other hand. The culture must be native of the human gastrointestinal tract, having the ability to ferment prebiotics, survives passage through the stomach and small bowel in adequate numbers, be capable of colonizing in site of action, and have beneficial effects on human health. In order to survive, the strain must be resistant to acidic conditions (gastric pH 1-4), alkaline conditions (bile salts present in the small bowel), enzymes present in the intestine (lysozyme) and toxic metabolites produced during digestion (Homayouni et al., 2008d). For example in traditional yoghurt production, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were used as starter culture. These bacteria do not belong to the indigenous intestinal flora, are not bile-acid resistant and do not survive passage through the gut. So, the traditional yoghurt culture is not to be considering as probiotic. In the case of dairy food product to be considered as a valuable alternative for delivery of probiotics, it

must to match definite necessities such as neutral pH, high enough total solids level, absence of oxygen and near to ambient temperatures (Homayouni et al., 2008b; Homayouni et al., 2008d; Homayouni et al., 2012). A number of dairy food bio-products have been employed and developed as delivery vehicles of probiotic bacteria. Around 80 bifido containing products are estimated to be on the world markets. Most of these products are from dairy origin including fresh milk, fermented milk, dairy beverages, ice cream, dairy desserts, cheese, cottage cheese and powdered milk (Tamime et al., 1995). Since the more interest in probiotics, different types of functional products were proposed as carrier foods for probiotic micro-organisms by which consumers can take in large amounts of probiotic bacteria for the therapeutic effects. Therefore, development of these products is a key research priority for food design and a challenge for both industry and science sectors. This chapter presents an overview of functional foods development with emphasizing probiotic dairy foods.

