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Mini Review

Non dairy probiotic beverages

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Abstract

The beneficial effects of food with added live microbes (probiotics) on human health are being increasingly promoted by health professionals. Probiotic products available in the markets today, are usually 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. And, owing to health considerations, from the perspective of cholesterol in dairy products for the developed countries, and economic reasons for the developing countries, alternative raw materials for probiotics need to be searched. Considering the above mentioned facts cereals, legumes, fruits and vegetables may be potential substrates, where the healthy probiotic bacteria will make their mark, both in the developing and the developed countries. This review aims at highlighting the research done on probiotic beverages from non dairy sources. These non dairy probiotic beverages can serve as a healthy alternative for dairy probiotics and also favor consumption by lactose intolerant consumers.

Introduction

Probiotics represent probably the archetypal functional food, and are defined as alive microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. Kollath in 1953, first defined the term “probiotic”, when he suggested the term to denote all organic and inorganic food complexes as “probiotics,” in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. Vergio, in his publication “Anti- und Probiotika”, compared the detrimental effects of antibiotics and other antimicrobial substances with favorable factors (“Probiotika”) on the gut microbiology. Lilly and Stillwell proposed probiotics to be “microorganisms promoting the growth of other microorganisms”. 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 nutrition’’. 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) (Guarner and Schaafsma, 1998; FAO/WHO, 2001).

Early scientific studies on microorganisms, during the second part of the 19th century which dealt with the interactions with the human host, suggested their benefits for digestion and the beneficial association of vaginal bacteria by production of lactic acid from sugars, thereby preventing or inhibiting the growth of pathogenic bacteria. These findings and other information on the early stages of development toward biotherapeutic concepts and the utilization of functional major metabolic product were generally grouped as “lactic acid bacteria” (LAB) (Escherich *et al*., 1886). Recent research has underlined the importance of a vital and “healthy” microbial population of the gastro intestinal tract (GIT). Increased research efforts during the last three decades, have confirmed the beneficial association of LAB with the human host. Metschnikoff suggested the longevity of the Caucasians to be related to the high intake of fermented milk, and considered that lactic acid production, resulting from sugar fermentation by LAB, to be particularly beneficial. The *Bifidobacteria*, another group producing lactic acid, phylogenetically distant but commonly accepted to form part of the LAB, were discovered in 1889 and described in the early 1900s by Tissier to be associated with the feces especially of breast-fed infants. When compared to

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formula-fed infants, a lower incidence of intestinal upsets was observed for infants receiving mother's milk. Thereby the assumption was made about the beneficial association of *Bifidobacteria* with the human GIT.

Functionality of probiotics

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children. However, some health benefits, e.g., immune modulation, may be achieved even with dead bacteria (Kalliomaki *et al*., 2001). Figure 1 shows the major health benefits conferred by probiotics these have been described here under.

Prevention of diarrhea

Several probiotic strains, especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhea, caused mainly by rotavirus. It is also well-established that some probiotic strains can both prevent and shorten antibiotic-associated disorders. (Fuller *et al*., 2008)

Stimulation of the immune system

Many human studies performed to investigate the effects of probiotic cultures on the immune system reveal that probiotic bacteria are able to enhance both innate and acquired immunity by increasing natural killer cell activity and phagocytosis, changing cytokine profiles, and increasing levels of immunoglobulins. Two probiotic strains have been developed with a particular focus on their enhancing effects on immune responses: *Bifidobacterium lactis* and *Lactobacillus rhamnosus*. Both strains have been demonstrated in several studies to enhance natural immune function in healthy people (Fuller *et al*., 2008).

Inflammatory bowel disease

There is growing evidence that probiotics have a potential therapeutic benefit for patients suffering from IBD. Controlled clinical studies have demonstrated that probiotics are efficacious in the maintenance of remission of pouchitis, prophylaxis of pouchitis after the formation of an ileoanal reservoir, maintenance of remission of ulcerative colitis, and treatment of Crohn's disease (Fooks *et al*., 2002).

