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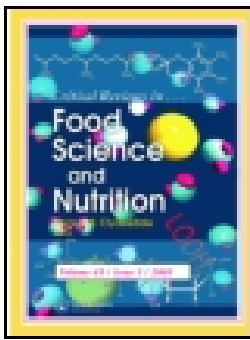


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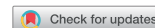
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## Non-dairy probiotic food products: An emerging group of functional foods

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### ABSTRACT

The functional food sector has shown tremendous growth in recent years with the application of probiotic bacteria as “food additives”. The utilization of probiotic bacteria in food presents many challenges related to their growth, survival, viability, stability and functionality in food processing, storage and consumption as well as changes of sensory characteristics of probiotic foods. Although dairy foods are currently the most common food carrier to deliver probiotics, an increasing number of non-dairy food matrices exhibit potential for delivery of probiotics. This review provides more recent insight into the emergence of non-dairy probiotics products, the interactions between probiotics and different food matrices and the challenges in developing such products. Some of the technical issues are also reviewed and discussed. These issues include the efficacy of probiotic bacteria in non-chilled, low pH or high water activity foods; the potential loss of bacterial viability, additionally unwanted fermentation and changes of the sensory characteristics of food products which may result in poor microbiological quality and low acceptability to consumers.

### KEYWORDS

Probiotics; functional food; non-dairy foods; viability; stability; sensory

### Introduction

The term functional food is credited to have been introduced in 1991 by the Japanese government, and refers to a food that is supplemented with extra ingredients such as vitamins, proteins, fibres, probiotic bacteria or other food additives which can contribute to human health and wellbeing (Granato et al. 2010a). In a recent study on the consumption of functional foods by older adults, probiotic yoghurt was the most popular product representing 56.0% of all consumed functional foods (Vella et al. 2013). People have been consuming fermented foods such as yogurt for thousands of years and some of those foods are still a part of our everyday diets. Scientific studies have demonstrated that fermentation contributes not only to the preservation of foods but also increases digestibility of some foods compared with the same non-fermented foods (Caplice and Fitzgerald 1999). Furthermore, lactic acid produced during fermentation can improve the value of fermented products by changing taste and texture of food matrices (Kun et al. 2008).

Probiotic food products are receiving a lot of attention largely due to their prospective health benefits and rapidly growing global markets. The reported health benefits of consuming probiotics include possible roles for the management and prevention of diarrhoea, inflammatory bowel disease, lactose intolerance, allergies, cancer, respiratory tract infections, constipation, urinary tract infections, helicobacter pylori infection and high blood cholesterol (Nagpal et al. 2012a). Although studies using animal models have shown that probiotics do not consistently show significant effects on fat depots, cholesterol or hormones levels, their ability to manipulate gut health and

the immune system are well-documented (Ali et al. 2004). Therefore, selection of suitable probiotic strains needs to be carefully considered for a certain health claim that should be based on available scientific evidence. The global sale of probiotic ingredients, supplements and foods is expected to reach US\$ 50.0 billion by 2020 with a compound annual growth rate of 8.0% from 2015 to 2020 (BBC Research 2016). Although the current probiotic market is dominated by dairy food products, non-dairy food products have some unique characteristics and advantages as alternatives to dairy probiotic food products. These advantages include meeting the needs of vegans and vegetarians; avoiding allergens present in dairy products; providing low cholesterol content products to consumers who suffer from cardiovascular diseases or obesity; suiting dietary habits of various ethnic groups; improving the aroma of soy products; increasing the nutritional value of non-dairy foods and preventing and inhibiting spoilage and growth of pathogens in meat and meat products (Granato et al. 2010b; Kołożyn-Krajewska and Dolatowski 2012; Nagpal et al. 2012a; Stadnik and Dolatowski 2014; Vasudha and Mishra 2013; Yeo and Liong 2010).

The most common probiotic bacteria utilised by the food industry are *Lactobacillus* spp. and *Bifidobacterium* spp. as they are typically granted “Generally Recognized As Safe” (GRAS) status by several regulatory agencies. Some yeasts such as *Saccharomyces cerevisiae* and *S. Boulardii* also possess potential probiotic properties (Figueroa-Gonzalez et al. 2011). Strains of *Lactobacillus acidophilus*, *Lactobacillus. casei*, *Lactobacillus. plantarum*, *Lactobacillus. rhamnosus* and *Bifidobacterium lactis*

