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Non-dairy Based Probiotics: A Healthy Treat for Intestine

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Dairy-based fermented products and yoghurts have been utilized as potential probiotic products since ancient times. However, recent upsurge in interest of consumers towards dairy alternatives has opened up new vistas for non-dairy probiotic research and development. Various matrices and substrates such as cereals, fruit juices, or mixture thereof are being utilized for delivering these beneficial microorganisms. Each matrix offers some advantages over the other. Vast knowledge available on a number of conventional fermented foods can also be utilized for future research in this area. The present review provides an insight on the recent research/developments in the field of non-dairy probiotic foods with particular reference to the foods consumed conventionally, in addition to their commercial availability and a way forward.

Keywords *Lactobacillus, Bifidobacterium*, probiotic foods, fermentation, functional foods

Food is considered a medium of sustenance that provides enough nutrients to meet metabolic requirements since time immemorial. Recent knowledge supports the hypothesis that “beyond nutrition diet may play detrimental or beneficial roles in some diseases and also affects various physiological functions” (Koletzko et al., 1998). In view of the increasing awareness about health, life expectancy, and the aspiration of people toward superior life quality these concepts become very important (Roberfroid, 2007). Some other important factors like hectic lifestyles, increased consumption of fast foods, limited physical activities; increased incidence of self-medication; increased awareness from media and health authorities on nutrition and the connection between diet and health; scientific developments in nutrition research; and a competitive and crowded food market, exemplified by limited margins has resulted in the rise of functional foods markets (Siró et al., 2008).

Probiotics, defined as “live microorganisms, which when administered in adequate amounts (10^6); confer a health benefit on the host” (FAO/WHO, 2002; Senok, 2005). The use of probiotics has been advocated (Saavedra, 2007) due to their ability of gut wall translocation, immune system stimulation (Mustafa et al., 2009), and epithelial function modulation (Martins et al., 2007), and producing substances that affect

intestinal pain receptors (Rousseaux et al., 2007; Zocco et al., 2007). The relatively rapid change to current diets compared with the slow change in human genomics, necessitated the supplementation of these beneficial microorganisms from external sources either as a part of regular diet or supplements because ancestral humans had a diet that was high in fibers (prebiotics) that promotes the growth of probiotic bacteria and fermentation processes were commonly used to prepare and preserve food in those times (Leach, 2007). The consumption of fermented milk is the main reason for longevity of Bulgarian peasants, this concept was given one hundred years ago by Nobel Laureate Eli Metchnikoff (Metchnikoff, 1908); that is also supported by a study on drosophila in which a linkage was found between long life and beneficial microflora of the gut (Brummel et al., 2004).

The increased scientific knowledge and evidences resulted in an upsurge in probiotic foods/supplements markets over last two decades and the growth rate in 2008 was between 5 and 30% depending on region and product type. The probiotic products are mostly marketed as food supplements and therefore escape the meticulous regulations regarding efficacy, safety, quality, labeling requirements, health claims validation, and consumer information as normally applied to pharmaceutical products. This limits the commercial exploitation of probiotics, since large scale production, consistent quality, health claims, or beneficial health effects remains unproven. Over half of probiotic market is with foods, i.e., 30–40% as supplements and only 10% as pharmaceuticals. The global market for overall probiotic products (drugs, supplements, and foods) was worth \$16 billion in 2008 and is projected to attain a

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growth of \$32.6 billion in 2014. *Lactobacillus* genus-based probiotics accounted for 61.9% of total sales in 2007, representing the major share (Anonymous, 2009).

Till last decade probiotics were commonly perceived to be derived from dairy-based products. Consumers generally link probiotic food products with yoghurt only because dairy products like fermented milk, yoghurt, and cheese, occupies the premier position in probiotic foods either due to more likingness by people or much availability. But knowledge enrichment in recent years has led to the scientific findings confirming the probiotic potential of isolates in many of the traditional non-dairy-based fermented foods. This has created a curiosity in the researchers to further explore the probiotic potential of various non-dairy-based fermented foods and creation of novel non-dairy-based probiotic foods. This paper reviews the present knowledge about various non-dairy-based probiotics available across the globe to give an insight to the subject and shows a way forward for future.

Dairy vs. Nondairy Probiotics

Dairy products are the most common formats in which probiotic foods are available. Among dairy, the major products are fermented milk, yoghurts, buttermilk, and kefir. Dairy products available in the market could be classified in to two categories: (A) fermented, which includes yoghurt, cheese, dahi, kefir, etc. (B) Nonfermented like sweet milk and ice creams. Live probiotic cultures (*Lactobacillus* and *Bifidobacterium*) have been added to ferment milk and different versions of probiotic yoghurts (plain or flavored) are available in a number of countries and accounts for largest share in the market. Example of fermented milk products are numerous such as Yakult in Japan, Actimeal in Paris, Suntory Bikkle in Japan, Nestle LC1 in Switzerland, Vifit in the Netherlands, and Nesvita in Switzerland. Europe has emerged as a global leader in dairy-based probiotic foods due to increased consumer familiarity with these beneficial microorganisms. In European market, dairy probiotic products share 65% of total functional food market. In comparison to Europe, US probiotic market is lagging behind. However, probiotic yoghurts are acquiring popularity owing to the increased likeness among the population. The growing demands for probiotic products in US market has resulted in the enhanced sales and this demand is further going to increase in future also. According to a report, the probiotic yoghurt market is estimated to grow at a compound annual growth rate (CAGR) of 11.1% till 2014 to occupy a market proportion of \$10.9 billion, whereas probiotic dietary supplements will reach around \$1.8 billion in 2014 growing at a CAGR of 8.6% (Markets and Markets, 2010).

With an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian or non dairy probiotic products. High cholesterol content of animal derived products and significant amount of lactose

intolerant population also necessitates the search for dairy alternatives.