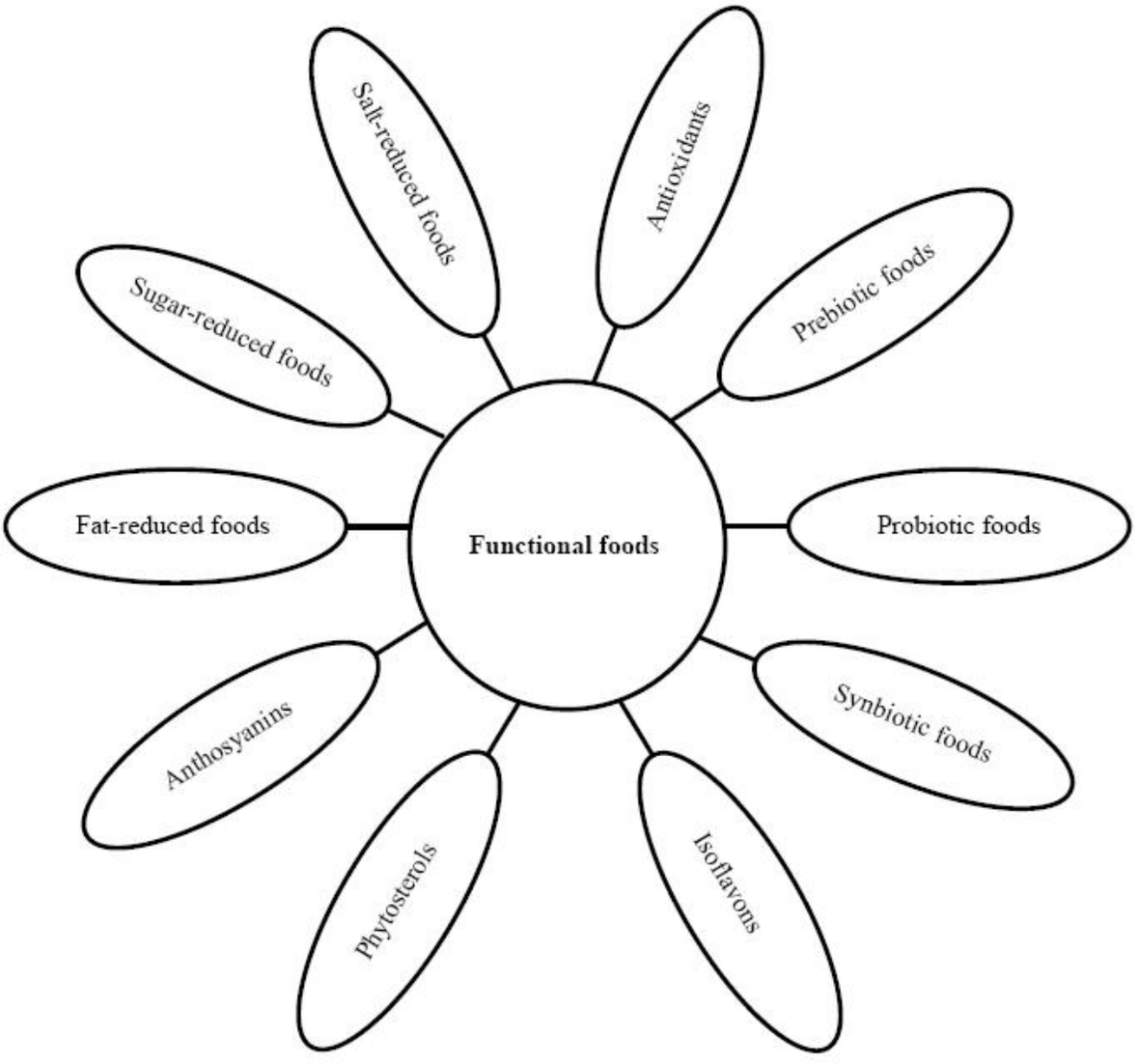

Figure 1. Classification of functional foods

2. Dairy probiotic foods

As mentioned before, dairy functional foods beyond its basic nutritional value has physiological benefits. Milk has an outstanding position in the development of functional foods because it has Omega-3, phytosterols, isoflavins, conjugated linoleic acid, minerals, and vitamins. Dairy products such as ice cream, cheese, yogurt, Acidophilus-Bifidus-milk, Ayran, Kefir, Kumis, Doogh containing probiotics and dairy beverages (both fermented and non-fermented) have long been considered as important vehicles for the delivery of probiotics. In fermentation process, acids such as lactic acid, acetic acid and citric acid are naturally produced. These acids are commonly used as organic acids to enhance organoleptic qualities as well as safety of food products. Lactic acid bacteria are found to be more tolerant to acidity and organic acids than most of the pathogens and spoilage micro-organisms.

2.1. Probiotic ice cream

Probiotic ice cream can be produced by incorporation of probiotic bacteria in both of fermented and unfermented mix (Homayouni et al., 2008b; Homayouni et al., 2012). Ice cream is ideal vehicle for delivery of these micro-organisms in the human diet (Akin et al., 2007; Kailasapathy and Sultana, 2003; Ravula and Shah, 1998; Homayouni et al., 2008d; Homayouni et al., 2012). *Lactobacillus* and *Bifidobacterium* are the most common species of lactic acid bacteria used as probiotics for fermented dairy products. Among the frozen dairy products with live probiotics, probiotic ice cream is also gaining popularity for its neutral pH. The pH of non-fermented ice cream is near to seven which is providing to survive probiotic bacteria (Akin et al., 2007; Christiansen et al., 1996; Homayouni et al., 2008b; Homayouni et al., 2008c; Homayouni et al., 2012). The high total solids level in ice cream including the fat and milk solids provides protection for the probiotic bacteria (Homayouni et al., 2012). Because the efficiency of added probiotic bacteria depends on dose level, type of dairy foods, presence of air and low temperature (Homayouni et al., 2008b), their viability must be maintained throughout the product's shelf-life and they must survive the gut environment (Kailasapathy and Chin, 2000). The therapeutic value of live probiotic bacteria is more than unviable cells; therefore, International Dairy Federation (IDF) recommends that a minimum of 10^7 probiotic bacterial cells should be alive at consumption time per gram/mililiter of product. Studies indicate, however, the bacteria may not survive in high enough numbers when incorporated into frozen dairy products unless a suitable method is used against freeze injury and oxygen toxicity (Dave and Shah, 1998; Kailasapathy and Sultana, 2003; Ravula and Shah, 1998; Homayouni et al., 2008d). The methods of increasing probiotic survival depend on type of food products. Selection of resistant probiotic strains to tolerate production, storage and gastrointestinal tract conditions, is one of the important methods (Homayouni et al., 2008d). Another way is to adjust the conditions of production and storage for more survival rates. The physical protection of probiotics by microencapsulation is a new method for increasing the survival of probiotics (Homayouni et al., 2007; Homayouni et al., 2008b). Encapsulation helps to isolate the bacterial cells from the adverse environment of the product and gastrointestinal tract, thus potentially reducing cell

loss. Encapsulation thus may enhance the shelf-life of probiotic cultures in frozen dairy products (Kebary et al., 1998; Shah and Ravula, 2000; Homayouni et al., 2008b). Selecting of suitable probiotic strains depends to ability survive simulated conditions of ice cream (high sucrose concentrations, high oxygen, refrigeration and freezing temperatures), acidic (to simulate gastric) and alkaline conditions (to simulate intestinal). Microencapsulation of probiotics can further protect these bacteria from the mentioned conditions (Homayouni et al., 2008d).

Homayouni et al. (2008d) studied the survival of probiotics in simulated ice cream and gastrointestinal conditions in order to select appropriate probiotic strains for use in probiotic ice cream. The growth and survival rate of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis* and *Bifidobacterium longum* in varying amount of sucrose concentrations (10, 15, 20 and 25%), oxygen scavengering components (0.05% L-cysteine and 0.05% L-ascorbate) and low temperatures (4°C and 20°C) during different periods of time (30, 60 and 90 days) in MRS-broth medium was studied. All of above stress factors have been able to influence the growth and survival of four probiotic strains. Results have demonstrated that it is possible to select the appropriate probiotic strains for use in probiotic ice cream. *Lactobacillus casei* (Lc01) and *Bifidobacterium lactis* (Bb12) had the highest resistance to simulated acidic, alkaline and ice cream conditions in comparison with other probiotic strains, making them suitable probiotic strains for use in probiotic ice cream (Homayouni et al., 2008b; Homayouni et al., 2008d).