are probiotics that can be incorporated into plant-based products (Martins et al. 2013). It is important to note that probiotic strains from gut or food origin show different tolerance to external stresses. For instance, the probiotic strains isolated from foods generally have higher tolerance to changes of temperature and pH during food product processing, but lower survival rate passing through the gastrointestinal tract (GIT) compared with probiotic strains isolated from the gut (Klein et al. 1998). Furthermore, the use of probiotic bacteria individually or in combination also needs to be carefully considered since the amounts and types of metabolites after fermentation could be variable thus affecting a final product quality (Champagne et al. 2010). To exert beneficial effects, it is recommended probiotic microorganisms should have at least  $10^6$  colony forming unit (CFU)/g or ml of viable cells in a product, to provide a daily dose of  $10^8 - 10^9$  of viable cells (Gomes and Malcata 1999). However, viability and stability of probiotic bacteria under detrimental environmental conditions during food processing, storage and consumption are still major challenges in the development of probiotic products. Studying the relationship between food substrates and probiotic bacteria can be an approach to develop survival-enhanced probiotic food products (Shori 2016).

Microencapsulation of probiotic bacteria cells has been suggested as a solution to the problem of viability loss. However, many technical issues need to be resolved when applying this technology to produce new probiotics foods. Selection of appropriate encapsulation technique, choosing safe and effective encapsulating material, and bacterial strain can affect efficiency of encapsulation or result in a decrease in bacterial viability (Huq et al. 2013). Currently, only a few microencapsulated probiotics have been developed as food products (Coghetto et al. 2016; De Prisco and Mauriello 2016). Microencapsulating cells do not always show a better survival than free cells. For instance, the viability of microencapsulated *L. reuteri* NCIMB 30242 and free cells was not significantly different in a fruit juice and a soy beverage for 8 weeks storage at 4°C or 8°C (Roy et al. 2016).

The environment within or adjacent to probiotic bacteria in a food matrix appears to be a significant factor for growth, survival, viability and functionality of probiotic bacteria. Living cells may adapt to suit a new environment that may be created during food production. Although dairy is currently the most common food carrier for probiotics, an increasing number of non-dairy food matrices show potential for the delivery of probiotics. A good example is that the release of citric acid from pomegranate juice was found to promote cell synthesis of *L. acidophilus* and *L. paracasei* (Mousavi et al. 2010). As well as stresses during processing, other factors such as culture preparation and preservation, food matrices, dietary habits, and host age and health can also affect the performance of probiotics (Marco and Tachon 2013). Importantly, strain-dependent properties of bacteria can raise the complexity of research into the interactions between probiotics and foods. The metabolic capabilities of probiotic microorganisms, such as *Bifidobacterium* spp. that utilize a wide range of mono- di and oligosaccharides, the “*bifidus* pathway” may influence the performance (Kun et al. 2008). Different nutritional needs for probiotics survival may partly explain the different consumption rates of

some media substrates, such as different sugars between different probiotic bacteria (Crittenden et al. 2002).

