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# **Non-Dairy Probiotic Products**

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## **Introduction**

The term probiotic was technically defined as “live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent nutrition” (FAO/WHO, 2001). This definition requires that the microorganisms must be alive and present in high numbers, generally more than  $10^9$  cells per daily ingested dose. Probiotic food products are considered as functional foods which are defined to contain health-promoting components beyond traditional nutrients and the addition of probiotic cultures is one approach in which foods could be modified to become functional.

The market for functional food is one of the fastest growing segments of the global food industry and estimated to worth a current value around US\$25 billion with a compound growth rate of 7.4%. There are a number of key drivers behind this unprecedented growth rate, including the increase in world population and changes in the demographics of that population (increase in aging population), advances in the understanding of the relationship between diet and health, and the demand for health and wellness food products from childhood to old age (Espin et al. 2007). Probiotic products have been widely promoted in the last decade for multitude of health benefits, and are well recognized by consumers as being “good for you” type products. The global market of probiotic ingredients, supplements and foods (dairy and non-dairy) reached nearly US\$23.1 billion in 2012 and is expected to reach US\$36.7 billion, with a compound growth rate of 6.2%, over the five-year period from 2013-2018. Up to 78% of current probiotic sales world-wide are mainly delivered through dairy products and the remaining 22% are mostly accounted for by non-dairy probiotic foods and beverages (bccresearch.com 2014, Hui and Özgül Evranuz 2012).

There is a growing interest in the development of non-dairy probiotic products due to issues such as lactose intolerance in many populations around the world and the unfavorable cholesterol content typically associated with fermented dairy products. The ongoing trend of vegetarianism and the requirements of cold storage environments for fermented dairy products are additional drivers for the consideration and development of non-dairy products as carriers of probiotic

agents. It is worth noting that the annual dairy consumption in Asia is considerably low ranging from 8-100 kg/capita in comparison to 251 kg in the USA, 310 kg in Australia and up to 330 kg for some European countries, thus suggesting the potential development of non-dairy probiotic products as another avenue for the growth of the functional food sector.(Sharma and Mishra 2013). Some commercial examples of non-dairy probiotic products are listed in Table 1.

Fruits, vegetables, legumes, cereal and meat have been utilized world-wide in the development of many traditional fermented food products as means of food preservation or nutritional enhancement. Additionally, plant products are characterized as being rich in minerals, vitamins, antioxidants and fibres, thus further enhancing their characteristics as candidates for the development of non-dairy probiotic products.

This chapter will provide an overview on the utilization of botanical and meat sources as substrates for probiotic growth highlighting throughout challenges and opportunities in utilizing such sources.

### **Cereal Grains and Soy Based Probiotic Fermentation**

Cereal grains and legumes are the world's largest food source, often called staple crops and constitute a major source of dietary nutrients for human and animal consumption. The global annual production of cereal grains is more than 2.3 billion metric tons and is grown on 73% of the world's total harvested area and remains the world's largest food yielding source (Serna-Saldivar 2010, Charalampopoulos et al. 2002). Although, in contrast to milk and meat, the nutritional quality of cereal grains is inferior due to essential amino acid deficiency such as lysine in cereals and methionine in legumes and the presence of antinutrients such as phytic acid and tannins, they are however considered as good substrates for the proliferation of probiotic bacteria due to their high content of non-digestible carbohydrates thereby acting as prebiotics. Lactic acid fermentation of cereals has been traditionally considered as the most

**Table 1:** Commercial examples of non-dairy probiotic products (Sharma and Mishra 2013, Gupta and Abu-Ghannam 2012, Prado et al. 2008)

