



Review

Trends in non-dairy probiotic beverages

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Received 30 May 2007; accepted 24 October 2007

Abstract

In recent times, there has been an increased interest to adapt healthy diets, which help in preventing diseases, and as a consequence, the study and development of new functional foods has gained much importance. Food additives as probiotics and prebiotics may exert positive effects on the composition of gut microbiota and are subject of intensive research. The allergy to dairy products affect negatively some persons. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Traditions and economic reasons that limit the use of dairy fermented products in some developing countries promote the idea of reduction of milk components as vehicles for the probiotic agents. At present, some non-dairy probiotic beverages are being commercialized and are discussed in this review. Probably, beverages such as fruit and vegetable juices would be the next food category where the healthy probiotic bacteria will make their mark.

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Keywords: Functional foods; Probiotics; Prebiotics; Fermented milks; Non-dairy beverages

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1. Introduction

The development of probiotics in the last two decades has signaled an important advance in the food industry. The number of scientific publications on probiotics has increased a lot stimulated by factors as exciting scientific and clinical findings using well-documented probiotic

organisms. Some concerns over the limitations and side effects of the pharmaceutical agents and consumers demand for the natural products have been discussed by Reid (2006).

Probiotic is a relatively new word meaning “for life” and it is generally used to name the bacteria associated with the beneficial effects for the humans and animals. The term probiotic was technically defined by an Expert Committee as “live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general

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Table 1
Probiotic microorganisms

Lactobacillus species	Bifidobacterium species	Others
<i>Lb. acidophilus</i>	<i>Bf. adolescentis</i>	<i>Bacillus cereus</i>
<i>Lb. amylovorus</i>	<i>Bf. animalis</i>	<i>Clostridium botyricum</i>
<i>Lb. brevis</i>	<i>Bf. breve</i>	<i>Enterococcus faecalis</i>
<i>Lb. casei</i>	<i>Bf. bifidum</i>	<i>Enterococcus faecium</i>
<i>Lb. casei</i> sp. <i>rhamnosus</i>	<i>Bf. infantis</i>	<i>Escherichia coli</i>
<i>Lb. crispatus</i>	<i>Bf. lactis</i>	<i>Lactococcus lactis</i> sp. <i>cremoriss</i>
<i>Lb. delbrueckii</i> sp. <i>bulgarius</i>	<i>Bf. longum</i>	<i>Lactococcus lactis</i> sp. <i>lactis</i>
<i>Lb. fermentum</i>		<i>Leuconostoc mesenteroides</i> sp. <i>dextranicum</i>
<i>Lb. gasseri</i>		<i>Pediococcus acidilactici</i>
<i>Lb. helveticus</i>		<i>Propionibacterium freudenreichii</i>
<i>Lb. johnsonii</i>		<i>Saccharomyces boulardii</i>
<i>Lb. lactis</i>		<i>Streptococcus salivarius</i> sp. <i>thermophilus</i>
<i>Lb. paracasei</i>		
<i>Lb. plantarum</i>		
<i>Lb. reuteri</i>		

Cited by Collins, Thornton and O’Sullivan (1998), Gorbach (2002), Holzapfel and Schillinger (2002) and O’Sullivan et al. (1992).

nutrition” (FAO/WHO, 2001; Guarner & Schaafsma, 1998). This means that the microorganisms must be alive and present in high numbers, generally more than 10⁹ cells per daily ingested dose. Each product should indicate the minimum daily amount required for it to confer specific health benefit(s). The probiotic microorganisms consist mostly of strains of the genera *Lactobacillus* and *Bifidobac-*

terium, but not exclusively (Table 1). These types of lactic acid bacteria have been used since recorded history in the production of fermented dairy products (FAO/WHO, 2001; Gorbach, 2002). No pathogenic, or virulence properties have been found for the lactobacilli, bifidobacteria, or lactococci (Aguirre and Collins, 1993). But, in terms of safety of the probiotics, FAO/WHO Expert Consultation believes that principles and practical criteria need to be generated to provide the guidelines as to how any given potential probiotic microorganism can be tested and proven to have a low risk of inducing, or being associated with the etiology of some disease, versus conferring a significant health benefit when administered to humans (FAO/WHO, 2001).

There are some ideal properties of the probiotic strains, which would benefit the human health and could be used in probiotic industry: resistance to acid and bile; attachment to the human epithelial cells; colonization in the human intestine; production of antimicrobial substances, called bacteriocins (Jack, Tagg, & Ray, 1995); good growth characteristics and beneficial effects on the human health, such as presented in Fig. 1. One of the most important characteristics of a probiotic strain is that it must be non-pathogenic and GRAS – Generally Regarded As Safe (Collins, Thornton, & O’Sullivan, 1998; Gorbach, 2002; Havenaar & Huis In’t Veld, 1992). The probiotic must also present some desirable characteristics, such as low cost, maintain its viability during the processing and storage, facility of the application in the products, resistance to the physico-chemical processing of the food.

There are different degrees of evidence supporting the health effects of the probiotics (FAO/WHO, 2001). These