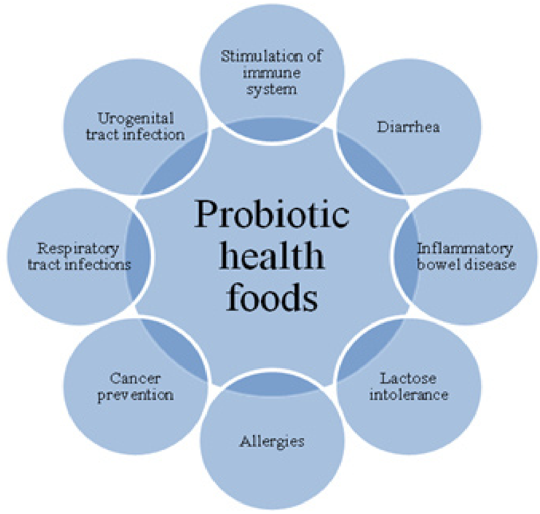

Figure 1. Probiotic beneficial effects on human health

Lactose intolerance

Bacterial cultures, yogurt starter cultures as well as some probiotic cultures are known to improve the lactose digestion in lactose maldigestors. Subjects suffering from lactose intolerance have a very low concentration of the lactose-cleaving enzyme β-galactosidase, and bacteria in fermented or unfermented food products release their β-galactosidase in the small intestine, where it supports lactose digestion (Li *et al*., 2012).

Allergies

Pelto *et al*. found that *Lactobacillus rhamnosus* GG confers an immunostimulatory effect in healthy adults. Probiotics have also been used successfully in the management of atopic eczema in infants. Furthermore, *Lactobacillus rhamnosus* GG proved to be effective in the prevention of early atopic disease in children at high risk. The *Lactobacillus rhamnosus* GG product when given prenatally to mothers and postnatally for 6 months to the mothers or to their infants directly, reduced the frequency of atopic eczema in the probiotic group to half that of the placebo group at the age of 2 years. The preventive effect was reconfirmed at the age of 4 years (Delcenserie *et al*., 2010).

Cancer

A few epidemiological studies indicate that consumption of fermented dairy products containing *Lactobacilli* or *Bifidobacteria*, lowers the incidence of colorectal cancer. However, there is some indirect evidence based on several markers applied in human studies (e.g., fecal enzyme activities, fecal mutagenicity and genotoxicity, immunological markers), which shows that probiotics reduce the risk of colon cancer. A case-control study conducted in

Japan with 180 cases and 445 controls revealed that habitual intake of lactic acid bacteria reduces the risk of bladder cancer (Rafter, 2004).

Respiratory tract infections

Respiratory effects of probiotics in animal models have included attenuating allergic airway responses and protecting against respiratory pathogens. Dendritic cells appear central to directing the beneficial immune response to probiotic bacteria and in translating microbial signals from the innate to the adaptive immune system, whereas regulatory T cells are emerging as potentially key effectors of probiotic-mediated responses, particularly in the reduction of allergic inflammation. Despite progress in basic research, clinical trials of probiotics in allergy/asthma and respiratory infection have been highly variable at best, leading to an undermining of confidence in this potential therapeutic strategy (Forsythe, 2011).

Constipation

Studies carried out on the effects of lactic acid bacteria on constipation and intestinal motility, have shown reduced severity of constipation and an improved bowel movement frequency and stool consistency in constipated but otherwise healthy people after consumption of a fermented milk drink containing *Lactobacillus casei* strain Shirota. Administration of *Bifidobacterium longum* BB536 to constipated women resulted in a significantly increased defecation frequency and stool softness. A positive influence of *Bifidobacterium longum* BB536 on the "regularity" was also reported for elderly people (Ouwehand *et al*., 2002).