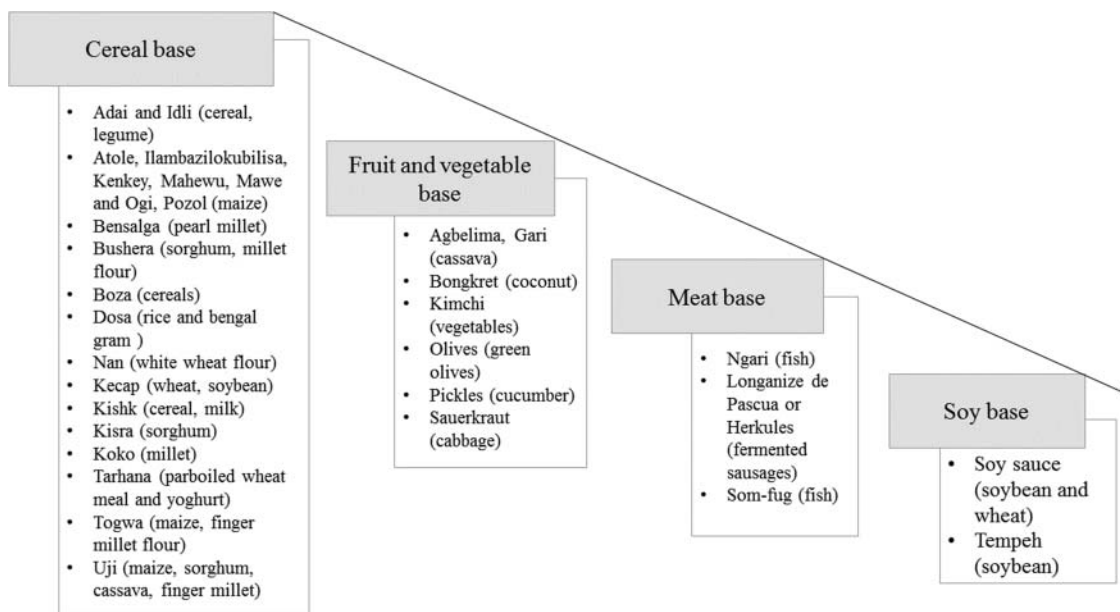
In recent few years some reviews have been published on non-dairy probiotic foods: Granato et al. (2010b) presented an overview of functional food development to emphasize the role of non-dairy foods in delivering probiotic bacterial strains; Vasudha and Mishra (2013) highlighted the research done on probiotic beverages from non-dairy sources; Kumar, Vijayendra, and Reddy (2015) compared trends in dairy and non-dairy probiotics products; and recently, Bansal et al. (2016) discussed non-dairy based probiotics with reference to microbiologically healthy intestine. The probiotics market is growing at a tremendous rate globally and could increase to US\$ 50 billion by 2020. Therefore, more frequent review of the scientific and technical developments in the probiotics sector will assist academics, researchers, industry and government agencies to keep a good track of the innovations and emerging challenges. This will assist with the design of new research and develop policies to support future growth. This review aims to provide a more recent scientific and technical insight into non-dairy probiotic products as emerging functional foods, mainly concentrating on four groups: fruit and vegetables, cereals, meat and meat products, and soy. In addition, a critical overview of the properties of different food matrices and probiotic bacteria, as well as examples of current technological challenges and advancements, functionality and sensory acceptances, and future directions for developing probiotic non-dairy foods have been included.

## Probiotic food products of non-dairy origin

Fruit and vegetables, cereals, soy and meat are non-dairy foods, which are rich sources of protein, minerals, vitamins, dietary fibres, antioxidants and other bioactive substances have been studied for suitability of probiotic survival and stability (Rivera-Espinoza and Gallardo-Navarro 2010). Additionally, the unique physiology of plants as well as fat constituents of meat are also able to protect probiotic bacteria from different stresses (Holck et al. 2011; Martins et al. 2013). Several traditional non-dairy fermented foods used as vehicles for probiotics delivery are presented in Figure 1. It is interesting to note that most traditional fermented foods are in the cereal based group, which is the least studied group in terms of developing innovative probiotic foods in current food industry. Conversely the most research interests in this field are in the sector of fruit and vegetables. To present a comprehensive view of probiotics applied to non-dairy foods, emerging and innovative non-dairy probiotic products are summarized in Table 1.

### Fruit and vegetable based probiotic food products

The richness in nutrients, unique physiological structures of fruit and vegetables, such as pores and irregularities occurring on the surface of intact fruit are likely to provide natural shelter for probiotic bacterial cells from stresses (Sapers 2001). For instance, a combination of *L. paracasei* LMGP 22043 and artichoke was found to positively influence the bacterial balance in the GIT partly due to micro-architecture of the artichoke surface (Valerio et al. 2011). The potential for fruit and vegetables



**Figure 1.** Traditional fermented foods that may contain potential probiotic bacteria. (adapted from Caplice and Fitzgerald 1999; Rivera-Espinoza and Gallardo-Navarro 2010; Vasudha and Mishra 2013).

as suitable vehicles to deliver probiotic bacteria has been extensively studied, and the development of such probiotic foods mainly falls into three categories; fermented or unfermented fruit juices, fermented vegetables, and minimally-processed fruit. A lot of work has been carried out to develop probiotic fruit and vegetable juices with high viable probiotic microbial cells loads incorporated into a wide range of juices and other forms of foods (Table 1).