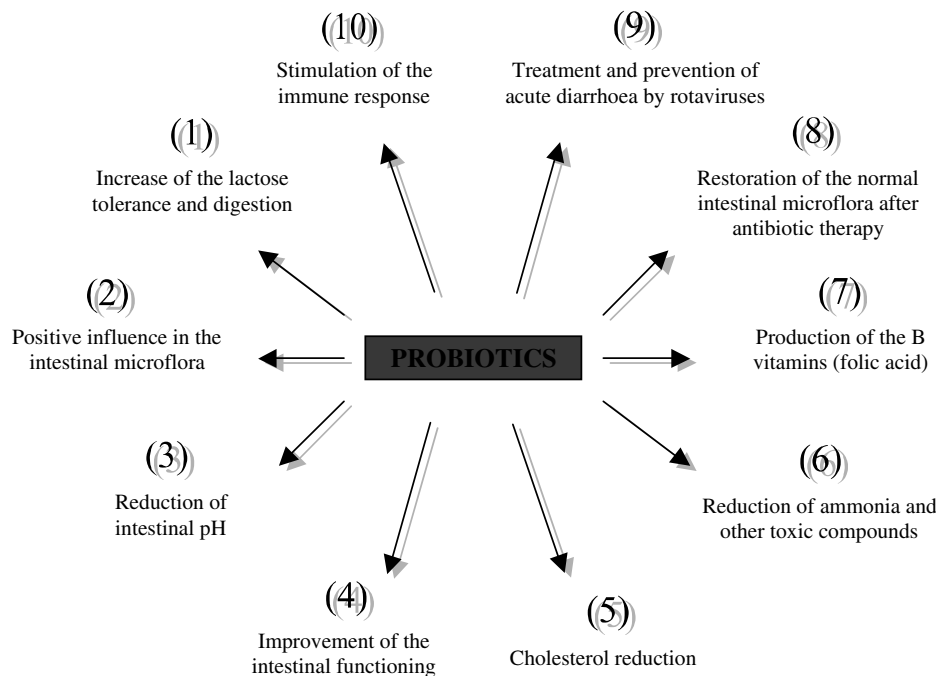


Fig. 1. Probiotic beneficial effects on human health. Adapted from Gibson & Roberfroid (1995).

Table 2
Examples of probiotic strains used in prevention and treatment of some diseases

Disorders	Strains	Clinical outcomes	References
Diarrhea caused by pathogenic bacteria and viruses	<i>Lb. rhamnosus</i> GG, <i>Lb. casei</i> Bf. <i>lactis</i> BB-12, <i>Bf. bifidum</i> Sc. <i>thermophilus</i>	Prevention and treatment of acute diarrhea caused by rotaviruses in children	Guarino, Berni, Spagnuolo, Albano, and Di Benedetto (1997), Guandalini et al. (2000), Isolauri, Juntunen, Rautanen, Sillanaukee, and Koivula (1991), Majamaa, Isolauri, Saxelin, and Vesikari (1995), Perdone, Bernabeu, Postaire, Bouley, and Reinert (1999), Saavedra, Bauman, Oung, Perman, and Yolken (1994), Shornikova, Isolauri, Burkanova, Lukovnikova, and Vesikari (1997) and Szajewska, Kotowska, Mrukowicz, Armanska, and Mikolajczyk (2001)
<i>Helicobacter pylori</i> infection and complications	<i>Lb. johnsonii</i> La1, <i>Lb. salivarius</i> , <i>Lb. acidophilus</i> LB	Inhibition of the pathogen growth and decrease urease enzyme activity necessary for the pathogen to remain in the acidic environment of the stomach	Aiba, Suzuki, Kabir, Takagi, and Koga (1998), Coconnier, Liévin, Hemery, and Servin (1998), Luo and Grayson (1995), Michetti et al. (1999) and Midolo, Lambert, Hull, Kabir et al. (1997)
Inflammatory diseases and bowel syndromes	<i>Lb. rhamnosus</i> GG	Remediation in inflammatory conditions through modulation of the gastrointestinal microflora	Giochetti et al. (2000), Gupta, Andrew, Kirschner, and Guandalini (2000) and Shanahan (2000)
Cancer in gastrointestinal tract	<i>Lb. rhamnosus</i> GG, <i>Lb. rhamnosus</i> LC-705, <i>Lb. casei</i> Shirota, <i>Lb. acidophilus</i> LA-2, <i>Bifidobacterium</i> sp., <i>Propionibacterium</i> sp.	Prevention or delay of the onset of certain cancers	Aso et al. (1995), El-Nezami, Mykkänen, Kankaanpää, Salminen, and Ahokas (2000), Hosada, Hashimoto, He, Morita, and Hosono (1996) and Oatley, Rarick, Ji, and Linz (2000)
Mucosal immunity	<i>Lb. casei</i> Shirota, <i>Lb. rhamnosus</i> HN001, <i>Lb. acidophilus</i> HN017, <i>Bf. lactis</i> HN019	Enhancement of immune parameters	Arunachalam, Gill, and Chandra (2000), Donnet-Hughes, Rochat, Serrant, Aeschlimann, and Schiffrin (1999), Gill, Rutherford, Prasad, and Gopal (2000), Gill, Cross, Rutherford, and Gopal (2001), Matsuzaki and Chin (2000), Sheih, Chiang, Wang, Chuh, and Gill (2001) and Perdigon, Vintini, Alvarez, Medina, and Medici (1999)
Allergic symptoms	<i>Lb. rhamnosus</i> GG, <i>Bf. lactis</i> BB-12	Prevention of allergic diseases onset	Isolauri, Arvola, Sutas, Moilanen, and Salminen (2000), Kalliomaki et al. (2001) and Majamaa and Isolauri (1996, 1997)
Cardiovascular disease	<i>Lactobacillus</i> sp.	Prevention and therapy of ischemic heart syndromes	De Roos and Katan (2000) and Oxman, Shapira, Klein, Avazov, and Rabinowitz (2001)
Bacterial and yeast vaginitis	<i>Lb. acidophilus</i> , <i>Lb. rhamnosus</i> GG	Eradication of vaginitis through restoration of dominated vaginal flora	Hilton, Isenberg, Alperstein, France, and Borenstein (1992), Hilton, Rindos, and Isenberg (1995), Reid et al. (2001a), Reid, Beuerman, Heinemann, and Bruce (2001b) and Sieber and Dietz (1998)
Urinary tract infections	<i>Lactobacillus</i> GR-1, <i>Lactobacillus</i> B-54, <i>Lactobacillus</i> RC-14	Lower risk of urinary tract infections through restoration of dominated vaginal flora	Reid, Bruce, and Taylor (1995) and Reid et al. (2001b)

Adapted from FAO/WHO (2001).

have been established by the scientific testing in the humans, performed by the legitimate research groups and published in peer-reviewed biomedical journals (Gorbach, 2002). Several examples are shown in Table 2, with some specific strains and clinical outcomes. Some of them are in the market (Table 3) and make good profits for the companies.