2.2. Probiotic cheese

Survival in processing conditions, presence of oxygen, degree of acidity, ability to grow well in milk-based products and to rapidly acidify milk, thus reducing the fermentation time and, consequently, contamination risk during preparation of inoculums are important factors for probiotic bacteria such as *Lactobacillus spp*. and *Bifidobacterium spp* in order to apply these bacteria in probiotic dairy products. Probiotic bacterial cells have to fulfill the basic technological necessities when used in commercial probiotic dairy products. Since probiotic bacteria have to be presented in sufficient numbers in product at consumption time, their survival have to be maintained up to shelf-life date. In addition, no adverse effects on taste and aroma of the product should be exerted by the probiotic organisms. Various types of cheese have a good potential to maintain the probiotic survival. So, it is a good vehicle to transfer probiotics to the human intestinal tract. There are two ways for development of probiotic cheese: in the first step, the manufacture processes of cheese products may have to be modified and adapted to the requirements of probiotics and in second step, appropriate probiotic strains may be applied or new cheese products may have to be developed. Dairy products containing living bacteria have to be cooled during storage. Cooling is necessary to guarantee high survival rates of probiotics and to bring sufficient stability of the product (Roy et al., 1997). In addition, oxygen content and water activity of the probiotic cheese have to be considered in prepackaged cheese (Dave and Shah, 1997a). Interaction of the live probiotic microorganisms with the components of the cheese have to

be inhibited by cooling of product. The degree of interaction depends on the kind and amount of carbohydrates available, degree of hydrolysis of milk proteins and thus availability of essential amino acids, and composition and degree of hydrolysis of milk lipids, determining the availability of short chain fatty acids (Fox et al., 1996). However, the proteolytic and lipolytic properties of the probiotic bacterial cells may have important effects on taste and flavor of the probiotic cheese (Kunji et al., 1996). The strength of interactions between probiotics and starter organisms in probiotic cheese depends on when the probiotics are added to the product. If they are added after fermentation, interactions may be kept to a minimum, since addition is possible immediately before or even after cooling below 8°C and metabolic activities of starters and probiotics are considerably reduced at refrigerated temperatures.

Antagonism between bacteria is often based on the production of metabolites that inhibit or inactivate more or less specifically other related starter organisms or even unrelated bacteria. While antagonism caused by bacteriocins, peptides, or proteins exhibiting antibiotic properties has been described as a limiting factor for combinations of starters and probiotics (Joseph et al., 1998), antagonism caused by hydrogen peroxide, benzoic acid, biogenic amines, and lactic acid may have considerable effects on probiotics in probiotic cheese. The physiological state of the probiotics may be of considerable importance for survival during ripening and/or storage if probiotics are added to the probiotic cheese after fermentation (Desmazeaud, 1996; Lankaputhra et al., 1996; Leuschner et al., 1998; Weber, 1996).

In probiotic cheese, probiotic cells must be able to grow and/or multiply in the human intestine and therefore should be able to survive during the passage through the gastrointestinal tract (GIT), which involves exposure to hydrochloric acid in stomach and bile in small intestine (Stanton et al., 2003). In fact, cheese provides a valuable vehicle for probiotic delivery, due to creation of a buffer against the high acidic environment in the gastrointestinal tract, and thus creates a more favorable environment for probiotic survival throughout the gastric transit, ought to higher pH. Moreover, the dense matrix and relatively high total solids as well as fat content of cheese may offer additional protection to probiotic bacteria in stomach (Bergamini et al., 2005; Ross et al., 2002). The presence of the prebiotics inulin and oligofructose can promote growth rates of *bifidobacteria* and *lactobacilli*, besides increased lactate and short chain fatty acids production in petit-suisse cheese (Cardarelli et al., 2007).

2.3. Probiotic yoghurt

Yoghurt has been historically recognized to be 'a healthy food' with therapeutically effects. There has been a considerable increase in the popularity of yoghurt especially probiotic yoghurt in recent years. The conventional yoghurt starter bacteria, *L. bulgaricus* and *Streptococcus thermophilus*, do not have ability to survive passage through intestinal tract and consequently so, they are not considered as probiotics. But the addition of *L. acidophilus* and *B. bifidum* into yoghurt can add extra nutritional and physiological values.

Similar processing to traditional yoghurt is applied for production of bio-yoghurt with incorporation of live probiotic starter cultures. Heat treated homogenized milk with an increased protein content (3.6–3.8%) is inoculated with the conventional starter culture at 45°C or 37°C and incubated for 3.5 and 9 h, respectively. The probiotic culture can be added prior to fermentation simultaneously with the conventional yoghurt cultures or after fermentation to cooled (4°C) product before packaging. Bio-yoghurt, containing *L. acidophilus* and *B. bifidum* is a potential vehicle for delivery of these probiotic cells to consumers. *L. acidophilus* and *B. bifidum* have to retain viability and activity in yoghurt as a probiotic at consumption time. Viability of probiotic bacteria in yoghurt products at refrigeration temperature is reported to be unsatisfactory over a long shelf life (Dave and Shah, 1997a). The survival of probiotic bacteria in fermented dairy products depends on the chemical composition of the fermentation medium (e.g. carbohydrate source), final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, strains used, interaction between species present, culture conditions, concentration of sugars (osmotic pressure), dissolved oxygen (especially for *Bifidobacterium* spp.), level of inoculation, incubation temperature, fermentation time and storage temperature. The lack of acid tolerance of some probiotic species and strains in fermented products based on milk is an important factor. During fermentation, pH levels decreases when the lactic acid content increases. 'Over-acidification' or 'post acidification' is due to decrease in pH after fermentation and during storage at refrigerated temperature. Excessive acidification is mainly due to the uncontrollable growth of strains of *L. bulgaricus* at low pH values and refrigerated temperatures. The 'overacidification' can be prevented to a limited extent by applying 'good manufacturing practice' and by using cultures with reduced 'over-acidification' behavior.