Brand/Trade Name	Description	Producer
Vita Biosa <sup>(c)</sup>	Mixture of aromatic herbs and other plants, which are fermented by a combination of lactic acid bacteria and yeast cultures	Biosa, Denmark
Jovita Probiotisch <sup>(c)</sup>	Blend of cereals, fruit and probiotic yogurt	H&J Bruggen, Germany
Proviva <sup>(c)</sup>	Fermented oatmeal gruel, malted barley containing <i>Lactobacillus plantarum</i> . The first probiotic food that does not contain milk, or milk constituents (1994).	Skane Mejerier, Sweden
Provie <sup>(c)</sup>	Fruit drink containing <i>Lb. plantarum</i>	Skane Mejerier, Sweden
Rela <sup>(c)</sup>	Fruit juice containing <i>Lactobacillus reuteri MM53</i>	Biogaia, Sweden
Grainfields	dairy-free, no genetically modified ingredients and no added sugar containing <i>Lactobacillus acidophilus</i> , <i>Lactobacillus delbrueckii</i> , <i>Saccharomyces boulardii</i> and <i>Sc. cerevisiae</i>	AGM Foods Pty, Australia
Wholegrain Liquid <sup>(c)</sup>		
Soytreat <sup>(c)</sup>	Kefir type product with six probiotics	Lifeway, USA
SOYosa <sup>(c)</sup>	Range of products based on soy and oats and includes a refreshing drink	Bioferme, Finland
Yosa <sup>(c)</sup>	Oat product flavoured with natural fruits and berries containing probiotic bacteria ( <i>Lb. acidophilus</i> , <i>Bifidobacterium lactis</i> )	Bioferme, Finland
Gefilus <sup>(c)</sup>	Fruit drinks containing <i>Lactobacillus rhamnosus</i> , <i>Propionibacterium freudenreichii</i>	Valio Ltd , Finland
Proflora <sup>(c)</sup>	Freeze -dried product containing <i>Lb. acidophilus</i> , <i>Lb. delbrueckii</i> , <i>Streptococcus thermophilus</i> and <i>Bifidobacterium</i>	Chefaro, Belgium
Bactisubtil <sup>(c)</sup>	Freeze -dried product containing <i>Bacillus sp.strain IP5832</i>	Synthelabo, Belgium
Snack Fibrá <sup>(c)</sup>	Snacks and bars with natural fibers and extra minerals and vitamins	Celigi' ueta, Spain
Bififlor <sup>(c)</sup>	Freeze -dried product containing <i>Lb. acidophilus</i> , <i>Lb. rhamnosus</i> , <i>Bifidobacterium</i>	Eko -Bio, The Netherlands
Hardaliye <sup>(c)</sup>	from the natural fermentation of the red grape, or grape juice with the addition of the crushed mustard seeds and benzoic acid	CDS Agro Ltd, Turkey
Biola <sup>(c)</sup>	Probiotic juice drinks containing <i>Lb. rhamnosus GG</i> , available in orange–mango and apple–pear flavors	Tine BA, Norway
GoodBelly <sup>(c)</sup>	Organic fruit juice-based probiotic beverage contains a patented <i>Lb. plantarum</i> 299v culture	Nextfoods, UK

simple and economical way for improving their nutritional value, shelf life, safety, sensory properties, and functional qualities (Charalampopoulos et al. 2002). Lactic acid bacteria (LAB) fermentation of cereals has been reported to enhance the degradation of phytase and release minerals such as manganese, iron, zinc and calcium that supports the growth of probiotic bacteria (Blandino et al. 2003). Additionally, fermentation of cereal grains by LAB cultures not only enhances the bio-availability of essential vitamins and minerals, but also reduces the level of non-digestible carbohydrates typically associated with cereals (Soccol et al. 2010).

Cereal grains are rich in native prebiotics like non-digestible carbohydrates which enhance acid and bile tolerance levels, resulting in better survival and protection of probiotic bacteria in the extreme environment of the gastrointestinal tract (Lamsal and Faubion 2009, Patel et al. 2004). Additionally, due to the presence of non-digestible carbohydrates, cereal grains can also act as synbiotics (exhibiting probiotic and prebiotic properties) which selectively stimulate the growth of probiotic bacteria in the colon (Charalampopoulos et al. 2002).

A multitude of non-dairy traditional fermented cereals products have been created throughout history for human nutrition worldwide, but only recently the probiotic activity of such products has been investigated (Rivera-Espinoza and Gallardo-Navarro 2010). Some of the interesting characteristics of the probiotic strains isolated from fermented cereal products is their ability to survive bile toxicity during their passage into the gastrointestinal system. Due to milk shortages in many parts of the world, cereals constitute the principal raw material for the development of multitude of non-dairy probiotic beverages, gruels and porridge. *Idli* (stem cooked fermented paste) and *Dosa* (fermented paste fried as pan-cake) are the most popular fermented foods in India made from black gram and rice respectively. Some of the LAB found to be responsible for the fermentation process are *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lactobacillus lactis* and *Pediococcus cerevisiae* (Blandinao et al. 2003).

Prado et al. (2008) provided a comprehensive review on fermented cereal beverages from around the world. For example *Boza* is a very popular colloid beverage consumed in Bulgaria, Albania,

Turkey and Romania and made from wheat, rye, millet, maize and other cereals mixed with sugar. It mainly consists of yeast and lactic acid bacteria in an average LAB/yeast ratio of 2:4. *Mahewu* is a sour beverage made from corn meal and is common in Africa and some Arabian Gulf countries where a maize porridge, sorghum, millet, malt are added for spontaneous fermentation by the natural flora, predominantly by *Lb. lactis*, at ambient temperature. *Pozol* is a beverage made from fermented corn dough consumed mainly in Mexico.

Charalampopoulos et al. (2002) identified a number of parameters that could influence the growth of probiotic strains on cereals such as: the composition and processing of cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage and the nutritional value of the final product. Variations in the capacity of different cereals, or their mixtures, to support the growth of probiotics have been reported, for example malt medium support a wide range of LAB bacteria including *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Lactobacillus reuteri* better than barley and wheat media. Also wheat and barley extracts exhibited a significant protective effect on the viability of *Lb. plantarum*, *Lb. acidophilus* and *Lb. reuteri* under a pH of 2.5 (Charalampopoulos 2002).

In a recent study by Rathore et al. (2012), malt, barley and a mixture of both was utilized as a substrate for the fermentation with *Lb. plantarum* (NCIMB 8826) and *Lb. acidophilus* (NCIMB 8821). The study reported increased growth in the malt substrate resulting in significant amounts of lactic acid production (0.5–3.5 g/L) along with 7.9 to 8.5 log CFU/mL cell populations being reached within 6 h of fermentation. The study concluded that the mixing of microorganism strains could give rise to the production of more flavour attributes thus offering potential opportunities for the developments of novel probiotic beverages from cereals.