Traditional Nondairy Fermented/Probiotic Foods

Lactic acid fermentations have been practiced since long and a number of conventional fermented products based upon nondairy substrates like cereals, vegetables, etc. are available. Due to less scientific evidences, their potential as probiotics remained unproven and they are not perceived as efficient vehicle for probiotic bacteria delivery due to numerous reasons (Molin, 2001). One such reason is that many fermented products are cooked before consumption leading to death of probiotic microorganisms like in case of Indian fermented products such as idli, dosa, dhokla, bhature, etc. Nondairy probiotics are available both in the form of beverages and fermented foods. These products may be based upon cereals, vegetables, or fruits. Figure 1 provides a glimpse of non-dairy-based fermented/probiotic foods consumed worldwide. Each product confers its unique and specific health benefits depending upon the substrate. The following text discusses some of the traditional, non-dairy-based fermented foods containing huge amounts of health promoting microorganisms.

Sauerkraut (fermented cabbage or acidic cabbage) directly translated from German: "sour herb" or "sour cabbage," made by naturally fermenting finely chopped, salted (2–3% salt) cabbage. Spontaneous fermentation is carried out by natural microflora of cabbage that includes lactic acid bacteria, including *Leuconostoc*, *Lactobacillus*, and *Pediococcus* (Farnworth, 2003). These bacteria ferment the sugars present in cabbage to yield lactic acid in large proportion in addition to some other acids in minor quantities. These components altogether result in typical texture and flavor of final product. Presence of lactic acid leads to enhanced shelf life and characteristic sensory attributes to sauerkraut. Sauerkraut production involves various steps, first of all mature cabbage heads are taken, washed, and finely chopped. The quality of heads is important and they are checked for diseases, cleanliness, vitamin C, and fructose content. To these sliced cabbage, salt is mixed generally at a concentration of 2.5%, but the salt concentration may vary depending upon a number of factors like dry mass and temperature, etc. High salt concentration results in expulsion of water containing nutrients from cabbage and also raises the dry mass of cabbage. The expelled fluid (rich in sugar and growth factors) provides nutrients to fermentation bacteria and supports their growth. In addition, many of the spoilage, harmful, and pathogenic bacteria are unable to grow at high salt concentration. Therefore, it provides a conducive environment for the growth of lactic acid bacteria. The cabbage is then transferred to fermentation tanks or air-tight containers but provision must be there for escape of gases that are produced during fermentation. The optimum temperature for fermentation is about 70°F. During the initial fermentation coliform bacteria like *Enterobacter cloacae*, *K. oxytoca*, and *Klebsiella pneumoniae*

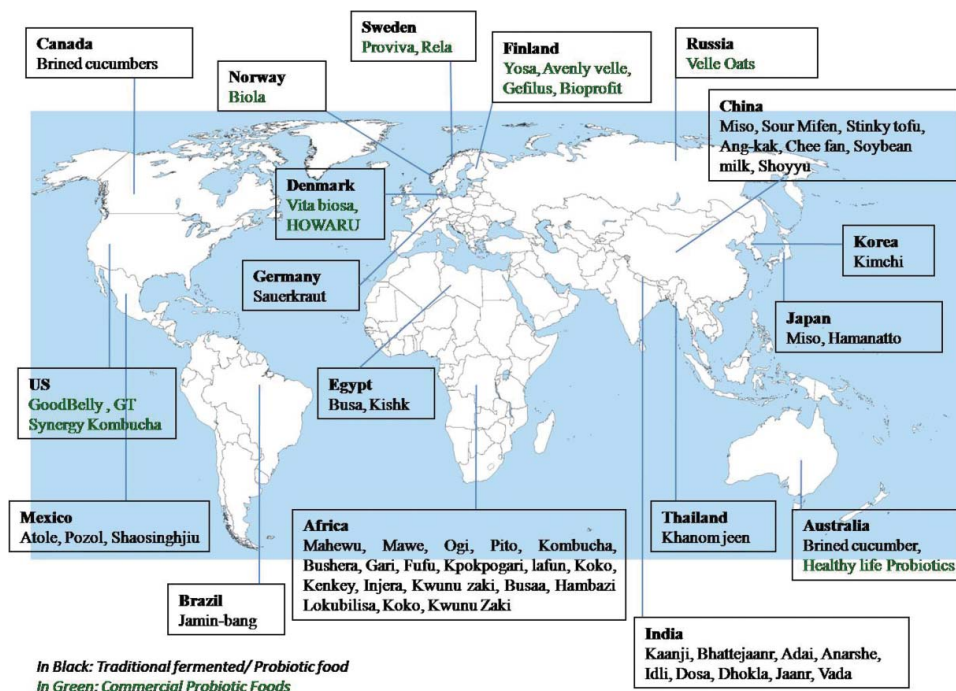


Figure 1 Different non-dairy-based fermented/probiotic foods consumed worldwide.

predominates. But once acid production is started, condition favorable for the growth of *Leuconostoc* starts building up. Fermentation is allowed to occur from 14 days to 4 months. Owing to the heterofermentative nature of *Leuconostoc*, carbon dioxide gas is also generated in addition to lactic acid production. There is continuous decline in the pH during the fermentation due to which *Lactobacillus* population develops. Beganović et al. (2011) have successfully attempted to produce improved probiotic sauerkraut by incorporating *Leuconostoc mesenteroides* LMG 7954 and *Lactobacillus plantarum* L4 probiotic cultures during controlled fermentation of cabbage. The probiotic count of the final product was $>10^6$ cfu/g.