Urogenital tract infections

Studies suggest that probiotic preparations given orally or intravaginally may provide a therapeutic source of *Lactobacilli* to help control urogenital infections in women. A case-control study with 139 females with acute urinary tract infection and 185 controls revealed that consumption of fermented milk products containing probiotic bacteria was associated with a decreased risk of recurrence of urinary tract infection (Dani *et al*., 2002).

Helicobacter pylori infection

Several probiotic strains have been shown to inhibit *Helicobacter pylori in vitro*, which has been associated with gastritis, stomach carcinoma, gastric ulcer, and lymphomas. Human studies confirmed this inhibitory effect on *Helicobacter pylori*, which seems to be independent of the viability of the bacteria (Hamilton-Miller *et al*., 2003).

High cholesterol

Many human studies have evaluated the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels with equivocal results. A fermented milk containing *Enterococcus faecium* and *Streptococcus thermophilus* was reported to produce a small but significant decrease in total and LDL-cholesterol in patients with primary hypercholesterolemia. (Pereira *et al*., 2002)

Why non dairy probiotics?

Most probiotic foods available today are milk based, but consumers' preference today lie more with botanical dietary supplements, which are either free from or have minimal cholesterol content. The above fact is highlighted by the trend in the U.S. functional food market, which is developing in a different fashion from that seen in Europe, with its functional food sector more broadly defined as neutraceuticals and consumer interest tending to lie more with botanical dietary supplements rather than fortification of foodstuffs. This trend however, is changing, as interest in immunity, cancer and heart health grows. Also, the market for functional foods is in its infancy in many countries; however, product innovation throughout a number of sectors, such as drinks, bakery and probiotics, is evident, with trends generally following those of the U.S. and U.K. (Luckow *et al*., 2004)

Asian diets are relatively low in meat and dairy foods, and plant-based foods contribute the core of the daily intake. Besides dietary habits, lactose intolerance discourages many Asian people from consuming milk. The average per capita dairy consumption (including fluid milk, butter, cheese, NFD, and WMP) in milk equivalent in the last decade for the major dairy markets was 10.2 kg in China, 71.8 kg in India, 7.8 kg in Indonesia, 97.6 kg in Japan, 67.8 kg in Malaysia, 24 kg in the Philippines, 80 kg in South Korea, 28.7 in Thailand, and 8.6 kg in Vietnam. This contrasts with per capita consumption of 330 kg in the EU-15, 310 kg per capita in Australia, and 251 kg per capita in the United States (Dong, 2006). Therefore, considering the above mentioned facts cereals, fruits and vegetable may be potential substrates, where the healthy probiotic bacteria will make their mark, both in the developing and the developed countries.

Current research trends in 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 *et al*, 2004; Yoon *et al*., 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. The non dairy probiotic beverages may be made from a variety of raw materials, such as cereals, millets, legumes, fruits and vegetables. Studies may be classified based on the source of raw material for the production of the non dairy probiotic beverage.

Cereals and legumes

Cereal grains are an important source of protein, carbohydrates, vitamins, minerals and fiber for people all over the world, and can be used as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon, thereby acting as prebiotics (Andersson *et al*., 2001). Cereals contain water-soluble fiber (such as β-glucan and arabinoxylan), oligosaccharides (such as galacto- and fructooligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept (Andersson *et al*., 2001; Shah, 2001). Strains of *Lactobacillus* have been recognized as complex microorganisms that require fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals to grow (Gomes and Malcata, 1999), and therefore fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial microorganisms. A multitude of non-dairy fermented cereal products has been created throughout history for human nutrition, but only recently probiotic characteristics of microorganisms involved in traditional fermented cereal foods have been reported.

Boza, a beverage consumed in Bulgaria, Albania, Turkey and Romania, is a colloidal 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. 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* (Gotcheva *et al*., 2000; Blandino *et al*., 2003).