Many studies have demonstrated the feasibility of probiotic bacteria incorporation into fruit and vegetables; however, their survival and stability in such matrices have been found to be highly strain dependent (Rivera-Espinoza and Gallardo-Navarro 2010). Three probiotic bacteria (*L. casei* A4, *L. plantarum* C3 and *L. delbrueckii* D7) in fermented cabbage juice were stored at 4°C without nutrient supplementation for 4 weeks (Yoon, Woodams, and Hang 2006). Although these strains showed high initial viable cell counts of 10<sup>9</sup> CFU/ml after fermentation, a loss of viability was observed at the end of cold storage. The viability of *L. plantarum* C3 and *L. delbrueckii* D7 had reduced to 4.1 × 10<sup>7</sup> CFU/ml and 4.5 × 10<sup>5</sup> CFU/ml, respectively whereas *L. casei* A4 did not survive in the fermented cabbage juice. Kun et al. (2008) evaluated the growth of *B. lactis* Bb-12, *B. bifidum* B7 and *B. bifidum* B 3.2 in fermented carrot juice. All strains showed high initial viable cell counts of 10<sup>10</sup> CFU/ml. However, their viabilities were not monitored during storage, thus offering little insight for further product development. Sharma and Mishra (2013) also reported high initial cell numbers of *L. acidophilus* NCDC 11, *L. plantarum* NCDC 414 and *Pediococcus pentosaceus* MTCC 2819 after fermentation of a vegetable juice mixture of bitter melon, bottle gourd and carrots, and then the cell numbers decreased gradually during storage at 4°C after 4 weeks. These findings are in contrary to the report of Pereira, Maciel, and Rodrigues (2011) where they found *L. casei* NRRL B-442 used to ferment cashew apple juice produced an initially high viable cell count of 10<sup>8</sup> CFU/ml and also maintained at 4°C for 42 days. A decrease in

probiotic viability during storage is a common finding in many studies, so selection of suitable techniques to enhance the stability of probiotics in this type of food during storage is a challenge.

Depleting the nutrient contents is a methodological approach to decrease the growth rate of probiotic cells, and another method is to decrease sugar content and pH (Nagpal, Kumar, and Kumar 2012b). However, in contrast to these approaches, Charalampopoulos, Pandiella, and Webb (2002) reported that probiotic bacteria treated with high concentrations of sugar and malt extract showed higher survival rates than untreated probiotics because the bacterial cells were protected from processing treatments as well as the sugar medium providing nutrients for rapid regeneration and growth. To investigate effects of sucrose, glucose, fructose, citric acid and ascorbic acid on viability of *L. plantarum* NCIMB 8826, a model preparation composed of those five compounds was designed, and compared with seven different commercial fruit juices as controls (Nualkaekul and Charalampopoulos 2011). It was suggested that high pH and citric acid concentration would have a positive impact on survival of NCIMB 8826. High viable counts in orange, blackcurrant, pineapple and grapefruit juices were reported, however viability in cranberry or pomegranate was compromised due to the effect of phenolic compounds. Mango and guava pulps also showed a negative effect on the viability of *L. acidophilus* La-5 and *B. animalis* Bb-12 in soy yoghurt (Bedani et al. 2014). Some antioxidants present in white grape seed extract, green tea extract and vitamin C have been found to protect the cell membrane of *L. rhamnosus* HN001, *B. lactis* HN001 and *L. paracasei* LPC 37 from lipid oxidation in the juices (Shah et al. 2010). However, *L. casei* T4 failed to maintain viability in cornelian cherry juice during refrigerated storage because of presence of low pH and phenolic compounds (Nematollahi et al. 2016). These findings indicated the importance of studying combinations of probiotic strains and food matrices.