Viability of both *Lactobacillus* and *Bifidobacterium* species reduces at low pH levels during refrigerated storage. So, strain selection and survival monitoring are necessary to produce high quality bio-yoghurt. Probiotic yoghurt contains metabolic products secreted by starter microorganisms, which influence the viability of *L. acidophilus* and *B. bifidum*. The inhibition of *bifidobacteria* in probiotic yoghurt is due to antagonism effects among starter bacteria rather than hydrogen peroxide or organic acids (Dave and Shah, 1997a). The ideal procedure for probiotic yoghurt manufacturing is growing the *Bifidobacterium* spp. separately, followed by washing out of free metabolites and the transfer of the cells to the probiotic yoghurt. Oxygen toxicity is a critical problem for *Bifidobacterium* spp. because they are strictly anaerobic. Low initial oxygen content in milk may obtain the low redox potential required in the early phase of incubation to guarantee *Bifidobacteria* growth. Oxygen easily dissolves in milk during yoghurt production and also permeates through packages during storage. It has been suggested to inoculate *S. thermophilus* and *Bifidobacterium* simultaneously during fermentation to avoid the oxygen toxicity problem. *S. thermophilus* has a high oxygen utilization ability, which results in reduction of dissolved oxygen in probiotic yoghurt and an enhancement in viability of *bifidobacteria*. Higher survival rates of lactic acid bacteria were obtained at lower storage temperatures (Foschino et al., 1996). Low storage temperature restricts the growth of *L. bulgaricus* and consequently also over-acidification. *Bifidobacteria* are substantially less tolerant to low storage temperature when compared to *L. acidophilus*.

2.4. Probiotic milk

Lactobacillus acidophilus does not rapidly grow in milk because it is an acid-loving bacterium. Therefore, it is essential to maintain the inoculum active by daily transfers of mother culture in acidophilus milk production. The probiotic milk is to market in liquid form. During fermentation, milk pH often goes beyond the narrow range of optimal pH of *Lactobacillus acidophilus* (5.5-6.0). This eventually leads to decrease these bacterial counts. In traditional acidophilus milk production, the milk is heated at 95°C for 1 h or at 125°C for 15 min (Vedamuthu, 2006). Such a high heat treatment stimulates the growth of *Lactobacillus acidophilus* by providing denatured proteins and released peptides. High-heat-treated milk is cooled to 37°C and kept at this temperature for a period of 3-4 h to allow any spores present to germinate. Then, milk is re-sterilized to destroy almost all vegetative cells. Unless skim milk is used, the heat-treated milk is homogenized and cooled down to inoculation temperature (37°C). *Lactobacillus acidophilus* is added as active bulk culture. The level of inoculation is usually 2-5% and the inoculated milk is left to ferment until pH 5.5-6.0 or ~1.0% lactic acid is obtained, with no alcohol (Surono and Hosono, 2002). The fermentation takes about 18-24 h under inactive conditions. After the fermentation, the number of viable *Lactobacillus acidophilus* colonies is about $2\text{-}3\times10^{9}$ cfu mL^{-1}, but this number decreases up to consumption time. In extended incubation period reduction in counts of *Lactobacillus acidophilus* may occur. To overcome this problem, replacement of 25% of *Lactobacillus acidophilus* culture by a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* can be used. Following fermentation, the warm product is rapidly cooled to <7°C before agitation and pumped to a filler where it is filled into bottles or cartons (Kosikowski and Mistry, 1997; Vedamuthu, 2006). Protein quality and total amino acid content are similar in both fermented and non-fermented milk. Acidophilus milk has higher free amino acids than milk. As the milk lactose is hydrolyzed by β-galactosidase of *Lactobacillus acidophilus*, acidophilus milk is more suitable for individuals suffering from lactose intolerance. It is also possible to enrich acidophilus milk with calcium, iron and vitamins. Undesirable sour milk flavor caused acidophilus milk is gained limited popularity by consumers. So, sweet acidophilus milk has been developed. When *Lactobacillus acidophilus* is incorporated into pasteurized milk at about 5°C and bottled aseptically, these bacteria are able to keep their viability up to 14 days without reducing the pH of milk due to it does not grow at low temperatures (<10°C). Freeze-dried cultures may keep their viability up to 58% after 23 days at 4°C in sweet acidophilus milk. *Lactobacillus acidophilus* remained viable in sweet acidophilus milk over 28 days at 7°C. Addition of 200 g of frozen culture concentrate to 2000 L of pasteurized milk is satisfactory to reach the target level of *Lactobacillus acidophilus* in the probiotic milk (Vedamuthu, 2006).