Suan Tsai or *Suan Cai* or Chinese fermented vegetables is a popular dish or seasoning food in some regions of China. As its name describes in Chinese, it is well known for its sour flavor. Suan-tsai can be made from Chinese cabbage, cabbage, or mustard. Most of these products are still produced by spontaneous fermentation even at household level, indicating the flexibility and simplicity of handling this kind of fermented food. After washing the leaves or boiling them for sterilization, the leaves are soaked with salt in an anaerobic environment and mechanical pressure is applied to extract the liquid. Fermentation is allowed to take place for one month at room temperature to get the final product. A novel probiotic species namely *Lactobacillus harbinensis* sp. nov. was isolated from conventional “Suan cai” of China (Miyamoto et al., 2005).

Kimchi, or gimchi (kimchee/kim chee), is also prepared from vegetables and is popular in Korea. Kimchi is served as a side dish with meal and generally eaten raw. A number of different types of kimchi can be formulated keeping one major

vegetable ingredient like cucumber, radish, cabbage, or green onion. Because the major ingredient is vegetable, kimchi contains less calories, high amount of dietary fibers, and overall high nutritional value. It also contains other functional ingredients like carotene and vitamin C (which can serve around 80% of recommended daily requirement), Vitamin B1/B2, vitamin A, iron, and calcium due to its ingredients red pepper, garlic, onion, and ginger. Owing to its health attributes kimchi has been listed in world’s top five healthiest foods by magazine “Health” (Anonymous, 2008). The health benefits which have been attributed to kimchi include: antitumor activity, lipid/hypercholesterolemia reducing activity, and antioxidant activity (Cho et al., 1997; Kim and Lee, 1997; Kim et al., 2002; Kim et al., 2004). In addition to its active ingredients, it also contains beneficial lactic acid bacteria which include different strains of *L. plantarum*, *Lactobacillus sakei*, and a distinct species named as *Lactobacillus kimchii*. These lactic acid bacteria provide additional health benefits like help in digestion. Lee et al. (2011) have isolated low acid producing microorganisms from kimchi and tested their probiotic potential and functional properties. They were successful in isolating potential probiotic strains which could be utilized to develop probiotic kimchi with consistent quality.

Brined cucumber or pickled cucumber is consumed in various countries like Canada, Australia, United States, and United Kingdom. In United Kingdom, pickled cucumbers are known as gherkins (gherkin or *Cucumis anguria* is the name of a particular cucumber species whose fruit size is comparatively small, approximately 5–7 cm). Brined cucumber is prepared from cucumber in a manner somewhat similar to that of

sauerkraut fermentation. Spices commonly added during pickle fermentation include garlic, white mustard seeds, oak cherry, salt, etc. The concentration of salt varies between 2 and 4%. The salted cucumber is allowed to go through natural fermentation in non-air-tight container to make them sour. The fermentation is uncontrolled and spontaneous thus depend upon natural bacteria present on the skin surface of cucumbers. Like other vegetable products, brined cucumber possesses less calories but it is high in sodium. It contains vitamin K in low amounts.

Fermented cassava is used to produce gari, a sour farinaceous meal and fufu, a fine paste starchy food which is made into porridge, in Nigeria. Other food types prepared from fermented cassava are candi, kpokpogari and lafun (Nigeria) kumkum, myiodo and atangana bread (Cameroon), agbelima and akyeke (Ghana), tapioca (Brazil), and foofoo (Congo) (Okafor, 1977; Odunfa, 1985; Oyewole, 1990; Obilie et al., 2004). Gari is a particularly popular food product because it comes in a ready-to-eat form. The production of gari is done by spontaneous fermentation following peeling and grating of cassava. The grated pulp is filled into cloth bags and tied securely. Heavy stones are placed on the sack to press out cassava juice. The cassava is left to ferment in this position for 3–5 days, the pulp is afterward roasted on a shallow metal pot, and the final product is a dry, farinaceous cream-colored powder (Ojo and Deane, 2002). In the process of fermentation of cassava, a number of microbiological and biochemical changes takes place. During fermentation, lactic acid bacteria metabolize the starch in the cassava pulp leading to production of organic acids, such as lactic acid, that lowers the pH (Odunfa, 1985; Okafor and Ejiofor, 1990; Coulin et al., 2006). The genera of lactic acid bacteria reported during gari production in West Africa include *Lactobacillus* and *Leuconostoc* (Kostinek et al., 2005).

Stinky tofu or *stinky soybean curd* is a famous and popular traditional fermented Chinese snack. It is a tofu that has been fermented in a mix of fermented milk and a vegetable, meat-, and fish-based brine, or some combination of the three. For truly smelly tofu, the brine should be weeks or even months old. Some consumers develop an increased appetite for it despite the strong odor. Stinky brine fermentation is an alkaline fermentation process since ammonia is produced and increases the pH of the product. The microbial strains found in stinky brine vary according to the specific ingredients of the manufacturers' adaptation (Liu et al., 2011). Huang and colleagues have isolated *Lactobacillus* genus and *Bacillus* genus from the fermented brine (Huang et al., 2009). Soybean is rich in protein and contains more than 10 different amino acids. Due to the activity of microbes, proteins in the fermented stinky tofu are further decomposed. The amino acids content, especially the essential amino acids, are enriched (Ma, 1996).

Nigerian ogi (fermented maize or sorghum porridge) and *Baba* (fermented millet) staple food of Nigerian population is prepared by lactic acid fermentation. Ogi is prepared by soaking sorghum or maize in water, wet-milling, and sieving them.

The paste is allowed to sediment and kept for fermentation (Banigo et al., 1974). Traditionally, the fermentation takes place spontaneously by various microorganisms, in which *L. plantarum* and *L. confusus* are the key players (Johansson et al., 1995). The major component in sorghum and maize is starch; therefore, the microorganisms must be able to ferment starch and produce amylases. Since the fermentation occurs naturally, therefore, the quality of end product is not consistent and varies from batch to batch. It depends upon the composition of the active microflora and amount of lactic acid produced. Thus, consistent quality can be obtained by practicing controlled fermentation with known starter cultures.