2. Probiotics as functional foods

The concern in consuming innocuous foods free of pathogenic microorganisms, or their toxins, or containing the microorganisms, which can alter their chemical components, has emerged in the developing countries. Moreover, they must not be contaminated with any undesirable substances or, at least, they must fulfill the maximum limits

of the residues allowed after exhausting studies demonstrating the risks. On the other hand, the interest of the consumers to ingest diets to maintain the health and prevent the degenerative, or chronic diseases such as diabetes, cancer, hypertension and others, has been influenced more and more, by the current of favorable opinions towards the consumption of the natural foods and limit processed foods. Since the 1980s, this and other factors have conducted to the study and development of the so-called functional foods. The interest in developing these products is rising day-by-day, driven by the market potential for the foods and beverages that can improve the health and well-being of the consumers (Hilliam, 2000).

The functional foods have also been termed as medicinal foods, nutraceuticals, prescriptive foods, therapeutic foods, super-foods, designer foods, foodiceuticals and medifoods

Table 3
Probiotic lactic acid bacteria marketed worldwide

Strains	Origin
<i>Lactobacillus casei</i> Shirota	Yakult, Japan
<i>Lactobacillus crispatus</i> CTV05	Gynelogix, USA
<i>Lactobacillus reuteri</i> MM53	BioGaia, Sweden
<i>Lactobacillus casei</i> F19	Arla Foods, Denmark/ Sweden
<i>Bifidobacterium lactis</i> HN019	Danisco, France
<i>Lactobacillus rhamnosus</i> GG	Valio, Finland
<i>Propionibacterium freudenreichii</i> ssp. <i>shermanii</i> JS	
<i>Lactobacillus acidophilus</i> NCFM	Rhodia, USA
<i>Lactobacillus acidophilus</i> NCFB 1748	
<i>Lactobacillus johnsonii</i> LA1 (NCC 533)	Nestlé, Switzerland
<i>Lactobacillus acidophilus</i> LA10 (NCC 90)	
<i>Lactobacillus fermentum</i> RC-14	Urex, Canada
<i>Lactobacillus rhamnosus</i> GR-1	
<i>Lactobacillus casei</i> DN-114 001	Danone, France
<i>Bifidobacterium animalis</i> DN-173 010	
<i>Lactobacillus plantarum</i> 299v	Probi AB, Sweden
<i>Lactobacillus rhamnosus</i> 271	
<i>Lactobacillus casei</i> CRL 431	Chr. Hansen, USA
<i>Bifidobacterium lactis</i> BB-12	
<i>Lactobacillus acidophilus</i> LA-5	
<i>Lactobacillus bulgaricus</i> LBY27	
<i>Streptococcus thermophilus</i> STY-31	

(Finley, 1996). Because of the good amount of information available currently, it is convenient to define the concept of the functional foods and differentiate them from other terms such as nutraceuticals, or fortified and special formulas. The functional food concept was developed during the 1980s in Japan and it gained legal status in 1991, being described as FOSHU, or “Foods for Specified Health Use”. The country has formulated a specific regulatory process for the functional food approval. And it has more than 100 products with FOSHU license (Sanders, 1998). Functional foods, as they are known in Japan, do not exist in either Europe, or the United States because in these countries, no regulation, or policy statements exist, specifically for them (Stanton et al., 2001). Legislation in Brazil is recent. Functional foods are regulated by ANVISA – National Agency of Sanitary Vigilance through Governmental Decree n. 15 e Resolutions n.16, 18 and 19 dated April 1999 (Brasil, 1999a, 1999b, 1999c, 1999d; Oliveira, Sivieri, Alegro, & Saad, 2002).

The norms that regulate functional foods must consider the following aspects in relation to the probiotics: the preparation should remain viable in large-scale production; it should be stable and viable during the storage and use; the preparation should be able to survive in the intestinal ecosystem; and, the host must get benefits to lodge probiotics.

Functional foods are defined as the foods that in addition to nutrients, supply the organism with components that contribute to cure the diseases, or to reduce the risk of developing them. They are foods like the conventional ones, which produce proved physiological effects and/or

reduce the risk of developing chronic diseases and could be similar in physical appearance to the conventional food, consumed as part of the daily dietary, but able to produce proved metabolic, or physiological effects, useful in the maintenance of a good physical and mental health and to able to help in the reduction of the risk of degenerative chronic diseases, besides to contribute to its basic nutritional functions. They could be foods with functional compounds, which have physiological effects on the consumer, besides their basic nutritive value. The main aim of these products is to prevent chronic diseases and to maintain the natural balance of vitamins and electrolytes of the body. According to the International Life Science Institute (ILSI), a food can be considered functional if it can demonstrate satisfactorily that it has a beneficial effect on one, or several specific functions in the organism, beyond the normal nutritional effects that improve the state of health and well-being, or reduce the risk of a disease. It is necessary to know what is meant by “beyond the normal nutritional effects”. The feeding defined in the recommended daily dose can contain, in addition to usual foods, restored foods (restoration of the initial content of vitamins and minerals) and enriched foods; the dietetic foods respond to the specify necessities of some consumers. The functional foods are beyond these basic necessities. However, they must continue being foods and must be consumed in compatible amounts with a balanced and diversified normal feeding. A functional food can be a natural one; or foods added of components; or foods of which component have been eliminated by means of technological or biotechnological procedures. It also can be a food, in which the nature of one or more of its component has been modified, or a food in which the bio-availability of one or more of its components has been modified, or any combination of the possibilities as above. The development of functional foods on the part of food industries and the clinical tests that are made to evaluate their effects will help the national and international sanitary authorities to establish clear criteria to classify this type of foods.

Nutraceuticals are defined as the foods manufactured from common foods and sold in the form of prepared like pills, capsules, powder, syrups, drinks, etc. They are medicinal formula, which are not associated generally with the foods and can exert a beneficial physiological effect, or assure protection against the chronic diseases. Fig. 2 shows a graphical representation of nutraceutical foods. Functional foods, design foods and nutraceuticals are expressions that can be used indifferently to talk foods, or isolated ingredients that provide determined non-nutritional beneficial physiological effects that can improve the health. The foods are composed by thousands of biologically active constituents that can have a beneficial effect on the health.