Survival, functionality and competition with other microorganisms are a number of challenges for the probiotic strain in any non-dairy substrate. In cereal-based probiotics, yeasts which could naturally be present on dried cereals or could be added deliberately to the fermentation process

could interact with the growth potential of the probiotic strains. The co-cultured organism may compete for growth nutrients or could produce metabolic products with inhibitory effects. Kedia et al. (2007) reported that the introduction of yeast in malt-based substrate fermentation had increased the growth of *Lb. reuteri* as compared to the pure LAB culture as yeast may produce vitamins that enhance the growth of LAB.

Probiotic cultures may require some growth promoters to initiate growth in cereals as reported by Helland et al. (2004). In these two studies, a malted brown rice media (cereal porridge) and cereal pudding of rice and maize were fermented with *Bifidobacterium longum* BB536 and *Bifidobacterium animalis* Bb12, respectively and the results obtained showed that the growth of Bifidobacterium is not possible unless the substrate was enriched with a growth promoter like milk. Helland et al. (2004) fermented a maize porridge made of a mixture of maize flour and barley malt with various probiotic strains and suggested that maize porridge supplemented with barely malt is a better medium for probiotic growth.

Oat-based substrates have been utilized successfully in probiotic fermentations due to their compositional structure such as the presence of special mixed links (1→3) and (1→4) β-glucan, arabinoxylans and cellulose dietary fibers along with comparatively high level of unsaturated lipids, proteins, vitamins and phenolic antioxidants (Awaisheh 2012). Whole-grain oat was fermented with *Lb. plantarum* to obtain a fermented drink that combined the health benefits of a probiotic culture with the oat prebiotic β-glucan and maintained a viable cell count of 10 log CFU/mL after 24 d of refrigerated storage at 4-6°C higher than that required for considerations as a probiotic drink (Angelov et al. 2006). Gupta et al. (2010) reported negligible changes in the β-glucan level after 21 d of refrigerated storage of a *Lb. plantarum* oat-based fermented drink. Oat-bran breakfast cereals were found to support the growth of *Lb. plantarum* within a moisture content of 50-58% indicating potential development of probiotic breakfast cereals (Patel et al. 2004). LAB fermentation of oat-based substrates combines the effects of β-glucan for cholesterol reduction and the effect of LAB benefits to maintain and improve the intestinal balance.

Soybean is one of the most important legumes in the traditional Asian diet and considered to be rich in high quality protein and oligosaccharides. However, wider utilization of soybeans has been limited by its typical beany flavour and the presence of raffinose and stachyose oligosaccharides which are not easily digested by the human digestive system (Socol et al. 2010). Traditionally, fermentation has been the most suitable option to increase the digestibility of soy products and to reduce their unacceptable off-flavour (Han et al. 2001). For example, a variety of fermented soybean based foods such as Turangbai, Hawaijar, Aakhuni, Bekangum, Pruyaan and Kinema have been produced and consumed by the ethnic people of North-Eastern India, Nepal and Bhutan (Tamang 2005). The utilization of probiotic bacteria was reported to enhance the level of free isoflavones in soy products, reduce the sugars that cause flatulence and also the level of off-flavour n-hexanal and pentanal organic compounds which are typically associated with the “beany” flavour of soy products (Champagne et al. 2005). Soymilk is considered as a good substrate for supporting the survival of a range of probiotic strains including *Lb. rhamnosus*, *Lb. reuteri*, *Lb. acidophilus* and *Lb. fermentum* possibly attributed to the oligosaccharides of soybeans acting as carbon sources (Wang et al. 2006). Several studies indicated that soy based products such as frozen soy desert, soy tempeh, soy yogurt, soy beverage and soy cheese worked as good substrates for the growth of probiotic bacteria showing total viable cell count as high as 8-9 log CFU/mL (Rivera-Espinoza and Gallardo-Navarro 2010, Wang et al. 2006). In particular *Lb. rhamnosus* strain seems to withstand a range of processing conditions of soybeans, suggesting its potential in the development of soy-based probiotic products.

Beans contain phenolic compounds that exhibit antioxidant activity with possible benefits to human health. LAB of soyfoods shows that this activity gets remarkably stronger in comparison to un-fermented beans (Wu and Chou 2009). Combining the health benefits of probiotic fermentation and the increased antioxidant capacity places soy-based products as a strong candidate for the development of non-dairy based probiotic products.

### **Fruits and Vegetables Based Probiotic Fermentation**

Fruits and vegetables could also be considered possible substrates for probiotic fermentation, being rich in several beneficial bioactive ingredients such as vitamins, dietary fibres and minerals that could support the growth of probiotic strains (Rößle et al. 2010). In the process of developing such products, fruits and vegetables are typically minimally processed (such as peeling and shredding) which results in the release of nutrients from the cellular component thus making a favourable environment for the growth of probiotic microorganisms without compromising much of the bioactive content of fruits and vegetables (Oliveira et al. 2011, Soccol et al. 2010).