Fermented rice noodle known as Sour Mifen in China and Khanom jeen in Thailand is widely consumed traditional food. These are not considered as potential probiotics since material is cooked after fermentation and as per the standard definition of probiotics, microorganisms must viable. For preparing fermented rice noodles, six months stored broken rice grains are soaked in water for about one hour and drained. They were allowed to ferment naturally for few days. After wet milling, precipitated rice flour is collected. Excess water is removed by putting the precipitated flour in cotton cloth and applying weight. Flour is then gelatinized and kneaded in warm water. The kneaded flour is extruded in hot water to make noodles. Extruded noodles are cooled immediately with water and kept for drying. In khamom jeen, fermentation is dominated by lactic acid bacteria but these lactobacilli are not consumed alive because the product is baked before consumption (Uchimura et al., 1991). Due to fermentation the lipid, protein, and ash contents are decreased whereas stringy mouth feel of the noodles is increase (Lu et al., 2003). A number of volatile compounds like 3-methylbutanoic acid, diacetyl, 2-methylpropanoic acid, etc. are synthesized due to metabolic activities of these microorganisms, which impart a characteristic flavor to khamom jeen/mifen (Keatkrai and Jirapakkul, 2010).

Sourdough bread is consumed commonly in European diet since centuries (Wahren, 1985). In addition to bread production, sourdough is being utilized for the manufacture of a range of products like cakes, crackers, etc. these days (Ottogalli et al., 1996; Foschino et al., 1999; Vogel et al., 1999; De Vuyst and Gaänzle, 2005). Sourdough fermentations are carried out by complex and diverse microflora that consist of yeasts and lactic acid bacteria mainly *Lactobacillus sanfranciscensis*. These complex fermentations impart the characteristic flavors and sensory attributes to the final bread. Horizons of sourdough utilization are still on expansion and it is finding its applicability to a range of different cereal flours in the world. About 30% of bakery products and 200 different kinds of sourdough breads in Italy utilize wheat sourdough (Ottogalli et al., 1996; INSOR, 2000). In some regions particularly southern Italy *Triticum durum* species of wheat is more commonly utilized for sourdough bread preparation whereas *Triticum aestivum*, the common species of wheat is used in several other parts of Italy (Corsetti et al., 2001). Rye cereal is extensively used in preparation of sourdough bread in various European

and Russian countries (Bushuk, 2001). Sourdough fermentation although imparts some beneficial effects but it is not considered as probiotics since microorganisms are killed during baking. A number of such fermented products are also consumed in various parts of India since ancient times like bhature made from fermented wheat flour in north India, idli and dosa made from fermented batter of rice/black lentils in south India, and dhokla from fermented batter of Bengal gram's flour in Gujarat.

Tanzanian togwa (fermented sorghum, maize, millet, or maize-sorghum beverage) is extensively consumed in Tanzania either in the form of porridge as weaning food or in diluted form as beverage. Togwa is prepared generally from sorghum, maize flour, and finger millet. In some parts rice and cassava flour is also utilized. Water is added to the cereal flour and it is cooked for 20 minutes to obtain gruel. To prepare malt flour, cereal grains are soaked in water for 12 hours, drained and allowed to germinate for three to six hours. The germinated grains are sun dried and milled to obtain malt flour. Malt flour is added to the gruel at a concentration of 5% (w/v). Inoculum is provided by adding 10% (v/v) old togwa to this mixture. In traditional method, fermentation is done by natural microflora, and therefore, the product characteristics vary. Due to uncertain product characteristics and unhygienic preparation practices togwa is less popular in high-income groups, and it is considered as food associated with poor people (Mugula et al., 2001). Some studies have been carried out on preparation of cereal gruel/porridge preparation utilizing known probiotic cultures (Kingamkono et al., 1994; Willumsen et al., 1997) or having improved stability/ characteristics (Cooke et al., 1987; Mokhorro and Jackson, 1995). Mugula et al., 2003 have isolated and characterized the bacteria from traditional togwa which includes numerous species of *Lactobacillus* among which *L. plantarum* was dominating, *Weissella confusa* and *Pediococcus pentosaceus*. Yeast species were found to be *Issatchenkia orientalis*, *Saccharomyces cerevisiae*, *Candida tropicalis*, and *Candida pelliculosa*. These isolates can further be utilized to develop starter culture in order to obtain togwa at commercial scale under hygienic conditions, having improved and consistent quality.

Ethiopian kombucha (fermented black tea or mushroom tea) is a sweetened black or green tea that is fermented with the help of yeasts and bacteria. The mass of yeast and bacteria forms a sac at the top of fermenting tea that appears like mushroom. The yeasts involved in kombucha fermentation are *S. cerevisiae*, *Torulaspora delbrueckii*, *Candida stellata*, *Schizosaccharomyces pombe*, *Brettanomyces bruxellensis*, and *Zygosaccharomyces bailii*. These yeasts convert sugar to alcohol which is further converted to acetic acid by bacteria. Kombucha is rich in vitamin B and antioxidants and commonly taken for therapeutic purposes. Although scientific evidences are unavailable in context of health benefits associated with kombucha but it is being consumed since centuries for health purposes. Kombucha can be prepared at home by using mother or so called mushroom cultures. Kombucha is also available in

the markets commercially (Table 2). Ernst (2003) has conducted an evidence based critical evaluation regarding efficacy and safety of kombucha. No clinical evidence was found in support of efficacy of kombucha. However, several case reports like metabolic acidosis, assumed liver damage, and cutaneous anthrax infections and one casualty are there which raise doubts regarding safety of kombucha. In light of these facts, kombucha cannot be recommended for medicinal purposes.