**Table 1.** Examples of non-dairy probiotic products reported in laboratory scale projects.

Category	Examples of products	References	
Fruit and vegetable	Vegetable juices including tomato, gourd, cabbage, carrot, andean tubers and <i>Moringa</i> leaves based beetroot	Nagpal et al. 2012; Yoon, Woodams, and Hang 2006; Kun et al. 2008; Mosso, Lobo, and Sammán 2016; Vanajakshi et al. 2015	
	Fruit juices including orange, pineapple, cranberry, apple, mandarin, grape, longan, passion fruit, cornelian cheery, water melon and cantaloupe	Luckow et al. 2006; Saarela et al. 2011; Nualkaekul and Charalampopoulos 2011; Nematollahi et al. 2016; Chaikham et al. 2013; Mestry, Mujumdar, and Thorat 2011; Russo et al. 2015; Farias, Soares, and Gouveia 2016	
	Fruit powders (apple, banana and strawberry), snacks (pear and peach)	Borges et al. 2016; Sohail et al. 2012	
	Fresh cut apple and papaya, cantaloupe, mango and guava pulps	Tapia et al. 2007; Russo et al. 2015; Bedani et al. 2014	
	Olives	De Bellis et al. 2010; Peres et al. 2012	
	Cashew juice	Pereira, Maciel, and Rodrigues 2011	
	Lupin-based yogurt	Hickisch et al. 2016	
	Sauerkraut	Beganović et al. 2011	
	Meat	Sausages including Iberian dry fermented, dry fermented, raw fermented, mutton fermented, dry-cured 'Longanize de Pascua', typical Czech fermented 'Herkules', low fat fermented	Muthukumarasamy and Holley 2006; Holko et al. 2013; Rubio et al. 2013; Trzaskowska et al. 2014; Ratanaburee et al. 2013; Sidira et al. 2015; Sidira et al. 2016
		Dry fermented pork loins	Stadnik and Dolatowski 2014
Salami		Coman et al. 2012	
Ham		Kołożyn-Krajewska and Dolatowski 2012	
Soybean		Okara	Bedani, Rossi, and Saad 2013
	Soy milk	Tang et al. 2007; Yeo and Liang 2009; Subrota et al. 2013; Espirito-Santo et al. 2014	
	Soy bar	Chen and Mustapha 2012	
	Fermented soy beverage	İcier et al. 2015; Champagne et al. 2009	
	Soy-based cream cheese	Liang et al. 2009	
	Frozen soy dessert	Heenan et al. 2004	
Cereal	Soy yoghurt	Pandey and Mishra 2015; Farnworth et al. 2007	
	Beverage from rice, barley, oats, wheat and malt	Mårtenssona, Öste, and Holst 2002; Chumphon, Sriprasertsak, and Promsai 2016; Russo et al. 2016; Angelov, Gotcheva, and Hristozova 2006	
	Cereal bar	Ouwehand, Kurvinen, and Rissanen 2004	
	Chocolate coated breakfast cereal	Saarela et al. 2006	

Some prebiotics derived from plant-based foods favour probiotic viability. The use of pear and pineapple to support growth of lactic acid bacteria has been investigated by Diaz-Vela et al. (2013). Carbohydrates from pear flour were consumed by bacteria more than those of pineapple flour or glucose probably due to different compositions of carbohydrates in these flours. Another study also found that oligosaccharide extracted from pitaya (dragon fruit) could support the growth of lactobacilli and bifidobacteria (Wichienchot, Jatupornpipat,

and Rastall 2010). Several prebiotics including xylo-oligosaccharides, xylan, galacto-oligosaccharide, fructo-oligosaccharide, polydextrose, lactitol, gentiobiose and pullulan added to pure culture fermentations were evaluated by Makelainen et al. (2010). They found that different bacteria selectively fermented different prebiotics. For example *B. lactis* strains and lactobacilli only fermented xylo-oligosaccharide and lactitol, respectively. Therefore, it is important to test the interactions of probiotics and prebiotics when considering using them as "synbiotic".

### Cereal based probiotic food products

Cereals such as wheat, maize, oat, barley and other grains, are abundant sources of dietary fibre some of which can have several beneficially physiological effects on the gut; can be used as prebiotics providing specific non-digestible carbohydrates and used as encapsulation materials to improve the viability and stability of probiotics (Capozzi et al. 2012).

Fermentation of cereals with lactic acid bacteria can increase the nutritional values and improve health-promoting properties of the final products such as beverages, bread, biscuits and breakfast cereals (Lamsal and Faubion 2009). Cereal grains normally contain dietary fibre-phenolic compounds (DF-PC) that are covalently bound to polysaccharides by ester bonds, where the ester bonds can be broken by fermentation to release some phenolic acids such as ferulic acid, thus promoting health benefits (Vitaglione, Napolitano, and Fogliano 2008). Fermentation of milled whole grain barley with three probiotics (*L. johnsonii* LA1, *L. reuteri* SD2112, and *L. acidophilus* LA-5) improved bioavailability of free phenolic acids (Hole et al. 2012). It has also been reported that the antioxidants in buckwheat, wheat germ, barley and rye increased after the fermentation with *L. rhamnosus* and *S. cerevisiae* (Đorđević, Šiler-Marinković, and Dimitrijević-Branković 2010). Changes in the viscosity of probiotic fermented oat-based foods were reported, the viscosity increased after the fermentation and then decreased throughout storage due to the utilization of oat  $\beta$ -glucan by *L. plantarum* strains (Russo et al. 2016).

Apart from the contribution of fermentation to improving survival of probiotics in cereal foods, cereal extracts showed a capacity to increase the tolerance of probiotic bacteria to harsh conditions. For instance; cereal extracts from malt, barley and wheat significantly improved the acid tolerance of three lactobacilli (*L. plantarum*, *L. acidophilus* and *L. reuteri*) to gastric acid (Charalampopoulos, Pandiella, and Webb 2003). Both soluble sugars and free amino acid nitrogen in the extracts were suggested to have a positive impact on the survival of these bacteria under the acid conditions. Moreover, these sugars were more effective in protecting the three bacteria than other components present in the same extracts. Michida et al. (2006) compared the influence of malt and barley extracts on the survival of *L. plantarum* in gastric and bile acids, and found the higher content of sugars in the malt extract enabled these bacteria to tolerate the acid conditions better than the barley extract (Salmerón, Thomas, and Pandiella 2015).