The structural composition of fruits and vegetable is typically characterized as having wide intercellular spaces thus allowing an easier entry and colonization by probiotic bacteria. In addition, fruits and vegetables are considered rich in indigestible cellulosic fiber thus providing a protective shield for probiotic bacteria to sustain their probiotic integrity while passing through the gastrointestinal tract resulting in augmented benefits for the host (Kourkoutas et al. 2006). In some cases, the rate of survival of probiotic bacteria in fruit and vegetable-based substrates was better than that observed in dairy-based probiotic products, with the additional advantage of lacking certain undesirable components associated with dairy products such as lactose and cholesterol (Prado et al. 2008).

However, it should be noted that the lack of certain essential amino acids and vitamins in some types of fruits and vegetables and their acidic nature could limit the growth of probiotic bacteria (Saad et al. 2011). The probiotic cell viability has been reported to depend upon the strains used, the characteristics of the substrate, oxygen content and final acidity of the product (Shah 2001). Based on the acid resistance capacity of probiotic strains, Sheehan et al. (2007) reported that *Lactobacillus* and *Bifidobacterium* strains survived for longer in orange and pineapple juice compared to cranberry juice. The same study reported that *Lb. casei*, *Lb. rhamnosus* and *Lactobacillus paracasei* displayed great robustness surviving at levels above 7 log CFU/mL in orange juice and above 6 log CFU/mL in pineapple juice for at least 12 wk. However, as juices are generally submitted to a thermal pasteurization step to enhance their safety and quality, this will subsequently reduce the viability of the probiotic bacteria when incorporated in such juices.

This was demonstrated in Sheehan et al. (2007) study where a combination of thermal pasteurisation at 76°C for 30 s, combined with high-pressure treatment of 400 MPa for 5 min showed that *Lb. casei*, *Lb. rhamnosus* and *Lb. paracasei* were not capable of withstanding the treatments required to achieve a stable juice at levels > 6 log CFU/mL. Shah (2007) reported that the optimum probiotic growth temperature is between 35-40°C, and the best pH is between 6.4 and 4.5, ceasing when a pH of 4.0-3.6 is reached. A number of suggested solutions to provide barriers against unfavourable conditions during probiotic fermentation included the immobilization of probiotic strains on agar, polyacrylamide, calcium pectate gel, chemically modified chitosan beads and alginates (Kourkoutas et al. 2005). Fruit juices have the added benefit of their sucrose content as demonstrated by Saarela et al. (2006) where sucrose-protected cells were reported to survive better than reconstituted skim-milk protected cells. Additionally, some of the techniques practiced to enhance the viability of probiotic bacteria include microencapsulation and vacuum impregnation. The various approaches described earlier were designed with the aim of not only protecting the probiotic bacteria from the extreme external processing conditions or the internal environment of the digestive system but also to increase their viability in the finished products (Prado et al. 2008, Yoon et al. 2006, Nedovic et al. 2011).

Nutrient supplementation was reported to be an important factor for initiating probiotic growth in some fruit and vegetable based substrates. In particular enriching with brewer's yeast autolysate prior to fermentation resulted in better survival of probiotic cultures in the typically low pH environment of fruits and vegetables. Rakin et al. (2007), reported that *Lb. plantarum* and *Lb. delbrueckii* were capable of surviving the low pH of beetroot juice that was supplemented with brewer's yeast autolysate, remaining at 5-7 log CFU/mL after 4 wk of storage at 4°C. *Lb. casei*, recognised for its low tolerance for low pH environments, lost cell viability completely after 2 wk.

The application of a heat treatment to the vegetable or fruit substrate prior to fermentation could enhance the growth rate and the viability of probiotic bacteria. For example, Yoon et al. (2005) reported that *Lb. plantarum*, *Lb. casei* and *Lb. delbrueckii* grew rapidly on sterilized cabbage

juice without nutrient supplementation and reached 8 log CFU/mL after 48 h of fermentation at 30°C. *Lb. plantarum* and *Lb. delbrueckii* produced significantly more titratable acidity than *Lb. casei*, suggesting that *Lb. casei* requires some essential growth nutrients that are deficient in cabbage juice. Jaiswal et al. (2012) studied the effects of blanching York cabbage at 95°C for 12 min as a means to inactivate surface microflora, reduce microbial competition, prior to fermentation with *Lb. plantarum*. The study showed that a growth of 9 log CFU/mL was attained after 36 h of fermentation and was sustained for a storage period of 15 d at 4°C. This study highlighted that the application of a relatively mild heat treatment to fruits and vegetables prior to fermentation could significantly improve the chances of probiotic growth and survival without compromising the nutritional content of such products. Jaiswal et al. (2012) study also highlighted that probiotic fermentation retained most of the polyphenolic content and the antioxidant activity of York cabbage unlike other processing methods known to reduce and negatively influence such health related properties. Similar approaches should be relevant to other types of fruits and vegetables where the initial heat treatment could be optimized to maximize growth and viability of probiotic strains.