Mahewu is a fermented, nonalcoholic maize beverage/gruel and is commonly consumed by all age groups in South Africa. To prepare mahewu maize, porridge is diluted and malted sorghum/millet flour or wheat flour is added. This mixture is left for about 24 hours at ambient temperature for spontaneous fermentation. *Lactococcus* (Lact.) *lactis* subsp. *lactis* was found to be the major microorganism in traditional fermentation (Steinkraus, 1996). To enhance the flavors, various fruit flavorings can be added after the fermentation (Edwards, 2003; Schweigart and Fellingham, 1963). McMaster et al., 2005 have developed a means of introducing *Bifidobacterium lactis* as a probiotic to the rural population of South Africa, using two existing traditional fermented foods, amasi and mahewu, by adding microcapsules containing viable *B. lactis*, at or in excess of recommended daily intake levels, after fermentation and pasteurization. Traditional mahewu is produced industrially using *Lactobacillus bulgaricus* var *delbrueckii* or *Lactobacillus brevis*. Mahewu is available at supermarket shelves of South Africa under brand names like Mageu, Powerforce Mahewu Banana (Zambezi Foods, Luton), Koka Simba Mahewu (Santooz). But the quality of these commercial mahewu is found to be inferior as compared to traditional, which requires development of starter cultures capable of imparting quality attributes to these industrial mahewu (Gadaga et al., 1999).

Boza is a Bulgarian traditional cereal-based fermented beverage with a pleasant sweet-sour, bread-like taste. It is also consumed in some areas of Turkey, Albania, and Romania. Different cereals (wheat, millet, and rye) can be used for boza production, and fermentation is caused by natural mixtures of yeast and lactic acid bacteria. Interactions between microorganisms are uncontrolled during the process, which leads to variability in the product quality. Hancioglu and Karapinar (1997) reported *Leuconostoc paramesenteroides*, which was predominant (26%), *Lactobacillus sanfrancisco*, *Lactobacillus coryniformis*, *Lactobacillus fermentum*, *Lactobacillus confusus*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, and *Leuconostoc oenos* in Turkish Boza. Albeit seven LAB species were identified in different proportions in Bulgarian Boza, of which *Lactobacillus* mainly *L. plantarum* was predominating at a level of 82% and rest 18% belonged to *Leuconostoc* (Gotcheva et al., 2000; Moncheva et al., 2003). The yeast species belonged to the genus *Saccharomyces* (47%), *Candida* (33%), and *Geotrichum* (20%).

Bushera is most common traditional beverage prepared in the Western highlands of Uganda. Bushera brewed from millet and sorghum with roots, has for years been produced by

natural fermentation at the household level. It is commonly consumed by all age groups either in the form of weaning food or as a refreshing beverage. Muyanja et al., 2003 have isolated and characterized lactic acid bacteria from household bushera which mainly belonged to *Lactobacillus* (*L. plantarum*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus delbrueckii* subsp. *Delbrueckii*), *Streptococcus thermophilus* and *Enterococcus* genera. Among the high-class society, bushera has maintained its popularity. But homemade bushera is preferred due to the apprehensions over hygiene in its preparation. Sensing its market potential, particularly in the Uganda, Peter Njawuzi started commercial production of bushera. Today, courtesy of Njawuzi's company, Multiline International located in Nyendo, Masaka district, bushera has graced shelves of up-market shops and even Uganda's leading supermarkets (<http://www.newvision.co.ug>).

Pozol is a refreshing nonalcoholic beverage, consumed in Southeastern Mexico since prehispanic times as an important part of the daily diet of different ethnic groups (Ulloa et al., 1987). Pozol 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 water and drunk. The Mestizos have modified the traditional Indian procedure by adding an extra cooking step to reduce the amount of solid sediment present in the beverage when the dough is suspended in water (Wacher et al., 2000). The lactic

acid bacteria included amylolytic strains (one strain under aerobic conditions and two under anaerobic conditions out of 25 from Indian pozol; three strains under aerobic conditions and five under anaerobic conditions, out of 19 from Mestizo pozol). Their presence is of interest, as starch is a major component of nixtamal. Yeast and mould microbiota were similar in both types of dough. Several of the yeast and mould strains isolated from the doughs (*Candida guilliermondii* var. *guilliermondii*, *Cladosporium cladosporioides*, and *Geotrichum candidum*) have been reported previously to occur in pozol (Ulloa et al., 1987). Gotcheva et al., 2002 have proved the probiotic potential of strains of *L. plantarum*, *Candida rugosa*, and *Candida lambica* which were isolated from traditional cereal-based fermented beverage of Bulgaria. These strains were capable of resisting up to 2% bile concentration. These can be exploited to develop probiotic pozol.

Nontraditional Nondairy Probiotics

During recent years several attempts have been made to develop probiotic foods from varied matrices like fruits, vegetables, cereal grains (Blandino et al., 2003), oilseeds, etc., including both fermented and non-fermented products (Table 1). Few non-dairy-based probiotic products are also available in various countries (Table 2). Given below are certain examples.

Fortified fruit juices represent a good alternative delivery vehicle for probiotic cultures. Fruit juices are consumed commonly therefore it is convenient to take full benefits of