Technology of bifidus milk and acidophilus-bifidus milk manufacturing is similar to acidophilus milk. Milk is standardized to desire protein and fat levels in both products. Then, for manufacture of bifidus milk, milk is heat-treated at 80-120°C for 5-30 min and rapidly cooled to 37°C. Heat-treated milk is inoculated with frozen culture of *Bifidobacterium bifidum* and *Bifidobacterium longum* at a level of 10% and left to ferment until pH 4.5. After fermentation, the product is cooled to <10°C and packaged. Final product has a slightly

acidic flavor and the ratio of lactic acid to acetic acid is 2:3. Milk used for acidophilus-bifidus milk production is usually enriched with protein prior to fat standardization and homogenization. The standardized milk is heat-treated at 75°C for 15 s or 85°C for 30 min. After cooling the milk to 37°C, frozen cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are inoculated and fermentation is allowed until pH 4.5–4.6 is reached (~16 h). Following fermentation, the fermented milk is cooled to <10°C. The shelf life of the product is about 20 days. Acidophilus-bifidus milk has a characteristic aroma and slightly acidic flavor. High viscosity of product cause to producing it in set form. It is also possible to produce probiotic milks by simply adding mix culture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* to cold pasteurized milk.

3. Development of functional dairy foods

Innovation is today's business demand and development of a new functional food is an expensive process and is very important for both food companies and consumers. Regulations should encourage food companies to follow functional food development. Development of dairy probiotic products requires detailed knowledge of both products and customers. It needs to manage customer knowledge effectively (Walzem, 2004; Jousse, 2008). Fundamental risks can affect the development of new functional food products and may leads to fail the development process. Development of new functional food products is very challenging and it has to complete the consumer's expectations for palatable and healthy products (Fogliano and Vitaglione, 2005; Granato et al., 2010; Shah, 2007). So, the development and commerce of functional food products is rather complex, expensive, and uncertain. Key points regarding for a successful functional food product development are consumer demands, technological conditions, and legislative regulatory background. However, consumer's knowledge of the health effects of specific ingredients can affect the acceptance of specific functional food. Therefore, functional ingredients that are in consumers mind for a long period of time, such as minerals, fiber, and vitamins, achieve considerably higher rates of consumer acceptance than new products, such as foods enriched with probiotics, prebiotics, flavonoids, carotenoids, and conjugated linilenic acid (CLA). Several ways to make a functional food product is to eliminating an allergenic protein, lactose, phenylanine and etc from the natural food product; by fortification with a micronutrient; by adding antioxidants, probiotics or prebiotics); by replacing a component, or by increasing bioavailability or stability of a component known to produce a functional effect or to reduce the disease-risk potential of the food (Roberfroid, 2000; Siro, et al., 2008; Granato, et al., 2010). Field of functional probiotic foods requires the cooperation of food technologists, nutritionists, medical doctors, and food chemists in order to obtain innovative products. In this way, these foods may be able to adjust physiological parameters related to health status or disease prevention in human. So, the design and development of functional probiotic foods is a scientific work (Hasler, 1998; Walzem, 2004; Fogliano and Vitaglione, 2005) which is an expensive and multistage process that takes into account many factors, such as sensory acceptance, physical and microbial stability, price, and chemical and other

intrinsic functional properties to be successful in the marketplace. Moreover, consumer attitude toward the functional probiotic product also needs to be understood and taken into consideration.

4. Consumer attitude toward functional dairy foods

The development of functional probiotic foods is increasing, as their market increases day by day, although the consumer's information about these foods is increasing without relation to gender, age, and educational or economic levels of the consumers. The therapeutical effect of a functional probiotic food may depend on the consumer's characteristics and the type of carrier and enrichment considered. For instance, yoghurt is most preferred by its enrichment with calcium and fiber. Ingredients such as vitamins and minerals applied in fortification of functional foods are widely recognized and accepted by consumers, but new functional ingredients such as probiotics and prebiotics are not common to them. So, there is a need for increasing the consumer knowledge with respect to these new special ingredients (Hillian, 2000; Luckow and Delahunty, 2004; Ares and Gambaro, 2007; Vianna et al., 2008).

The sensory properties of prebiotic functional foods in comparison with conventional products can lead to different acceptance level. Oligofructose provides some suitable sensory properties such as rounder mouth feel, reduced aftertaste, and slight sweetness to the products. These properties are responsible for high score values for taste, creaminess, and overall acceptability of functional food products. The first important marker in choosing a functional food is flavor, and health consideration is in the second order. If the ingredients added give unpleasant flavors to the product, consumers are not interested in consume such functional probiotic food even if this results in health advantages. This means that flavor is correlated to intrinsic sensory properties of the product such as overall acceptability. In general, as functional products consumption increases, the acceptance of such products may increase, even if the sensory profiles are different from conventional products. When functional ingredients such as probiotics are added to dairy foods, consumers must be aware of probiotics health benefits in order to recognize the functional probiotic foods as being more beneficial than the conventional ones. Functional probiotic food industry should communicate with consumer in a clear way and this is one of the most important aspects for success (Tepper and Trail, 1998; Matilla-Sandholm et al., 1999; Roberfroid, 2000; Tuorila and Cardello, 2002; Nicolay, 2003; Vieira, 2003; Homayouni, 2008a).