Apart from pH reduction and lactic acid production, probiotic fermentation of plant-based products have the potential for enhancing the prebiotic characteristics of the developed product, thus further enhancing the overall health benefits. Vergara et al. (2010) fermented cashew apple juice to produce prebiotic oligosaccharides. The prebiotic effect of cashew apple juice fermented with *Lactobacillus mesenteroides* was demonstrated by the better growth of *Lactobacillus johnsonii* in fermented cashew apple juice when compared to the observed growth in culture media containing only glucose and fructose as the carbon source.

A number of innovative vegetable and fruit-based fermented products have been recently investigated. di Cagno et al. (2011) developed a protocol for the manufacture of fermented smoothies, in which white grape juice and aloe vera extract were mixed with red fruits (cherries, blackberries, prunes and tomatoes) or green vegetables (fennels, spinach, papaya and kiwi) and were fermented using a mixed culture of *Lb. plantarum*, *Weissella cibaria* and *Lactobacillus*

*pentosus* strains. The previous study reported an enhancement in the antioxidant capacity and the sensory properties of the developed product.

The sensory properties of probiotic based products are an essential component into the success of such products and it is worth noting that not all plant-based products would show an enhancement in their sensory properties upon probiotic fermentation. For example the sensory impact study by Luckow and Delahunty (2004) showed that consumers prefer the sensory characteristics of conventional orange juices to those containing probiotics, but if the associated health benefits were provided, then consumer preference increases over the conventional orange juices. The same study suggested the addition of tropical fruits in percentages not exceeding 10% (v/v) to mask perceptible off-flavours of probiotics when incorporated in juices.

Overall, reports have been indicating that tomato, carrot, cabbage, artichokes and red beet juices were proven to be particularly suitable for probiotic fermentation, allowing rapid growth of the strains in addition to the production of a viable cell population above 8 log CFU/mL (Rivera-Espinoza and Gallardo-Navarro 2010). Probiotic growth can be further enhanced by the incorporation of essential nutrients.

Daily intake of fruits and vegetables is estimated to be lower than the doses (400 g) recommended by the World Health Organization (WHO), and the Food and Agriculture Organization (FAO). Fruits and vegetables can either be consumed as fresh or industrially processed. Minimal processing, while attractive to consumers due to freshness attributes and high sensory properties, have short shelf life due to rapid microbial growth. Other methods of preservation such as canning or pasteurization do enhance the shelf-life of fruits and vegetables but can result in alterations in the physical and chemical properties of such products. The consumer trend towards fresh-like, health-promoting and rich flavour ready-to-eat or drink foods and beverages is increasing. Lactic acid fermentation presents one of the most suitable approaches for increasing daily consumption of fresh-like vegetables and fruits (di Cagno et al. 2011). Probiotic juices have become the most studied and suitable substrate for the growth and viability of probiotic strains. Due to their taste,

flavour and nutrient content, probiotic juices have the potential to be widely accepted and consumed by a wide range of consumers.

### **Meats and Seafood Based Probiotic Fermentation**

Meat and seafood based products are considered as basic sources of high quality proteins and amino acids, numerous minerals and a good source of vitamins such as A, D, E and B complex (Kołożyn-Krajewska and Dolatowski 2012). Due to its composition and structure, meat serves as an excellent medium for probiotic growth and has been reported to protect the probiotic bacteria against bile during the passage through the GI tract (Khan et al. 2011).

The preservation of meat by LAB fermentation is an ancient practice that provides considerably stable meat products with acceptable quality and sensory characteristics. Meat fermentation typically relies on native lactic acid bacteria that are usually present at low numbers on raw meat surfaces prior to fermentation. LAB fermentation is known for favourable modifications in flavour and texture in addition to substantial improvements in the product shelf-life and consumer convenience (Xu et al. 2008, Tu et al. 2010). In particular, dry fermented type sausages, without thermal processing, lend themselves to be appropriate candidates for the development of probiotic meat-based products. Such products tend to acquire their characteristics fermentation flavours earlier on during the preparation stage, so that the later incorporation or addition of probiotic cultures provides better chances for survival without altering the sensorial properties of the final product. As part of its manufacturing process, dry fermented sausages contain high numbers of LAB, but they are not regarded as probiotics (Leroy et al. 2006).

Typical LAB strains commonly utilized in meat starter cultures to develop the taste, flavour and texture, associated with fermented meats, include *Lb. casei*, *Lb. plantarum*, *Lb. sakei*, *Lactobacillus pentosus* and *Pediococcus acidilactici*. The inclusion of functional starter cultures in fermented meats such as *LB. reuteri* and *B. longum* could offer further benefits in addition to those offered by classical processing starter cultures. This approach could open up a new range of meat products characterized as being healthier and without compromising their traditional

sensory characteristics (Amor and Mayo 2007). Research has highlighted so far that probiotic organisms tend to survive poorly in fermented foods in general, and encapsulation has been suggested as a mean for protection especially in high acidic environments that typically characterize acid foods. In the case of fermented meats, the meat and fat matrix could provide a natural “encapsulate” for probiotic organisms, thus rendering meat products as better candidates to support probiotic organisms than fruits, vegetables and cereal products. Muthukumarasamy and Holley (2007) reported that encapsulation could reduce the inhibitory action of probiotic organisms against pathogenic bacteria. Accordingly, products intended for probiotic inclusion should be of a very good microbiological quality in order to realize the full health potential of probiotic organisms. The suitability of probiotic cultures will have to be verified for each individual sausage type with the emphasis on using dominant strains of probiotics due to their adaptation to the meat environment. For example, *Lb. plantarum* and *Lb. pentosus*, which are naturally present in Scandinavian-type fermented sausage, were considered as appropriate candidates for probiotic meat starter cultures (Klingberg et al. 2005).