Table 1 Unconventional nondairy probiotic products

Substrate	Probiotic microorganisms	Viable cell count	Reference
Fermented banana puree	Immobilized <i>Lactobacillus acidophilus</i>	10 ⁸ CFU/mL	Tsen et al., 2009
Fermented tomato juice	<i>Latobacillus acidophilus</i> LA39, <i>Lactobacillus plantarum</i> C3, <i>Lactobacillus casei</i> A4, <i>Lactobacillus delbrueckii</i> D7	10 ⁶ –10 ⁹ CFU/g	Yoon et al., 2004
Fermented beet juice	<i>L. plantarum</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus delbrueckii</i>	10 ⁹ CFU/mL	Yoon et al., 2005
Fermented cabbage juice	<i>Lactobacillus plantarum</i> C3, <i>Lactobacillus delbrueckii</i> D7	10 ⁷ CFU/mL	Yoon et al., 2006
Probiotic-enriched dried fruits	<i>Lactobacillus casei</i> (spp. <i>rhamnosus</i>)	10 ⁷ CFU/g	Betoret et al., 2003
Probiotic table olives	<i>Lactobacillus</i> GG, <i>Lactobacillus paracasei</i>	10 ⁷ –10 ⁸ CFU/g	Lavermicocca et al., 2005
Saccharified rice yoghurt	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>	7.6 × 10 ⁷ CFU/g	Boonyaratankornkit et al., 2000
Water-based pudding	<i>Lactobacillus rhamnosus</i> GG	10 ⁸ CFU/g	Helland et al., 2005
Fermented peanut milk	<i>Bifidobacterium pseudocatenulatum</i> G4	10 ⁸ CFU/g	Kabeir et al., 2009
Probiotic Noni juice	<i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i> , <i>Bifidobacterium longum</i>	10 ⁹ CFU/mL	Wang et al., 2009
Fermented soymilk	<i>Lactobacillus acidophilus</i> (LAFTI L10 and La4962), <i>Bifidobacterium lactis</i> LAFTI B94 and longum B1536), <i>Lactobacillus casei</i> (LAFTI L26 and Lc279), <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> Lb1466, <i>Streptococcus thermophilus</i> St1342, <i>Lactococcus lactis</i> (LL3)	10 ⁸ CFU/mL	Donkor et al., 2007; Saris et al., 2003
Nonfermented frozen vegetarian dessert	<i>Lactobacillus acidophilus</i> MJLA1, <i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> Lp-01, <i>Bifidobacterium lactis</i> BDBB2, and <i>Bifidobacterium lactis</i> Bb-12	10 ⁷ cfu/g	Heenan et al., 2004
Oat-based probiotic drink	<i>Lactobacillus plantarum</i> B28; <i>Lactobacillus reuteri</i> ATCC 55730	9.3 × 10 ⁹ CFU/mL; 10 ⁸ CFU/mL	Martensson et al., 2002; Angelov et al., 2006
Probiotic mahewu/amasi	Microencapsulated <i>Bifidobacterium lactis</i>	~10 ⁹ CFU/mL	McMastera et al., 2005

Table 2 List of commercially available non-dairy-based probiotic products

Probiotic Product	Type	Probiotic microorganisms	Company	Country
GoodBelly drink	Fruit juice	<i>Lactobacillus plantarum</i> 299v	NextFoods	Colorado, US
Velle oats	Drink-type and yogurt-type	<i>Lactobacillus plantarum</i> , <i>Leuconostoc mesenteroides</i> , <i>Pediococcus cerevisiae</i> , <i>Saccharomyces cerevisiae</i> , and <i>Bacillus subtilis</i>	Velle	Russia
Proviva	Fermented fruit drink With oatmeal	<i>Lactobacillus plantarum</i> 299v	Skane dairy	Sweden
Yosa	Oat bran yoghurt	<i>Lactobacillus acidophilus</i> La-5, <i>Bifidobacterium bifidum</i> Bb-12	Bioferme Oy	Finland
Healthy life probiotics	Fruit juice	<i>Lactobacillus paracasei</i> 8700:2, <i>Lactobacillus plantarum</i> Heal9	Golden circle	Australia
HOWARU Bifido		<i>Bifidobacterium lactis</i> HN019	Danisco	Denmark
HOWARU Rhamnosus		<i>Lactobacillus rhamnosus</i> HN001	Danisco	Denmark
Avenly velle	Oat based drink	<i>Lactobacillus</i> and <i>Bifidobacterium</i>	Avenly Oy Ltd.	Finland
Biola	Fruit juice	<i>Lactobacillus rhamnosus</i> GG	Tine BA	Norway
Rela	Fruit juice	<i>Lactobacillus reuteri</i> MM53	Biogaia	Sweden
GT synergy kombucha	Black tea	–	Millennium Products Inc.	California, US
Grainfields wholegrain liquid	Grains, beans, and seeds	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus delbreukii</i> , <i>Saccharomyces boulardii</i> , <i>Saccharomyces cerevisiae</i>	AGM Foods Pvt. Ltd.	Australia
Vita biosa	Aromatic herbs and other plants	<i>L. acidophilus</i> , <i>L. casei</i> , <i>Bifidobacterium bifidum</i>	Biosa Inc.	Denmark
Gefilus	Fruit juice	<i>Lactobacillus rhamnosus</i> GG	Valio Ltd.	Finland
Bioprofit	Fruit juice	<i>Lactobacillus rhamnosus</i> GG, <i>Propionibacterium freudenreichii</i> ssp. <i>Shermanii</i> JS	Valio Ltd.	Finland

probiotics through their consumption. Besides, fruit juices are also being marketed as functional drinks after calcium and vitamin fortification and are quite popular. Typically low pH in the range of 2.5–3.7 of fruit juices poses a challenge in probiotic product development. Since most of the bacteria are sensitive to such low pH conditions. Therefore, it is necessary to choose such strains that can withstand highly acidic conditions and have acceptable viability and shelf life. In order to achieve health benefits from probiotics, the recommended concentration of viable probiotics at the time of consumption should be $>10^6$ cfu/mL (Kurmann and Rasic, 1991; Kailasapathy and Chin, 2000; Boylston et al., 2004). Several studies have been conducted to select probiotics suitable for fruit juice matrix. In one such study conducted by Shah et al., 2010 on survival of HOWARU *Lactobacillus rhamnosus* HN001, HOWARU *Bifidobacterium lactis* HN001, and *Lactobacillus paracasei* LPC 37, showed that none of the strains tested could grow in such harsh environments. The stability of probiotic strains could be improved by vigilant strain selection, mutation/selection of strains against stress, applying protective materials or encapsulation in suitable matrices. Sheehan et al., 2007 have tested the viability of *Lactobacillus salivarius* ssp. *salivarius* UCC118 and UCC500, *Lactobacillus paracasei* ssp. *paracasei* NFBC43338, *Lactobacillus rhamnosus* GG, *Lactobacillus casei* DN-114 001 and *Bifidobacterium lactis* Bb-12 in various fruit juice matrices like pineapple, orange, and cranberry juice. Survival was better in orange and pineapple juices as compared to cranberry juice. *Lactobacillus casei* DN-114 001,