Table 1. Traditional probiotic beverages from cereals and pulses

Name of the beverage	Source	Strains	References
Boza	Wheat, rye, millet, maize and other cereals	*Lb. plantarum, Lb. acidophilus, Lb. fermentum, Lb. coprophilus, Leuconostoc reffinolactis, Leuconostoc mesenteroides, Lb. brevis, Saccharomyces cerevisiae, Candida tropicalis, Candida glabrata, Geotrichum penicillatum, Geotrichum candidum*	Blandino *et al*., 2003 Gotcheva *et al*., 2000
Bushera	Sorghum, millet flour	*Lactobacillus, Lactococcus, Leuconostoc, Enterococcus and Streptococcus. Lb. brevis*	Muianja *et al*., 2003
Mahewu	Maize, sorghum, millet malt, wheat flour	*Lactococcus lactis subsp. lactis*	Blandino *et al*. 2003, Gadaga *et al*., 1999
Pozol	Maize	-	Wacher *et al*., 2000
Togwa	Maize flour and finger millet malt	*Lactobacillus, Streptococcus, Lb. plantarum* A6	Giraud *et al*., 1993, Parada *et al*., 1996
Bushera	Sorghum, millet flour	*Lactobacillus, Lactococcus, Leuconostoc, Enterococcus and Streptococcus. Lb. brevis*	Muianja *et al*., 2003
Mahewu	Maize, sorghum, millet malt, wheat flour	*Lactococcus lactis subsp. lactis*	Blandino *et al*. 2003, Gadaga *et al*. 1999

Bushera is a traditional beverage prepared in the Western highlands of Uganda, 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 *et al*., 2003).

Mahewu (amahewu) is a sour beverage made from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. It is consumed in Africa and some Arabian Gulf countries. 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* (Gadaga *et al*., 1999; Blandino *et al*., 2003).

Pozol a refreshing beverage, consumed in the Southeastern Mexico, is 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 the 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, a starch-saccharified traditional beverage consumed in Africa, 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 refreshment as well as a weaning food (Oi and 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* (Parada *et al*., 1996), for example, Lb. plantarum A6, isolated by Giraud *et al*. (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.

Santos (2001) developed a probiotic beverage with the fermented cassava flour using mixed culture of *Lb. plantarum*, which were amylolytic 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 x 10^9 cells/mL of lactic amylolytic bacteria and 2.3 x 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 *et al*. (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 β-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 and 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 β-glucan content in the drink of 0.31–0.36% remained unchanged throughout fermentation and storage of the drink. The viable cell counts reached at the end of the process were about 7.5 x 10^{10} CFU/mL. The shelf life of the oat drink was estimated to 21 days under refrigerated storage.

Since few decades ago, soybean has received attention from the researchers due to its protein quality. Soymilk is suitable for the growth of the lactic acid bacteria, especially *Bifidobacteria* (Matsuyama *et al*., 1992; Chou and Hou, 2000). 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 (Wang *et al*., 2002; Shimakawa *et al*., 2003; Fuchs *et al*., 2005).

Fruits and vegetables

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 and 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 and Delahunty, 2004).

Current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content (Heenan *et al*., 2004). Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way (Betoret *et al*., 2003). This could make them ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants (Yoon *et al*., 2004), while lacking the dairy allergens that might prevent consumption by certain segments of the population (Luckow and Delahunty, 2004). There is a genuine interest in the development of fruit juice based functional beverages with probiotics because they have taste profiles that are appealing to all age groups and because they are perceived as healthy and refreshing foods (Tuorila and Cardello, 2002; Yoon *et al*., 2004; Sheehan *et* *al*., 2007). However, unsuitable contents of aromas (perfumery, dairy) and flavors (sour, savory) have been reported when *Lactobacillus plantarum* is added to juices (Luckow and Delahunty, 2004). The sensory impact study by Luckow and Delahunty (2004) showed that consumers prefer the sensory characteristics of conventional orange juices to their functional counterparts (juice containing probiotics) but if their health benefits information is provided the preference increases over the conventional orange juices.