Despite the popularity of fermented meat products, the commercial production of probiotic meat products is still not common and only very few manufacturers offer fermented meat products enriched with probiotics (Kołożyn-Krajewska and Dolatowski 2012). Germans and Japanese producers were the pioneers in incorporating probiotic bacteria into meat products launching for example a probiotic salami (fermented with intestinal *Lb. acidophilus*, *Lb. casei*, and *Bifidobacterium* spp.) and probiotic meat spread (fermented with *Lb. rhamnosus* FERM P-15120) (Arihara 2006). Currently, probiotic meat products are still a relatively new product concept for the meat industry despite a high demand by health conscious consumers. Meat products such as dry fermented sausages, ham or lion have the advantage of being considered as an appropriate substrate for probiotic growth as their production usually requires slight or no heat processing, thus providing the appropriate conditions essential for the survival of probiotic cultures (Ammor and Mayo 2007). However, because of the mild processing conditions these products are subjected to, other indigenous meat micro-flora could grow on meat surfaces and

subsequently competing with the potential survival and growth of probiotic bacteria on such products and the overall progress of the fermentation process. Accordingly, there is a need for the selection of appropriate probiotic strains with a high viability when incorporated in fermented meat matrixes (Tu et al. 2010). Starter cultures are modified to withstand the anaerobic atmosphere, high salt concentrations, low temperatures and low pH that prevail in fermented meat products. Immobilization of probiotic cultures in order to enhance their chances of survival and also to withstand certain heat treatments that meat is typically exposed to has been reported. Kanellaki and Kourkoutas (2013) studied the effect of immobilization on the cell viability of *Lb. casei* ATCC 393 during probiotic fermentation of dry-fermented sausages. The sausages were fermented with free or immobilized *Lb. casei* ATCC 393 bacteria on wheat, and after 66 d of ripening it was observed that the viable cell counts in sausages produced with immobilized culture were higher than that required for the characterization as a probiotic product. The results of the previous study also indicated that the same product had an improved profile of aroma-related compounds with a total of 124 volatile compounds including esters, organic acids and carbonyl compounds being identified.

The incorporation of probiotic cultures in other types of meat products is receiving an increasing interest. In this case, raw meat products are now believed to be an appropriate matrix for the growth of probiotic microorganisms. For instance, Neffe-Skocińska et al. (2011) studied the impact of probiotic culture incorporation in pork sirloin meat upon storage at different temperatures (16, 20, and 24°C) and a sensory evaluation was conducted after the completion of an aging period of 21 d. During this aging period, the viable counts of probiotic bacteria were at their highest (7-8 log CFU/g) at 20°C while the lowest viable counts (4-6 log CFU/g) were recorded at 16°C. The results concluded that the probiotic pork sirloins meat product, stored at 20°C with 21 d aging period were considered to be the higher quality product from a microbiological and sensorial perspective.

In the case of seafood, the application of probiotic bacteria is only limited to increase the shelf life with little or no impact on the final sensory characteristics of the product. In contrast to meat,

LAB are usually not considered as natives of fish or seafood, but certain strains of *Lactobacillus* and *Lactococcus*, have been found to be associated with fishery products (Ghanbari et al. 2013). The antimicrobial compounds released by LAB such as lactic acid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), diacetyl (2,3-butanedione) and bacteriocins are basically responsible for killing the harmful bacteria and extending the shelf-life of seafood (Nes 2011). For example, Fall et al. (2010) inhibited the growth of *Brochothrix thermosphacta* bacteria in cooked shrimp and improved its sensory properties by using the probiotic strain *Lactococcus piscium* CNCM I-4031. The incorporation of probiotic strains of lactic acid bacteria in meat products will open up new opportunities for manufactures and consumers and could alleviate some of the health concerns typically associated with meat consumption.

### **New Possible Sources for Probiotic Fermentation**

Several plant food processors produce high levels of agro-industrial by-products globally. Disposal of this waste incurs considerable costs to processors and represents a significant environmental hazard. However there is a growing awareness that this material could represent a valuable resource in the development of value-added food products. Brewers' spent grain (BSG) is a classical example of an agro-industrial waste resulting from brewing, where millions of tons of this waste material are produced annually and typically ending up either in animal feed or in landfills with very little application for human consumption. Due to the presence of polysaccharides and proteins, BSG has been used as a substitute to expensive carbon sources for industrial production of lactic acid. Production of 4 g/L lactic acid was reported by *Lb. delbrueckii* using BSG (Mussatto et al. 2007). Interest in the addition of BSG as a means to enhance the quality of food products for human consumption has increased due to its high content of oligosaccharides and phenolic compounds. In a study reported by Gupta et al. (2013) BSG was utilized in the development of a fermented nutraceutical liquid product using *Lb. plantarum* ATCC 8014 in a 7L bioreactor. BSG in water was autoclaved at 121°C for 1 min in order to release nutrients and breakdown its lignocellulosic material. A growth of 10 log CFU/mL was reported after 19 h of fermentation and was maintained for a storage period of 30 d at 4°C. *Lb.*

*plantarum* of BSG additionally resulted in the release of sugars, antioxidants and phenolic compounds in the broth medium. These attributes opens up new opportunities and possibilities for the development of new probiotic beverages that utilizes agro-industrial by-products which would be both economically and environmentally attractive and viable.