The first generation of functional foods involved supplementation of the constituents such as calcium, or vitamins, for their recognized health attributes (Ziemer & Gibson, 1998). Fermented dairy products can be included in the cat-

egory of functional foods because of their content of calcium (which can reduce the osteoporosis, hypertension and colon cancer) and other health-enhancing components. In the recent decades, there is a greater interest in the potential beneficial effects of the fermented milk on the health, resulting in the increase of the available variety and amount consumed around the world. Dairy products have been used traditionally like vehicle for the probiotic bacteria in humans. Lactic acid bacteria are the more important group of microorganisms used in the fermented milk elaboration and much of them are considered probiotics. Fermented milks are result of the metabolism of lactic acid bacteria that grow in milk. Its excellent nutritional quality can be attributed mainly to the milk, which offers an important source of calcium, proteins, phosphorus and riboflavin. The additional benefits on the health are result of the fermentation process, which supplies fermented milk with a large number of microorganisms and products of fermentation. Fermented milks have probiotic effect because their consumption takes to the ingestion of high amounts of live bacteria that exert benefits in the balance of the intestinal microflora and the health, beyond the basic nutrition. Probiotic dairy foods containing health-promoting bacteria are an important segment of the functional-food market (Batish & Grover, 2004). It represents a strong area in the growth within the group of functional foods and intense investigation to develop the dairy products with the probiotic microorganisms is in active progress.

In fact, the concept of functional foods has become more directed towards food additives that may exert a positive effect on the gut microbiota composition. This has largely concentrated on the probiotics, but more recently interest in prebiotics has increased (Ziemer & Gibson, 1998). Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of bacteria in the colon. This definition overlaps with the definition of dietary fiber, with the exception of its selectivity for certain species (Gibson & Roberfroid, 1995; Schrezenmeir & De Vrese, 2001). Food ingredients classified as prebiotics must not be hydrolyzed or absorbed in the upper gastrointestinal tract, need to be a selective substrate for one, or a limited number of colonic bacteria, must alter the microbiota in the colon to a healthier composition and should induce luminal, or systematic effects that are beneficial to the host health (Gibson & Roberfroid, 1995). Peptides, proteins and lipids contain prebiotics characteristics, but some carbohydrates have received the most attention, including lactulose, inulin, and a range of oligosaccharides that supply a source of fermentable carbohydrate for the beneficial bacteria in the colon (Crittenden, 1999; Ziemer & Gibson, 1998). The inulin and oligofructose are carbohydrates stored in the plants, presented in the foods as garlic, bananas, cereals, onions, and in high amounts in the root of the chicory. Oligofructose is a natural component of inulin (De Bondt, 2003). Symbiotics are

mixtures of the probiotics and prebiotics that benefit the host by improving the survival and implantation of the selected microbial supplements. Because of the nutritional benefits associated with microflora management approaches, foods are the main vehicle for pro-, pre- and symbiotics (Gibson & Roberfroid, 1995; Ziemer & Gibson, 1998).

3. Achievements of dairy probiotic beverages

Fermented beverages make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology, which preserves the food, improves its nutritional value and enhances its sensory properties (Gadaga, Mutukumira, Narvhus, & Feresu, 1999). Traditional fermented beverages have been documented in different countries, especially in Africa. *Kule naoto*, or *Kwerionik*, for example, are fermented milks produced and consumed in Kenya and Uganda, respectively (Pinto, Franz, Schillinger, & Holzapfel, 2006). *Amasi* (*mukaka wakakora* or *zifa*) is a traditional fermented milk consumed in Zimbabwe. It is produced by leaving fresh raw bovine milk to ferment naturally at ambient temperature in earthenware pots, or any other suitable containers. The microorganisms inherent in the milk, the container and the surrounding air are assumed to ferment the milk within 1–3 days, depending on the ambient temperature. From these, bacterial strains belonging to *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus* have been identified (Gadaga et al., 1999).

Ergo is traditional fermented milk produced in Ethiopia. It is thick, smooth, of uniform appearance and has some resemblance to yoghurt. Usually it has a white milk color when prepared carefully. The product is semi-solid and has a pleasant odor and taste. Several microorganisms are present in *ergo* such as Gram-positive bacteria. Yeasts and moulds were also isolated but were not identified. The LAB included *Streptococcus thermophilus*, *Streptococcus acidominus*, *Streptococcus bovis*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Enterococcus faecalis* var. *liquefaciens*, *Lactococcus cremoris*, *Lactococcus lactis*, *Leuconostoc dextranicum*, *Leuconostoc lactis* and *Lactobacillus xylosus*

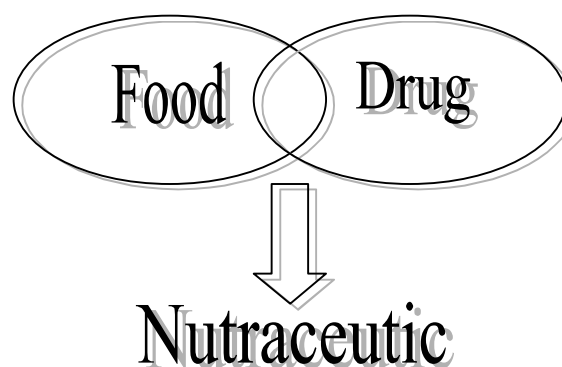


Fig. 2. Graphical representation of a nutraceutic.

(Gonfa, Fite, Uрга, & Gashe, 1999; Gonfa, Foster, & Holzapfel, 2001).

The original observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff, the Russian born Nobel Prize winner working at the Pasteur Institute at the beginning of the last century, who suggested that “the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in human bodies and to replace the harmful microbes by useful microbes” (FAO/WHO, 2001). Almost at the same time, the first formulae for deliberate administration of live lactic acid bacteria was launched in Paris, which was a yogurt based on a culture of *Sc. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. With this successful introduction, the incorporation of probiotic bacteria in foods was focused on milk-based products (Molin, 2001).