According to Sheehan *et al*. (2007), when adding *Lactobacillus* and *Bifidobacterium* orange, pineapple and cranberry juice, extensive differences regarding their acid resistance were observed. All of the strains screened survived for longer in orange and pineapple juice compared to cranberry. *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei* display a great robustness surviving at levels above 7.0 log cfu/ml in orange juice and above 6.0 log cfu/ml in pineapple juice for at least 12 weeks. However, after thermal pasteurization at 76^{o}C for 30 s and 90^{o}C for 1 min in addition to a high-pressure treatment of 400MPa for 5 min, *Lb. casei*, *Lb. rhamnosus* and *Lb. paracasei* were not capable of withstanding the treatments required to achieve a stable juice at levels >6.0 log cfu/ml (Sheehan *et al*., 2007).

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^{o}C and consumed either fresh, or aged. The lactic acid bacteria found in the beverage were *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus casei* subsp. *pseudoplantarum*, *Lactobacillus brevis*, *Lactobacillus pontis*, *Lactobacillus acetotolerans*, *Lactobacillus sanfransisco* and *Lactobacillus vaccinostercus*. This characterization allowed the selection of appropriate strains for the manufacture of hardaliye using pasteurized, or sterile filtered grape juices (Arici and Coskun, 2001).

Yoon *et al*. (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^{o}C. The lactic acid cultures reduced the pH to 4.1 and the viable cell counts reached nearly (1.0– 9.0) x 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^{o}C. Yoon *et al*. (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^{o}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^{o}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^{o}C. The cultures (*Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7) grew well on cabbage juice and reached about 1 x 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^{o}C, the viable cell counts of *Lb. plantarum* and *Lb. delbrueckii* were 4.1 x 10^7 and 4.5 x 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 *et al*. (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 and Von Stocar, 1990), reduction of the time of fermentation and enrichment of the vegetable juices with amino acids, vitamins, minerals and antioxidants (Chae, Joo, and 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).

Evidently, there are already some relatively new nondairy 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. delbreukii*, *Saccharomyces boulardii* and *Sc. cerevisiae*. The liquid is dairy-free, contains no genetically modified ingredients and has no added sugar (Superfoods, 2006). Vita Biosa 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 Biosa 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 Biosa 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 barley 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*. This formula is used as the active ingredient in the food product in which 5% of the oatmeal gruel is mixed with a fruit drink. The consumer product contains 5×10^{10} CFU/mL (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. 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, 2005). 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).

Scope and future challenges

The applicability of probiotics in food products depends in general on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH, mechanical stress, salt content, and content of other harmful or essential ingredients. For many products, excess water activity is a critical parameter that increases the death rate of bacteria. Products with an unfavorable water activity are, for example, cereals, chocolate, marmalade, honey, and toffees. These products are too "dry" for applying live bacteria and too "wet" for the application of freeze-dried bacteria. Freeze-dried bacteria could be applied in these products if the bacteria could be protected from moisture, as small amounts of moisture can be very detrimental to the dried culture. In addition to dairy products, fruit juices have been shown to be suitable carriers for probiotics. The limiting factor for many of the probiotic strains is the low pH of the juices. There is growing interest in consumers towards healthier foods and probiotic fruit, vegetable juices and cereal based beverages can serve as a good option. But, the application of probiotic cultures in non-dairy products and environments represents a great challenge and needs to be researched at the industrial level for commercial production of these healthy products (Mattila- Sandholm *et al*., 2002).

Conclusion

Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way. This could make them ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population. Probiotics products available in the markets today are mainly milk based; however, the increase in consumer vegetarianism and demand for cholesterol free probiotics has encouraged scientists and researchers to explore newer matrices as vehicles for probiotics particularly vegetable and fruit juices. Vegetables contain beneficial nutrients, such as minerals, vitamins, dietary fibers, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population, thus making them ideal substrates for probiotic cultures.

Vegetables and fruits are reported to contain a wide variety of antioxidant components, including phytochemicals. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation. There is a genuine interest in the development of non dairy based functional beverages with probiotics because they serve as a healthy alternative for dairy probiotics, are cholesterol free and also favor consumption by lactose intolerant consumers.

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