Marine underutilized resources, mainly algal materials were also investigated as possible substrate candidates in the development of probiotic products, as virtually most of the research has been limited to terrestrial plants. Gupta et al. (2011) utilized *Lb. plantarum* in the fermentation of three species of edible Irish brown seaweeds; *Himanthalia elongata*, *Laminaria digitata* and *Laminaria saccharina*. Heat treatment at 95°C for 15 min considerably enhanced the growth of *Lb. plantarum* due to sugar release as compared to non-heat-treated seaweeds. In particular, the *Laminaria* species is rich in laminaran polysaccharides which can be fermented by LAB. A maximum 10 log CFU/mL was achieved at the end of 16-24 h of fermentation for *L. digitata* and *L. saccharina*. Gupta et al. (2011) study indicated the fermentative capability of seaweeds as a sole source of nutrition for the growth of *Lb. plantarum*. Seaweeds are a vast untapped resource of nutraceuticals and its potential incorporation in the development of probiotic plant-based products merits the importance of further needed research.

Table 2 illustrates a list of non-dairy probiotic products recently explored from cereals, legumes, soy, fruits, vegetables, meat and other possible sources.

**Table 2:** Potential non-dairy probiotic fermented products recently developed.

Products	Probiotic culture	Substrate/Matrix	Viability of the probiotics in the product		References
			Abundance	Storage time	
<b><i>Cereal grains and soy based</i></b>					
Beverage	<i>Lb. plantarum</i>	Rice, barley, oats, wheat, soy flour and red grape juice	8.4 log CFU/g	30 d	Coda et al. (2012)
Beverages	<i>Lb. plantarum</i> <i>Lb. acidophilus</i>	Malt, barley and a mixture of both	Above 8 log CFU/mL	6 h	Rathore et al. (2012)
Soybean bar	<i>Lb. acidophilus</i>	Soybean	Above 8 log CFU/g	8 wk	Chen and Mustapha 2012
Oat based drink	<i>Lb. plantarum</i>	Oat	Above 10 log CFU/mL	21 d	Gupta et al. (2010)
<b><i>Fruit and vegetable based</i></b>					
Pineapple beverage	<i>Lb. casei</i>	Pineapple juice	6.03 log CFU/mL	42 d	Costa 2013
Vegetable drinks	<i>Lb. acidophilus</i> <i>Lb. plantarum</i>	Bitter gourd, bottle gourd and carrot	8 log CFU/mL	72 h	Sharma and Mishra 2013
Carrot juice	LAB	Pasteurized carrot juice	9-10 log CFU/mL	4 wk	Tamminen et al. (2013)
Snack product	<i>Lb. acidophilus</i>	Apple, mandarin and pineapple grape juice	Above 7 log CFU/g	3-15 d	Betoret et al. (2012)
Apple beverage	<i>Lb. casei</i> <i>Lb. acidophilus</i>	Fuji and Gala apples	Above 7.6 log CFU/mL	28 d	de Souza Luciana Neves Ellendersen et al. (2012)
Cantaloupe beverage	<i>Lb. casei</i>	Cantaloupe juice	8.3 log CFU/mL	42 d	Fonteles et al. (2012)
Table olives	<i>Lb. pentosus</i>	Olives	6 log CFU/ mL	7-14 d	Aponte et al. (2012)
Pear Juice	<i>Lb. acidophilus</i>	Pear	6 to 7 log CFU/mL	72 h	Ankolekar et al. (2012)
Beverage	<i>Lb. plantarum</i> <i>Lb. paracasei</i> <i>Lb. fermentum</i>	Shalgam (black carrot, turnip)	6 to 8 log CFU/mL	10 d	Tanguler and Erten 2012
Probiotic products	<i>Lb. plantarum</i>	York cabbage	10.3 log CFU/mL	15 d	Jaiswal et al. (2012)

Products	Probiotic culture	Substrate/Matrix	Viability of the probiotics in the product		References
			Abundance	Storage time	
<b><i>Meat and seafood based</i></b>					
Dry-fermented sausages	<i>Lb. casei</i>	Dry-sausages	8 log CFU/g	6 mon	Wójciak et al. (2012)
Dry-fermented sausages	<i>Lb. fermentum</i> <i>P. acidilactici</i>	Iberian dry-sausages	Above 7 log CFU/g	50 d	Ruiz-Moyano et al. (2011)
Meat product	<i>Lb. casei</i>	Pork sirloins	7 to 8 log CFU/g	21 d	Neffe-Skocińska et al. (2011)
Som-fug	LAB	Fish	Above 8 log CFU/g	15 d	Riebroy et al. (2007)
<b><i>Others sources</i></b>					
Nutraceutical drink	<i>Lb. plantarum</i>	Brewers' spent grain	10.4 log CFU/mL	30 d	Gupta et al. (2013)
Seaweed beverage	<i>Lb. plantarum</i>	Brown seaweed	10 log CFU/mL	72 h	Gupta et al. (2011)