Macedo (1997) developed a probiotic beverage with a mix of 35% buffalo cheese whey, 30% soymilk and 35% cow milk fermented by a mixed culture of *Lactobacillus casei* Shirota and *Bifidobacterium adolescentis*. The concomitant use of these two strains resulted both in a pleasant taste and flavoured product besides an expected association with health-promoting effects. The fermentation was carried out at 37 °C for 8 h keeping a 1:5 proportion between the lactic acid and bifidobacteria in a 5% inoculum amount. The fermented beverage with a light extra-flavoring with vanilla essence was evaluated during a 28 days storage period at 4 °C, thus confirming the preservation of its chemical, microbiological and sensorial stabilities, although, a slight variation in the acidity profile the desirable balance for the viable cells of the bacterial strains (6.8×10^8 cells/mL for *Lb. casei* Shirota and 2.3×10^7 cells/mL for *Bf. adolescentis*) was found in the sensorial characteristics of the fermented milk beverage allowing its good acceptability in all time course of the storage period.

Vinderola, Gueimonde, Delgado, Reinheimer, and De Los Reyes-Gavilán (2000) evaluated the carbonation of pasteurized milk as a method for improving the fermentation conditions and/or bacterial viability in the fermented milk added with *Lactobacillus acidophilus* and/or *Bifidobacterium bifidum*. The fermentation time was significantly lowered in carbonated milk. The use of CO₂ had no detrimental effects on the sensory properties of the fermented milk and did not exert any influence on the viability of *Lb. acidophilus*. Oliveira et al. (2002) studied the combined effect of milk supplementation and culture composition on acidification, textural properties, and the microbiological stability of the fermented milks containing probiotic bacteria. Whey, casein hydrolysate and milk proteins were tested as supplementation. Two strains of probiotic bacteria, *Lb. acidophilus* LA5 and *Lactobacillus rhamnosus* LC35, were used in pure culture, and in mixed culture with *Sc. thermophilus* (ST7). The results showed that fermented products texture was not dependent on the culture composition, but strongly dependent on milk supplementation. Sodini, Lucas, Tissier, and Corrieu (2005) studied the physical

properties and the microstructure of the yoghurts containing probiotic bacteria and supplemented with milk protein hydrolysates. Three casein hydrolysates and three whey protein hydrolysates were added to the milk. The milks were fermented with either of the two different cultures and compared to the control yoghurt without supplementation. For both the cultures, the addition of the hydrolysates reduced the fermentation time but negatively affected the texture, decreasing the complex viscosity and graininess in the yoghurts.

The ingestion of the selected strains of the probiotic bacteria and the nutraceuticals has the potential to benefit the consumer but little information is available regarding the effect of nutraceuticals on the viability of probiotic species of bacteria in a foodstuff. Awaishah, Haddadin, and Robinson (2005) studied the effect of different concentrations of three nutraceuticals (ω -3-fatty acids, isoflavones and phytosterols) on the viability of two probiotic cultures (*Lactobacillus gasseri* and *Bifidobacterium infantis*), which were incorporated into the yoghurt. The sensory qualities of the yoghurt and the viability of the probiotic species were evaluate during the storage at 4 °C. The combination of the three nutraceuticals gave significantly higher counts than the control milk. In particular, *Bf. infantis* count was higher in the combination than in the presence of individual nutraceuticals.

Some strains of LAB as *Lactobacillus* ssp. SLH6 and *Sc. thermophilus* ST4 were used as inocula to ferment experimental mixtures of milk powder (3 g%) plus cassava starch (6 g%), or inulin (6 g%) as prebiotic. The use of this type of milk–oligosaccharides mixtures offers the possibility to reduce the use of milk to 1/3 of that used in regular yogurts, which can also serve as vehicles for the probiotic and prebiotic agents. The product is yogurt-like and the taste is fairly good. It can be offered to those countries that have shortage of milk and are good producers of cassava starch and other starchy products. The bioavailability of many nutrients in vegetable diets is usually low, and this will significantly contribute to the nutritional inadequacy. In poor countries animal foods and milk although of excellent nutritional value, are not available in enough quantity to these populations, due to their customs and high coasts (Parada, Sambucetti, Zuleta, & Rio, 2003).

The effect of raffinose family oligosaccharides (RFOs) addition on probiotic viability of *Bifidobacterium lactis* Bb-12 and *Lb. acidophilus* La-5 in the fermented milk was evaluated by Martínez-Villaluenga, Frías, Gómez, and Vidal-Valverde (2006) during 21 days of storage at 5 °C. Viability retention of both strains were higher in the fermented milk with RFOs, which had beneficial effects on the survival of these probiotic cultures in the dairy products. Stored products containing the probiotics and the prebiotics have synergistic actions in the promotion of health.

Evolus® (Valio, Finland) is the first blood pressure reducing fermented milk drink developed in Finland in October 2000. Its beneficial effect is due to the bioactive

peptides that are generated when *Lactobacillus helveticus* decomposes milk casein. A host of clinical tests have shown these peptides to have a positive influence on lowering the blood pressure (Valio, 2006).

Faced with the market saturation in the third world and stock dumping in advanced countries, dairy organizations that produce traditional fermented products must explore new avenues of the product diversification. New trends are becoming apparent with regard to consumers acceptance of the fermented milks. The selection of the microorganisms used as starters in the manufacture of the fermented milks is now based on the interaction with the gastrointestinal microflora and the metabolic properties. Genetic engineering of the microorganisms and new technological possibilities is expected to create new fermented milks in which desired properties would be emphasized. New products should be developed and traditional ones should be maintained and improved. Hence, the introduction of new technologies assumes added significance. Other potential areas include special fermentation products, such as probiotic milk foods with immunomodulating activity. These products could receive greater consumer attention as the market demand for the value-added fermented products is expected to be very high in the near future. Similarly, *Bifidobacterium*-derived fermented products have shown excellent physiological activity in infant digestion metabolism and nutrient utilization. Milk-based formulae with bioactive peptides of immunological significance can be prepared by fermentation process to mimic the biological attributes of breast milk.

These examples are only few applications of the potentiality of probiotics. However, other vehicles such as non-dairy raw materials may be used to deliver into the host the benefic lactic acid bacteria and other microorganism. Evidently, the fermented milk products continue to be subject of intensive researches to enhance their inherent nutritional and healthful attributes. Unsoundly, these products represent an exciting area of commercial interest for the dairy industry. The commercial stability of the fermented milks allows the expansion and coverage of the needs of different consumer categories. With the growing interest of consumers in health-related foods, the market for probiotic health microorganisms would have a bright and promising future (Batish & Grover, 2004).