Lactobacillus rhamnosus GG and *Lactobacillus paracasei* NFBC43338 were able to survive for about 12 weeks at concentration of $>10^7$ cfu/mL in pineapple juice and $>10^6$ cfu/mL in orange juice. These strains were further tested for their ability to survive pasteurization conditions, but none was found suitable. So these cultures were recommended to be used as probiotics in orange or pineapple juice matrix, but they must be added before pasteurization. In another study by Saarela et al., 2006, the stability of differently produced (variables being fermentation time, pH during drying, and cryoprotectant) freeze dried *Bifidobacterium animalis* subsp. *lactis* E-2010 (Bb-12) cells in fruit juice and low-fat milk were tested. Cells produced involving different production/processing steps had indifferent stability in milk, whereas in case of juice, sucrose-protected cells survived better than other cells. This poor viability in fruit juices can be attributed to the low pH of juices in comparison of neutral pH of milk. *Bifidobacteria* are generally more sensitive to acidic environments and have poor survival at pH values <4.6 (Boylston et al., 2004). Among these *Bifidobacterium animalis* strains were found to be more acid resistant than the strains of other *Bifidobacterium* species (Matto et al., 2004). To improve the stability of probiotic microorganism either some growth supporting substrates like oat meal, fructose, sucrose, etc. are added or the bacteria is covered in protective material like microencapsulation with alginate, etc. Most of the commercially available non dairy probiotics are based upon fruit juices (Table 2).

Sweden was the first country to launch the first probiotic functional food that does not contain milk or milk constituents in 1994 with name ProViva manufactured by Skane Dairy, Sweden. The base substrate is oatmeal gruel that is fermented by lactic acid bacteria *L. plantarum* 299v. Malted barely is added to enhance the liquefaction of the product. The final product contains 10^{12} cfu/L. This active ingredient, i.e., fermented oatmeal gruel was mixed at a concentration of 5% with various fruit drinks like rose hip, black currant, blueberry, strawberry, or tropical fruits. The consumer product contains 5×10^{10} cfu/l of *L. plantarum* 299v/L. The bacteria were found to be highly resistant to the low pH of the fruit drink (pH < 2.8–3.4), which depends on the type of fruit juice used and remained viable for ≥ 1 month under refrigerated conditions. GoodBelly, a similar product based upon oatmeal and *L. plantarum* 299v was first nondairy probiotic drink that came in US market in the year 2006.

Helland et al. (2005) have developed cereal-based puddings without milk and compared it with milk based puddings. To prepare puddings, rice flour and maize flour were taken in a ratio of 3:1. Salt and fructose were added to flour mixture. Since rice and maize do not contain much amount of fermentable sugar fructose was added. Puddings were prepared in water with or without adding a prebiotic product Litesse (Danisco Sweeteners Ltd., UK). Out of the four strains (*Lactobacillus acidophilus* La5, *Lactobacillus acidophilus* NCIMB 701748 *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* Bb12) tested only *Lactobacillus rhamnosus* GG showed good viability (8 log cfu/g) in water-based puddings. Addition of prebiotic Litesse did not show any effect on growth and metabolism of probiotic bacterial strains.

Saccharified Rice Yoghurt was developed by Wongkha-laung and Boonyaratanakornkit (2000). Saccharified rice was fermented to produce yoghurt like product. The substrate was Jasmine rice which was saccharified at 55°C with the help of amylase enzymes. Fortification of this saccharified rice was done with 3% soybean oil, 0.4% calcium lactate and 3% casein to enhance the nutritional value and taste. Rice milk obtained from this fortified saccharified rice is then inoculated with mixed cultures of *L. casei* subsp. *rhamnosus* and *Lactobacillus acidophilus*. Palatability can further be enhanced by adding pectin and strawberry. Bacterial count of yoghurt was found to be 7.6×10^7 CFU/g. This can be stored for at least 20 days at 4°C. Sensory evaluation revealed that rice-based yoghurt with strawberry was well accepted when compared with commercial strawberry yoghurt. Sensory acceptability of this rice-based yoghurt was good as compared to commercial strawberry yoghurt.

During recent years, efforts are being made on development of multifunctional foods like *synbiotics* which contains pre as well as probiotics potential simultaneously (Bielecka et al., 2002). A prebiotic can be defined as a complex oligosaccharide that is not digested by the human in the upper gastrointestinal tract but can selectively stimulate the growth of one or more species of beneficial bacteria present in the gut (Gibson

and Roberfroid, 1995). This type of products can be developed either by utilizing different cereals or supplementing the product with different oligosaccharides like fructose oligosaccharide, inulin, galactose oligosaccharide, etc. Cereals contain water-soluble fibers such as arabinoxylan and β -glucan, oligosaccharides (galacto- and fructooligosaccharides), and resistant starch, which can act as a prebiotic. In addition to prebiotic effects, these cereal constituents like starch can further be utilized for microencapsulation of probiotic bacteria, which would lead to increased survival of probiotic bacteria under adverse conditions like low pH of fruit juices and unfavorable environment of the gastrointestinal tract.