## **Enhancing the Viability of Probiotics in Plant Based-Products**

International standards stipulate that fermented products claiming health benefits should contain a minimum of  $10^7$  viable probiotic bacteria per gram of the product when sold (Shah 2001). Probiotic viability could be defined as surviving environmental and processing conditions and reaching the site of action and producing a beneficial health effect to the host (De Vos et al. 2010). Generally, probiotic bacteria suffer from poor survival in probiotic products mainly attributed to nutrients composition, low pH conditions and the type of the probiotic strain utilized. These factors have to a greater extent been controlled and optimized so survival rates of probiotics could be enhanced during the storage of such products. In fact, survival of probiotics in the extreme acidic pH of the human gastro-intestinal system is the actual challenge for developing probiotic products generally, and particularly plant-based probiotic products which tend to be more on the acidic side thus limiting the types of probiotic strains that could be utilized to acid-resistant types, and potentially losing out on strains that could contribute better to flavour and texture or could confer more health benefits to the host. Probiotic encapsulation, or providing probiotic living cells with a physical barrier, has been proposed as an efficient technology to improve viability during long-term storage and to preserve the metabolic activity in the gastrointestinal tract (Zuidam and Nedovic 2010). Encapsulation is defined as a process that entraps a substance into another substance, producing particles in the nanometer (nanoencapsulation), micrometer (microencapsulation) or millimetre scale (Burgain et al. 2011). Carrier material should isolate and protect bacterial cells from the effects of hostile environments, be safe for consumption with Generally Recognised As Safe (GRAS) status and cost effective in order to minimize the influence on the final product. Low cost carrier materials include starches, inulin, pectin and most carbohydrates (De Vos et al. 2010). Food-grade polymers such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatine and pectin have been widely used for various microencapsulation techniques.

Currently there is a range of well-established microencapsulation technologies for the protection of probiotic bacteria (Burgain et al. 2011). The selection of an encapsulation method depends on

a number of factors such as the required particle size, physical and chemical properties of the carrier material, the applications of the encapsulated material, and the required release mechanism and cost. Recently, research is directed towards the development of carrier materials that offer multiple delivery and other benefits, in addition to protecting probiotic bacteria, including functional, nutraceutical and prebiotic properties. Nanoencapsulation has the potential to provide delivery of probiotic bacteria to certain parts of the gastro-intestinal tract where they could interact with specific receptors to maximize the delivery of their health capacity. *In vivo* studies are required using human subjects to confirm the efficacy of encapsulation in delivering probiotic bacteria and their controlled release in the gastro-intestinal system.

### **Health Claims of Probiotic Products**

The European Food Safety Authority (EFSA) has not released a favourable opinion in relation to live organisms other than for live cultures in yoghurt, which were shown to improve the digestion of lactose in yoghurt in individuals with lactose maldigestion. The unfavourable opinion of EFSA was mainly because microorganisms were not properly characterized in the health claims submitted or due to the poor evidence of beneficial effect. The current situation as stated by EFSA requires further research to support a beneficial physiological effect of probiotics specifically in humans (Pravst 2012). It is expected that the same argument would be also applied to non-dairy probiotic products

### **Concluding Remarks**

- The application of non-thermal methodologies should be considered for the elimination/reduction of surface microflora of non-dairy substrates in order to reduce competition upon inoculation with the probiotic culture. In the production of fermented dairy products, the primary raw material milk is typically pasteurized to eliminate pathogenic and reduce spoilage microorganisms. Non-thermal treatments will enhance the viability of the probiotic cultures with minimal losses in the nutritional attributes of the non-dairy substrates.

- Lactic acid bacteria, including some probiotic strains, could inhibit the growth of pathogenic bacteria resulting in an extension of the microbiological shelf-life, thus adding an element of safety in addition to nutrition for non-dairy fermented products.
- Mixed strains of probiotics could offer a range of flavours, thus creating opportunities for novel probiotic products particularly masking the “beany flavour” that is typically associated with cereal and legume- based products.
- There is a significant potential for the incorporation of probiotics in bakery products given their wide range and high consumption rates. In this case, the probiotic cultures should be encapsulated for protection against the high temperature applied to baked products.
- While encapsulation could significantly help towards increasing the viability and availability of probiotic cultures, on the negative side, encapsulation would restrain the inhibitory action of probiotic cultures against some pathogenic microorganisms. However, this could be counteracted by utilizing good microbiological quality raw material.
- Combining genetic engineering of microorganisms with novel processing technologies will be expected to create new fermented products in which desired properties would be emphasized. New products should be developed and traditional ones should be maintained and improved.
- Probiotic fermentation of plant-based products has the potential to improve the nutraceutical properties of the final products by enhancing the levels of phenolic content and antioxidant activity.
- The exploitation of lactic acid fermentation through the selection of controlled fermentation processes and starter cultures could be considered as an approach for enhancing the consumption of fresh-like vegetable and fruits among the world population.

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