4. Non-dairy probiotic beverages

Probiotic products are usually marketed in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products (Heenan, Adams, Hosken, & Fleet, 2004; Yoon, Woodams, & Hang, 2006). There are a wide variety of traditional non-dairy fermented beverages produced around the world. Much of them are

non-alcoholic beverages manufactured with cereals as principal raw material.

Boza is a beverage consumed in Bulgaria, Albania, Turkey and Romania. It is a colloid suspension, from light to dark beige, sweet, slightly sharp to slightly sour, made from wheat, rye, millet, maize and other cereals mixed with sugar, or saccharine. *Boza* has become a very popular beverage consumed daily by people of all ages due to its pleasant taste, flavour and high nutritional values. Microflora identification of Bulgarian *boza* shows that it mainly consists of yeasts and lactic acid bacteria, in an average LAB/yeast ratio of 2.4. The lactic acid bacteria isolated were *Lactobacillus plantarum*, *Lb. acidophilus*, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostoc reffinolactis*, *Leuconostoc mesenteroides* and *Lactobacillus brevis*. The yeasts isolated were *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *Geotrichum candidum* (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Gotcheva, Pandiella, Angelov, Roshkova, & Webb, 2000).

Bushera is the most common traditional beverage prepared in the Western highlands of Uganda. The product is consumed by both the young children and the adults. The sorghum, or millet flour from the germinated sorghum and millet grains is mixed with the boiling water and left to cool to ambient temperature. Germinated millet or sorghum flour is then added and the mixture is left to ferment at ambient temperature for 1–6 days. The lactic acid bacteria isolated from *Bushera* comprised of five genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus*. *Lb. brevis* was more frequently isolated than other species (Muianja, Narvhus, Treimo, & Langsrud, 2003).

Mahewu (*amahewu*) is a sour beverage made from the corn meal, consumed in Africa and some Arabian Gulf countries. It is prepared from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. The predominant microorganism found in African *mahewu* is *Lactococcus lactis* subsp. *lactis* (Blandino et al., 2003; Gadaga et al., 1999).

Pozol is a refreshing beverage, consumed in the South-eastern Mexico, made by cooking maize in an approximately 1% (w/v) lime solution, washing with water, grinding to make a dough known as *nixtamal*, shaping into balls, wrapping in banana leaves and leaving to ferment at ambient temperature for 0.5–4 days. The fermented dough is suspended in he water and drunk. Some fibrous components are not completely solubilized by *nixtamalization* and sediment is present in the beverage when the dough is suspended in the water (Wacher et al., 2000).

Togwa is a starch-saccharified traditional beverage consumed in Africa. In the southern part of Tanzania, *togwa* is usually made from the maize flour and finger millet malt. In this region, it is consumed by the working people and

also used as a refreshment as well as a weaning food (Oi & Kitabatake, 2003). *Togwa* is also regularly consumed by the young children. The cereal, or cassava flour is cooked in the water. After cooling at 35 °C, starter culture (old togwa) and cereal flour from the germinated grains are added. The fermentation process finishes at pH 4.0–3.2 (Molin, 2001).

Lactic acid bacteria can rarely convert starch into lactic acid. However, some strains of *Lactobacillus* and *Streptococcus* can do it (Parada, Zapata, De Frabrizio, & Martinez, 1996). For example, *Lb. plantarum* A6, isolated by Giraud, Grosselin, Parada, and Raimbault (1993), showed extracellular amylase activity. Sour cassava starch is obtained by a natural fermentation and this product is largely appreciated in Africa, South America and other developing countries. *Lb. plantarum* D34 was isolated in Colombia, which showed an amyolytic property. Santos (2001) developed a probiotic beverage with the fermented cassava flour (liquid phase + solid) using mixed culture of *Lb. plantarum* A6, which were amyolytic strains of *Lb. casei* Shirota and *Lb. acidophilus*. The best parameters of the fermentation were 8% inoculation rate, incubation temperature and period as 35 °C and 16 h, respectively, and 20% of cassava flour. At the end of the fermentation, the amount of bacteria was 2.8×10^9 cells/mL of lactic amyolytic bacteria and 2.3×10^9 cells/mL of probiotic bacteria. The final lactic beverage had 36% of guava juice, 10% of sugar and 54% of fermented lactic beverage. The lactic beverage maintained its microbiological and physico-chemical quality for 28 days storage period at 4 °C.

Angelov, Gotcheva, Kuncheva, and Hristozova (2006) produced a symbiotic functional drink from the oats by combining a probiotic starter culture and whole-grain oat substrate. The oats and barley are the cereals with highest content of beta-glucan, recognized as the main functional component of the cereal fibers. Studies have indicated the hypocholesterolemic effect of this compound, leading to 20–30% reduction of LDL-cholesterol, and to an expected overall effect of reduced cardiovascular disease risk (Stark & Madar, 1994; Wrick, 1994). The substrate was fermented with *Lb. plantarum* B28. The levels of starter culture concentration, oat flour and sucrose content were established for completing a controlled fermentation for 8 h. The addition of aspartame, sodium cyclamate, saccharine and Huxol (12% cyclamate and 1.2% saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during the product storage. The beta-glucan content in the drink of 0.31–0.36% remained unchanged throughout fermentation and storage of the drink. The viable cells counts reached at the end of the process were about 7.5×10^{10} CFU/mL. The shelf life of the oat drink was estimated to 21 days under refrigerated storage.

Since few decades ago, the soybean has received attention from the researchers due to its protein quality. Because of their functional properties, it has a great application potential in the food industry. The soymilk is suitable for

the growth of the lactic acid bacteria, especially bifidobacteria (Chou & Hou, 2000; Matsuyama et al., 1992). Several studies have mentioned the production and use of the fermented soymilk drinks as probiotic, mainly soybean yogurt, which further can be supplemented with oligofructose and inulin (Fuchs, Borsato, Bona, & Haully, 2005; Shimakawa, Matsubara, Yuki, Ikeda, & Ishikawa, 2003; Wang, Yu, & Chou, 2002).