5. Conclusion

The future success of functional probiotic dairy foods in marketplace depends on consumer acceptance of such products. The consumers must be convinced by its health claims through clear, honest, and definite messages to agree to pay the cost associated with functional probiotic dairy foods. Development of probiotic dairy products is a key research priority for food design and a challenge for both industry and science sectors. Among the functional foods, the dairy probiotic products, especially ice cream and cheese are good vehicle to

transfer probiotics to the human intestinal tract. Additional way to keeping up the probiotic cells in the gut is to entering prebiotics into the intestine through the regular consumption of food containing these components. It is clear that versus probiotics the amounts of prebiotics do not changes during the passage from upper intestinal tract.

Author details

Aziz Homayouni
Department of Food Science and Technology, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, I.R. Iran

Maedeh Alizadeh
Pediatric Nursing, Faculty of Nursing and Midwifery of Maragheh, Tabriz University of Medical Sciences, Tabriz, I.R. Iran

Hossein Alikhah
Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, I.R. Iran

Vahid Zijah*
Department of Dentistry, Behbood Hospital, Tabriz University of Medical Sciences, Tabriz, I.R. Iran

6. References

Akin, M. B., Akin, M. S., and Kirmaci, Z. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream, *Food Chemistry* 104 (2007), pp. 93-99.

Ares, G., and G´ambaro, A. Influence of gender, age and motives underlying food choice on perceived healthiness and willingness to try functional foods, *Appetite* 49 (2007), pp. 148-158.

Bausserman, M. and Michail, S. (2005). The use of Lactobacillus GG in irritable bowel syndrome in children: a double-blind randomized control trial. The Journal of Pediatrics, 147(2): 197-200.

Bergamini, C. V., Hynes, E. R., Quiberoni, A., Sua´rez, V. B., and Zalazar, C. A. Probiotic bacteria as adjunct starters: influence of the addition methodology on their survival in a semi-hard Argentinean cheese, *Food Research International* 38(5) (2005), pp. 597-604.

Cardarelli, H. R., Saad, S. M. I., Gibson, G. R., and Vulevic, J. Functional petitsuisse cheese: Measure of the prebiotic effect, *Anaerobe* 13 (2007), pp. 200-207.

Christiansen, P. S., Edelsten, D., Kristiansen, J. R., and Nielsen, E. W. Some properties of ice cream containing Bifidobacterium bifidum and Lactobacillus acidophilus, *Milschwissenschaft* 51 (1996), pp. 502-504.

Cummings, J. H., Macfarlane, G. R., and Englyst, H. N. Prebiotic digestion and fermentation, *American Journal of Clinical Nutrition* 73 (2001), pp. 415-420.

* Corresponding Author

Dave, R., and Shah, N. P. Viability of probiotic bacteria in yoghurt made from commercial starter cultures, *International Dairy Journal* 7 (1997a), pp. 31-41.

Dave, R. I., and Shah, N. P. Effect of cysteine on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures, *International Dairy Journal* 7 (1997b), pp. 537-545.

Dave, R. I., and Shah, N. P. Ingredient supplementation effects on viability of probiotic bacteria in yogurt, *Journal of Dairy Science* 81 (1998), pp. 2804-2816.

Desmazeaud, M. (1996). *Growth inhibitors of lactic acid bacteria, in Dairy Starter Cultures, Cogan, T.M. and Accolas*. New York: VCH Publishers, (pp. 131–155).

Ejtahed, H. S., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M., Mofid, V. and Akbarian-Moghari, A. (2011). Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. Journal of Dairy Science, 94: 3288-3294.

Ejtahed, H. S., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M. and Mofid, V. (2012). Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition, 28: 539- 543.

Faber, S. M. (2000). Comparison of probiotics and antibiotics to probiotics alone in treatment of diarrhea predominant IBS (D-IBS), alternating (A-IBS) and constipation (C-IBS) patients. Gastroenterology, 118: 687-688.

Food and Agriculture Organization of United Nations; World Health Organization. FAO/WHO (2002). *Guidelines for the evaluation of probiotics in food, Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report*. Available from. http://www.who.int/foodsafety/publications/fs_management/probiotics2/en.

Fogliano, V., and Vitaglione, P. Functional foods: planning and development, *Molecular Nutrition and Food Research* 49 (2005), pp. 256-262.

Foschino, R., Fiori, E., and Galli, A. Survival and residual activity of Lactobacillus acidophilus frozen cultures under different conditions, *Journal of Dairy Research* 63 (1996), pp. 295-303.

Fox, P. F., Wallace, J. M., Morgan, S., Lynch, C. M., Niland, E. J., and Tobin, J. Acceleration of cheese ripening, *Antonie van Leeuwenhoek* 70 (1996), pp. 175-201.