Efficacy and Safety of Probiotics

Although the commercial and research interest is on rise about utilizing the probiotic benefits, there have been uncertainties and apprehensions regarding efficacy and safety of probiotics. Their presence as mammalian intestinal microflora and established use in a diversity of traditional fermented foods worldwide is best evidence regarding their safety. A detailed list of microorganisms that have a documented and long history of safe use and are being used in various food products has been prepared by European Food and Feed Cultures Association and International Dairy Federation (www. effca.com). As per the list *Bifidobacterium*, *Lactobacillus*, and *Lactococcus* are categorized as GRAS (generally recognized as safe) because they have long history of safe use. However, concerns are emerging with use of *Enterococcus* as probiotics because many strains have been found vancomycin resistant and cause of nosocomial infections. Few other reports of infections linked with the consumption of commercial products containing *Lactobacillus rhamnosus*, *Saccharomyces* and *Bacillus* are also there, though not supported by substantial evidences (Rautio et al., 1999; Mackay et al., 1999; Hennequin et al., 2000; Spinosa et al., 2000; Oggioni et al., 1998; Richard et al., 1988). Therefore, manufacturer owes the responsibility to prove that a particular probiotic strain can be safely consumed. FAO/WHO has formulated a criterion for ensuring safety of probiotics and recommended that a minimum of following tests must be conducted to characterize the probiotic strains:

1. Determination of antibiotic resistance/susceptibility patterns
2. Deleterious metabolic and hemolytic activities
3. Assessment of side-effects and adverse incidents in humans
4. Surveillance of toxin production
5. Evaluation of infectivity in immunocompromised animal models

In most of controlled studies with probiotics performed with adults, children, and even with immunocompromised

patients, no undesirable effects have been observed (Apostolou et al., 2001; Rayes et al., 2005; Michail et al., 2006). Nevertheless, the safety of probiotic organisms is an important criterion for their use into food products and if safety information about a particular strain is unavailable it should be tested as per guidelines.

Different countries have different guidelines regarding assessment of efficacy and safety of probiotic strains, but no international regulatory consensus regarding probiotic food products is available till date. The probiotic products available in the market possess a number of health claims like effective against pathogens, anticarcinogenic, immunomodulating, blood cholesterol reducing, etc. but these are not supported by clinical evidences. These products are even not properly labeled in context to type, viable count, and shelf life of probiotic microorganisms (Weese, 2002; Hamilton-Miller and Shah 2002; Weese, 2003; Drago et al., 2004; Weese and Martin, 2011). As per the recommendations given by FAO/WHO in 2002 (FAO/WHO, 2002), following information should be mentioned on the label of probiotic products: genus, species, and strain designation, minimum viable count of each probiotic strain at the end of the shelf life, suggested serving size to deliver the effective dose of probiotics related to the health claim(s), proper storage conditions, and corporate contact details for consumer information. According to FAO/WHO guidelines, probiotic organisms used in food should be able to survive in the gastrointestinal tract under low pH conditions and resistant to bile and gastric juices. In addition, they must be able to colonize the gastrointestinal tract. A number of dairy yoghurts that contains probiotic strains are now available worldwide, and particularly in Europe, these products are

leading functional foods. In the United States, a "Live Active Culture Seal" has been commenced by National Yoghurt Association to discriminate between fermented and probiotic refrigerated/frozen yoghurt products. It is given on the products containing at least 10^7 cfu of lactic acid bacteria/g at the time of manufacture (<http://www.aboutyogurt.com/lacYogurt/>). However, these are cumulative counts of starter cultures and probiotic microorganisms thus do not reflect the true probiotic potential. There are a number of health claims associated with probiotics consumption like effective in gastrointestinal/genitourinary infections, bowel disorders and allergies, all of which upset a significant amount of the population all over the world. Nevertheless, extensive research is required to confirm these benefits. Before commercialization, an organized characterization approach should be adopted by manufacturers/scientists based upon the guidelines provided by the target country.

Future prospects

Fermented foods constitute an essential part of routine life. With the development of scientific knowledge on the microbial composition and characteristics of traditionally fermented foods new probiotic products for people who can not consume dairy probiotics due to few reasons like lactose intolerance, cholesterol content, etc. or when the milk products are hard to find, could be developed. Individual requirement (depending upon the age group) and preference for a particular food can also be a driving force for future probiotic research and development. Table 3 provides a glimpse of different food formats based upon their active constituents and health benefits in

Table 3. Potential substrates and their active constituents providing health benefits beyond probiotic potential

Active constituent	Substrates	Type of probiotic food	Health benefits in addition to probiotic potential
Carbohydrate	Dairy	Yoghurts, ice creams, fermented milk	Provides energy to body
	Cereals like wheat, rice, sorghum, maize	Porridges, beverages, gruel blended with fruit juices	
	Fruits	Fermented/nonfermented juices	
Protein	Dairy	Yoghurts, cheese	Builds and repairs muscle and other tissues
	Soybean, groundnut	Soy/groundnut yoghurts, ice-creams	
Calcium	Dairy	Cheese	Improves bone health
	Soybean	Tofu	
	Finger millet	Porridges, beverages	
Iron	Pearl millet, barnyard millet	Porridges, beverages	Prevents anemia
	Apricots, coconut	Fermented milk/dry fruits	
	Spinach, olives	Fermented vegetables	
Vitamins	Dairy	Yoghurts, ice creams, fermented milk	Required for proper functioning of various enzymes
	Fruits	Fermented/nonfermented juices	
	Vegetables	Fermented vegetables	
	Millets	Porridges, beverages	
Antioxidants	Fruits	Fermented/nonfermented juices	Neutralize free radicals, reduce risk of certain cancer
	Vegetables	Fermented vegetables	
	Millets	Porridges, beverages	
	Tea	Fermented tea	
Fibers	Oats, millets	Porridges, beverages	Reduce risk of colon cancer, lower LDL, and total cholesterol

addition to their probiotic potential, which can be explored further. The choice of suitable matrix, composition and processing of substrate, the growth capability, productivity and stability during processing/storage of the probiotic strain, the organoleptic properties, and the nutritional value of the final product are the main researchable issues. Besides, nondairy probiotic foods should conform to the accepted standards established by the regulations of target markets. Large-scale production requires constant quality throughout the batches hence mixed fermentations with high variability should be replaced by pure cultures.

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