Despite potential sensory challenges, there is a genuine interest in the development of fruit-juice based functional beverages, fortified with the probiotic and prebiotic ingredients. The fruit juices have been suggested as an ideal medium for the functional health ingredients because they inherently contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups, and because they are perceived as being healthy and refreshing (Tuorila & Cardello, 2002). The fruits and vegetables are rich in the functional food components such as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow & Delahunty, 2004a).

Hardaliye is a lactic acid fermented beverage produced from the natural fermentation of the red grape, or grape juice with the addition of the crushed mustard seeds and benzoic acid. This beverage can be found in the Thrace region of Turkey. It is very well known and has been produced and consumed since ancient times. The mustard seed's eteric oils affect the yeasts and also give flavor to the final product. Benzoic acid inhibits, or decreases alcohol production by affecting the yeast. Once fermented, the *hardaliye* is stored at a temperature of 4 °C and consumed either fresh, or aged. The lactic acid bacteria found in the beverage were *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus casei* subsp. *pseudopantarum*, *Lactobacillus brevis*, *Lactobacillus pontis*, *Lactobacillus acetotolerans*, *Lactobacillus sanfransisco* and *Lactobacillus vaccinoferus*. This characterization allowed the selection of appropriate strains for the manufacture of *hardaliye* using pasteurized, or sterile filtered grape juices (Arici & Coskun, 2001).

Yoon, Woodams, and Hang (2004) determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *Lb. acidophilus* LA39, *Lb. plantarum* C3, *L. casei* A4 and *Lb. delbrueckii* D7. The tomato juice was inoculated with a 24 h-old culture and incubated at 30 °C. The lactic acid cultures reduced the pH to 4.1 and the viable cell counts reached nearly $(1.0-9.0) \times 10^9$ CFU/mL after 72 h fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 CFU/mL after 4 weeks of cold storage at 4 °C. Yoon, Woodams, and Hang (2005) also evaluated the potential of red beets as the substrate for the production of probiotic beet juice by the above four species of lactic acid bacteria. All the lactic cultures were capable of rapidly utilizing the beet juice for the cell synthesis and lactic acid production. *Lb. acidophilus* and *Lb. plantarum* produced higher amount of lactic acid than other cultures and reduced the pH of the fermented beet juice from

an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30 °C. Although the lactic cultures in fermented beet juice gradually lost their viability during the cold storage, the viable cell counts of these bacteria, except for *Lb. acidophilus*, in the fermented beet juice still remained at 10^6 – 10^8 CFU/mL after 4 weeks of cold storage at 4 °C.

Yoon et al. (2006) also developed a probiotic cabbage juice using lactic acid bacteria. Cabbage juice was inoculated with a 24 h-old lactic culture and incubated at 30 °C. The cultures (*Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7) grew well on cabbage juice and reached about 1×10^9 CFU/mL after 48 h of the fermentation. *Lb. casei* produced a lower amount of titratable acidity, expressed as lactic acid, than *Lb. delbrueckii*, or *Lb. plantarum*. After 4 weeks of the cold storage at 4 °C, the viable cell counts of *Lb. plantarum* and *Lb. delbrueckii* were 4.1×10^7 and 4.5×10^5 CFU/mL, respectively. *Lb. casei* did not survive at low pH and lost cell viability completely after 2 weeks of the cold storage. The fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Rakin, Vukasinovic, Siler-Marinkovic, and Maksimovic (2007) enriched beetroot and carrot juices with the brewer's yeast autolysate before lactic acid fermentation with *Lb. acidophilus*. The addition of the autolysate favorably affected the increase of the number of lactic acid bacteria during the fermentation (Aeschlimann & Von Stocar, 1990), reduction of the time of fermentation and enrichment of the vegetable juices with amino acids, vitamins, minerals and antioxidants (Chae, Joo, & In, 2001). The use of spent brewer's yeast from the brewery was important for the economic optimization of the fermentation. A mixture of beetroot and carrot juices has optimum proportions of pigments, vitamins and minerals (Rakin et al., 2007).

Luckow and Delahunty (2004a) evaluated the consumer's acceptance for the appearance, aroma, texture and flavour of the probiotic fruit juices. Novel blackcurrant juices containing the probiotic cultures were compared with the conventional blackcurrant juices by the descriptive analysis. The probiotic juices contained aroma and flavors characteristic of the functional ingredients. Subsequent testing took place in a local shopping center, where the consumers were presented with two randomly coded blackcurrant juice samples. One of the products was a natural blackcurrant juice, and the other was a commercially processed blackcurrant juice containing probiotic cultures. The consumers were instructed that one of the juice samples contained "special ingredients" designed to improve their health. They were asked to assess their overall impression of both the juices, and to rate their acceptance of the sensory characteristics. Furthermore, based on their overall impressions and guided by their individual expectations, the consumers were asked to identify the juice they perceived to be the 'healthiest' (e.g., containing the "special ingredients"). The juice preference was dependent on the gender and the age. In general, the consumers selected their most preferred juice product as the 'healthiest' sample.

Luckow and Delahunty (2004b) examined the sensory impact of functional ingredients (e.g., probiotics, prebiotics, vitamins, and minerals) on the aroma and taste of orange fruit juices. A trained panel ($n = 10$) performed a descriptive sensory analysis on four functional orange juices and seven conventional orange juices. The functional juices were described as possessing perceptible "dairy", "medicinal" and "dirty" flavors, distinguishing them from the conventional juices. Subsequently, 100 consumers participated in a preference test, whereby five orange juices (three conventional and two functional) were ranked in order of the consumer preference. The ranking decisions were based solely on the sensory characteristics of the juices, since product information was not provided. On an overall basis, the consumers preferred the sensory characteristics of conventional juices. However, cluster analysis identified a small consumer segment (11%) that significantly preferred the sensory attributes of functional juices.