Granato, D., Castro, I. A., Ellendersen, L. S. N., and Masson, M. L. Physical stability assessment and sensory optimization of a dairy-free emulsion using response surface methodology, *Journal of Food Science* 73 (2010), pp. 149-155.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., and Shah, N. P. Probiotic dairy products as functional foods, *Comperhensive reviews in Food Science and food safety* 9 (2010), pp. 455-470.

Halpern, G. M., Prindville, T. and Blankenburg, M. (1996). Treatment of irritable bowel syndrome with Lacteol forte: A randomized, double-blind, crossover trial. The American Journal of Gastroenterology, 91: 1579-1585.

Hansen, L. T., Wojtas, P. M. A., Jin, Y. L. and Paulson, A. T. Survival of Ca-alginate microencapsulated Bifidobacterium spp. In milk and simulated gastrointestinal conditions, *Food Microbiology* 19 (2002), pp. 35-45.

Hasler, C. M. Functional foods: their role in disease in developing new food products for a changing prevention and health promotion, *Food Technology* 52 (1998), pp. 57-62.

Haynes, I. N., and Playne, M. J. Survival of probiotic cultures in low-fat ice-cream, *Australian Journal of Dairy Technology* 57 (2002), pp. 10-14.

Hillian, M. Functional food: how big is the market? *World Food Ingredients* 12 (2000), pp. 50-53.

Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S., and Razavi, S. H. Effect of lecithin and calcium chloride solution on the microencapsulation process yield of calcium alginate beads, *Iranian Polymer Journal* 16(9) (2007), pp. 597-606.

Homayouni, A. (2008a). *Therapeutical effects of functional probiotic, prebiotic and symbiotic foods*. (1st ed.). Tabriz: Tabriz University of Medical Sciences.

Homayouni, A., Azizi, A., Ehsani, M. R., Razavi, S. H. and Yarmand, M. S. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream, *Food Chemistry* 111 (2008b), pp. 50-55.

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., and Yarmand, M. S. Spectrophotometrically evaluation of probiotic growth in liquid media, *Asian Journal of Chemistry* 20(3) (2008c), pp. 2414-2420.

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., and Yarmand, M. S. Growth and survival of some probiotic strains in simulated ice cream conditions, *Journal of Applied Sciences* 8(2) (2008d), pp. 379-382.

Homayouni, A. Letter to the editor, *Food Chemistry* 114 (2009), pp. 1073.

Homayouni, A., Azizi, A., Javadi, M., Mahdipour, S. and Ejtahed, H. (2012). Factors influencing probiotic survival in ice cream: A Review. International Journal of Dairy Science, doi: 10.3923/ijds.2012.

Joseph, P. J., Dave, R. I., and Shah, N. P. Antagonism between yogurt bacteria and probiotic bacteria isolated from commercial starter cultures, commercial yogurts, and a probiotic capsule, *Food Australia* 50 (1998), pp. 20-23.

Jousse, F. Modeling to improve the efficiency of product and process development, *Comprihensive Review of Food Science and Food Safety* 7 (2008), pp. 175-181.

Kailasapathy, K., and Rybka, S. L. acidophilus and Bifidobacterium spp. Their therapeutic potential and survival in yoghurt, *Australian Journal of Dairy Technology* 52 (1997), pp. 28-35.

Kailasapathy, K., and Chin, J. Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp., *Immunology and Cell Biology* 78 (2000), pp. 80-88.

Kailasapathy, K., and Sultana, K. Survival and β-D-galactosidase activity of encapsulated and free Lactobacillus acidophilus and Bifidobacterium lactis in ice cream, *Australian Journal of Dairy Technology* 58 (2003), pp. 223-227.

Kebary, K. M. K., Hussein, S. A., and Badawi, R. M. Improving viability of bifidobacterium and their effect on frozen ice milk, *Egyptian Journal of Dairy Science* 26 (1998), pp. 319-337.

Kim, H. J., Camilleri, M. and McKinzie, S. (2003). A randomized controlled trial of a probiotic, VSL 3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. Alimentary Pharmacology and Therapeutics, 17: 895-904.

Koletzko, B., Aggett, P.J., and Bindels, J.G. Growth, development and differentiation: a functional food science approach, *Brazilian Journal of Nutrition* 80 (1998), pp. 35-45.

Kosikowski, F. V. and Mistry, V. V. (1997). *Cheese and fermented milk foods, in Origin and Principles: Fermented Milks*. Westport: F.V. Kosikowski, (pp. 57-74).

Kunji, E. R. S., Mierau, I., Hagting, A., Poolman, B., and Konings, W. N. The proteolytic systems of lactic acid bacteria, *Antonie van Leeuwenhoek* 70 (1996), pp. 187-221.

Lankaputhra, W. E. V., Shah, N. P., and Britz, M. L. Survival of bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide, *Milchwissenschaft* 51 (1996), pp. 65-70.

Leuschner, R. G., Heidel, M., and Hammes, W. P. Histamine and tyramine degradation by food fermenting microorganisms, *International Journal of Food Microbiology* 39 (1998), pp. 1-10.

Luckow, T., and Delahunty, C. Consumer acceptance of orange juice containing functional ingredients, *Food Research International* 37 (2004), pp. 805-14.