Evidently, there are already some relatively new non-dairy probiotic beverages in the market. Grainfields Wholegrain Liquid[®] is a refreshing, effervescent liquid that delivers active, friendly lactic acid bacteria and yeasts as well as vitamins, amino acids, and enzymes. It is made from organic ingredients including the grains, beans, and seeds such as the malted organic oats, maize, rice, alfalfa seed, pearl barley, linseed, mung beans, rye grain, wheat, millet. The liquid is fermented to achieve high levels of active probiotic bacteria sustained in a liquid medium immediately available for the use within the digestive system. Grainfields Wholegrain Liquid[®] is fermented with lactobacilli and yeasts cultures: *Lb. acidophilus*, *Lb. delbrueckii*, *Saccharomyces boulardii* and *Sc. cerevisiae*. The liquid is dairy-free, contains no genetically modified ingredients and has no added sugar (Superfoods., 2006).

Vita Bios[®] is a mixture of aromatic herbs and other plants, which are fermented by a combination of lactic acid and yeast cultures. This beverage contains no sugar and can produce carbon dioxide. The bacteria are selected based on the criterion of their providing the microflora of the intestines with the best possible conditions. During the fermentation, the lactic acid formed produces a low pH value of about 3.5. The low pH prevents the development of harmful bacteria in the finished product. Vita Bios[®] also contains a high number of antioxidants. The reason for this beverage becoming so popular is its ability to quickly restore the natural balance in the digestive system. The liquid restricts harmful bacteria and thus gives the beneficial bacteria a better possibility to multiply and create healthy intestinal flora. Vita Bios[®] is manufactured in Denmark (Superfoods, 2006).

Proviva[®] was the first probiotic food that does not contain milk, or milk constituents. It was launched in Sweden by Skane Dairy (Sweden) in 1994. The active component comprises lactic acid bacteria fermented oatmeal gruel. Malted barely is added to enhance the liquefaction of the product and *Lb. plantarum* 299v carries out the fermentation. The final product contains 1×10^{12} colony-forming

units (CFU) of *L. plantarum*/L. This formula is used as the active ingredient in the food product in which 5% of the oat meal gruel is mixed with a fruit drink. The consumer product contains 5×10^{10} CFU/L (Molin, 2001).

Valio Ltd. began developing non-dairy drinks with *Lb. rhamnosus* GG in 1996 and the first product was launched in 1997. GEFILUS® fruit drinks have a shelf life of 5 weeks when refrigerated (Leporanta, 2005a, 2005b). The latter was from Valio's commercial Bioprofit® product, with *Lb. rhamnosus* GG and *Propionibacterium freudenreichii* ssp. *shermanii* JS (Daniells, 2006). Biola® juice drink is a new probiotic juice manufactured by Tine BA in Norway. It contains added *Lb. rhamnosus* GG. Tine is using this strain under license from Finnish dairy company Valio Ltd. The juice drinks contain more than 95% fruit and no added sugar and are available in orange–mango and apple–pear flavours (Leporanta, 2005b). Rela® is a fruit juice with *Lactobacillus reuteri* MM53 manufactured by the Biogaia, Sweden.

In recent years, the consumer demand for non-dairy-based probiotic products has increased (Shah, 2001) and the application of probiotic cultures in non-dairy products and environments represents a great challenge (Mattila-Sandholm et al., 2002). Juice manufacturers in particular were considered to lead new product development activities for gut benefit beverages as line extensions of existing functional drinks such as Valio's GEFILUS®. Fruit juice-based probiotic drinks would become an increasingly important category in future years (Dairy Industries International, 2004; Leatherhead Food Research Association, 2004).

5. Perspectives of probiotic beverages

There is every reason to believe that beverages would be the next food category where the healthy bacteria will make their mark. Likely candidates are chilled fruit juices, bottled water, or fermented vegetable juices. The probiotic microorganisms also have been directly incorporated into the drinks. The key to the development of this second generation of the probiotic products is a special Direct Liquid Inoculation system. It allows food producers to add the probiotic bacteria directly to the finished food product. The technology uses Tetra Pak's aseptic dosing machine Flex Dos that allows the bacteria to be added to liquids just before they are filled into the cartons. The innovation is expected to significantly boost the market for the probiotic beverages, which have so far been restricted by the delicate nature of the ingredient and concerns over the contamination. The probiotic bacteria must be live to exert their health benefits but they can be destroyed by a number of processing situations. In beverages, for example, the heat treatment in a standard production run would kill the live bacteria (Chr Hansen, 2006; Shah, 2001).

Adding probiotics to the juices is more complex than formulating in the dairy products because the bacteria need protection from the acidic conditions in the fruit juice. However, with microencapsulation technologies, the probi-

otics can become an important ingredient in the functional foods, expanding the probiotic application outside the pharmaceutical and supplement industries. Microencapsulation technologies have been developed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment (Del Piano et al., 2006). It is the process by which small particles or droplets are surrounded by a coating to produce the capsules in the micrometer to millimeter range known as microcapsules. The microencapsulation allows the probiotic bacteria to be separated from its environment by a protective coating. Several studies have reported the technique of the microencapsulation by using gelatin, or vegetable gum to provide protection to acid-sensitive *Bifidobacterium* and *Lactobacillus* (Chandramouli, Kailasapathy, Peiris, & Jones, 2004; Lee, Cha, & Park, 2004; O'Riordan, Andrews, Buckle and Conway, 2001; Sultana et al., 2000).

New product development requires detailed knowledge of both the products and the customers. The high reported failure rates for new international functional beverages suggest a failure to manage the customer knowledge effectively, as well as a lack of the knowledge management between the functional disciplines involved in the new product development process. The methodologies that advance both a firm's understanding of the customer's choice motives and the value systems, and its knowledge management process, can increase the chances of new product success in the international functional beverages market (Bogue & Sorenson, 2006). The commercial success of the probiotic products ultimately depends on taste and appeal to the consumer (Heenan et al., 2004; Yoon et al., 2006). The consumer needs to receive a comprehensible and reasonable message about probiotics, without appearing to be exaggerated. The "health claims" must be defensible when placed under the scrutiny by the controlling authorities. In the coming years of the new Millennium, changes would occur in the interface between the scientific studies and the acceptance by the consumers (Gorbach, 2002). With increasingly competitive markets, the functional foods and the beverages manufacturers have targeted functionality as an extremely important marketing tool to create competitive advantages in the marketplace (Sorenson & Bogue, 2005).

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