Matilla-Sandholm, T., Blum, S., Collins, J.K., Crittenden, R., DeVos, W., Dunne, C., et al. Probiotics: towards demonstrating efficacy, *Trends in Food Science and Technology* 10 (1999), pp. 393-399.

Meurman, J. H. (2005). Probiotics: do they have a role in oral medicine and dentistry? Europian Journal of Oral Sciences, 113(3): 188-196.

Mirzaei, H., Pourjafar, H. and Homayouni, A. (2012). Effect of calcium alginate and resistant starch microencapsulation on the survival rate of Lactobacillus acidophilus La5 and sensory properties in Iranian white brined cheese. Food Chemistry, 132: 1966-1970.

Mountzouris, K. C. and Gibson, G. R. Colonization of the gastrointestinal tract, *Annales Nestle* 61 (2003), pp. 43-54.

Nicolay, C. Language is a key to marketing digestive health products, *Functional Foods and Nutraceuticals* 6 (2003), pp. 20-22.

Ravula, R. R. and Shah, N. P. Viability of probiotic bacteria in fermented frozen dairy desserts, *Food Australia* 50 (1998), pp. 136-139.

Roberfroid, M. (2000). *Inulin-type fructans*. Boca Raton: CRC Press.

Roberfroid, M.B. Concepts and strategy of functional food science: the European perspective, *American Journal of Clinical Nutrition* 71 (2000), pp. 1660-1664.

Ross, R. P., Fitzgerald, G., Collins, K., and Stanton, C. Cheese delivering biocultures: probiotic cheese, *Australian Journal of Dairy Technology* 57(2) (2002), pp. 71-78.

Roy, D., Mainville, I., and Mondou F. Bifidobacteria and their role in yogurt-related products, *Microecology Therapy* 26 (1997), pp. 167-180.

Saggioro, A. (2004). Probiotics in the treatment of irritable bowel syndrome. Journal of Clinical Gastroenterology, 38: 104-106.

Shah, N. P., and Ravula, R. R. Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts, *Australian Journal of Dairy Technology* 55 (2000), pp. 139-144.

Shah, N. P. Functional cultures and health benefits, *International Dairy Journal* 17 (2007), pp. 1262-1277.

Siro, I., Kapolna, E., Kapolna, B., and Lugasi, A. Functional food: product development, marketing and consumer acceptance-A review, *Appetite* 51 (2008), pp. 456-467.

Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P. B., and et al. Market potential for probiotics, *American Journal of Clinical Nutrition* 73 (2001), pp. 4765-4835.

Stanton, C., Desmond, C., Coakley, M., Collins, J. K., Fitzgerald, G., and Ross, R. P. (2003). Challenges facing development of probioticcontaining functional foods. In E. R. Farnworth (Eds.). *Handbook of fermented functional foods* (pp. 27-58). Boca Ranton: CRC Press.

Surono, I.S. and Hosono, A. (2002). Fermented milks: Types and standards of identity. In H. Roginski, J. Fuquay, and P.F. Fox (Eds.). *Encyclopedia of Dairy Microbiology*. (pp. 1018-1023).

Tamime, A. Y., Marshall, V. M. E., and Robinson, R. K. Microbiological and technological aspects of milks fermented by bifidobacteria, *Journal of Dairy Research* 62 (1995), pp. 151-187.

Tepper, B., and Trail, A. Taste or health: a study on consumer acceptance of corn chips, *Food Quality and Preference* 9 (1998), pp. 267-272.

Tuorila, H., and Cardello, A. V. Consumer responses to an off-flavour in juice in the presence of specific health claims, *Food Quality and Preference* 13 (2002), pp. 561-569.

Vedamuthu, E.R. (2006). Other fermented and culture-containing milks. In R. Chandan, C.H. White, A. Kilara, and Y.H. Hui (Eds.), *Manufacturing Yogurt and Fermented Milks* (pp. 295-308). Blackwell Publishing.

Vernazza, C. L., Rabiu, B. A., and Gibson, G. R. (2006). Human colonic microbiology and the role of dietary intervention: Introduction to prebiotics. In G. R. Gibson and R. A. Rastall (Eds.), *Prebiotics: Development and application* (pp. 1-12). England: John Wiley and Sons Ltd.

Vianna, J. V., Cruz, A. G., Zoellner, S. S., Silva, R., and Batista, A. L. D. Probiotic foods: consumer perception and attitudes, *International Journal of Food Science and Technology* 43 (2008), pp. 1577-1580.

Vickers, Z., Mullan, L., and Holton, E. Impact of differences in taste ratings on the consumption of milk in both a laboratory and a foodservice setting, *Journal of Sensory Studies* 14 (1999), pp. 249-262.

Vieira, P. How to create brand awareness for new products, *Functional Foods and Nutraceuticals* 6 (2003), pp. 38-40.

Walzem, R. L. Functional foods, *Trends In Food Science and Technology* 15 (2004), pp. 518.

Weber, H. (1996). Starter cultures in dairy industry. In H. Weber (Eds.), *Mikrobiologie der Lebensmittel: Milch und Milchprodukte (in German)* (pp. 105-152). Hamburg: Behr's Verlag.