Utilisation of different carbohydrates derived from oat to culture different bacteria indicates specificity of sugars towards microbial survival. Kontula, von Wright, and

Mattila-Sandholm (1998) compared  $\beta$ -gluco-oligosaccharides and xylo-oligosaccharides, which were derived from oat. The latter selectively supported the growth of *L. rhamnosus* GG and *L. lactis* but not of *L. plantarum*. In another study, a range of dietary fibres including  $\beta$ -glucan, xylan, xylo-oligosaccharides and arabinoxylan were investigated for their influence on the fermentation of several *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococci* spp., *Bacteroides* spp., *Clostridium* spp. and *Escherichia coli* (Crittenden et al. 2002). The authors found bifidobacteria could utilize arabinoxylan as a carbon source but  $\beta$ -glucan derived from barley had no effect on the growth of any of the tested bacteria. However,  $\beta$ -glucan derived from oat could be consumed by *B. bifidum* DSM 20456 (Mårtenssona, Öste, and Holst 2002). In another study, barley  $\beta$ -glucan used as an encapsulation material successfully protected the cells of *L. casei*, *L. brevis* and *L. plantarum* from simulated gastrointestinal digestion, heat treatment and storage (Shah et al. 2016).

### Meat based probiotic food products

Fermentation of meat products can produce many substances including lactic acid, acetic acid, alcohols, aldehydes, ketones and bacteriocins; which impact product quality, flavour, safety and shelf life (Kołożyn-Krajewska and Dolatowski 2012; Sidira et al. 2016). During the production of dry-cured fermented meat products, proteolysis can enhance colour, taste and aroma of the final product because the muscle structure of meat breaks down, and proteins are degraded into small peptides and free amino acids (Stadnik and Dolatowski 2014). Furthermore, some species of *Bifidobacterium* and *Lactobacillus* produce health-promoting components such as conjugated linoleic acid (CLA) and increase the functional characteristics of food (Kołożyn-Krajewska and Dolatowski 2012). Besides, different probiotic delivery systems such as encapsulation and entrapping probiotics in gelled dispersions enhanced the viability of probiotics in meat products after heat treatments during processing and cooking (Cavalheiro et al. 2015).

Fermented sausage ingested without cooking is regarded as a good vehicle to transfer probiotics into the intestine because the cells can be embedded within protein and fat in the sausage matrix (Rubio et al. 2013). Rivera-Espinoza and Gallardo-Navarro (2010) evaluated the population of probiotics in sausages and found the initial inoculum of  $10^5$  CFU/g of *L. plantarum* 299V increased to  $10^8$  CFU/g after fermentation. A comparison of probiotic bacteria counts (loading) and sensory evaluation of mutton sausages between *L. acidophilus* CCDM 476 and *B. animalis* 241a were investigated by Holko et al. (2013). The authors observed a higher viability of *L. acidophilus* CCDM 476 in the final product and after 60 days of storage compared with the viability of *B. animalis* 241a. Both texture and typical smell of mutton were improved by addition of the probiotic strains. In addition, 1% of orange fibre added into the formulation of Spanish non-fermented dry-cured sausage (*Longanize de pascua*) was found to favour the growth of *L. casei* CECT 475 and to improve sensory and safety of the final product (Sayas-Barberá et al. 2012).

The concentration of volatile compounds of dry-fermented sausages after heat treatment is highly correlated to the concentration of the starter culture (Sidira et al. 2015). Two different strains of *L. casei* were investigated for the microbiological quality after inoculation into raw-fermented sausages and after storage for 6 months (Trzaskowska et al. 2014). The aroma of cured meat, the taste of dried meat and muscle tissue fragmentation after fermentation were acceptable, but bitter and fatty-like taste, and acrid odour was also present in the final products. Although the number of the two strains of *L. casei* was similar after fermentation, *L. casei* LOCK 0900 exhibited a better capacity to inhibit the growth of *Staphylococcus aureus*, *E. coli* and *Enterobacteriaceae*. Increased trichloroacetic acid (TCA) soluble peptides and free amino acid content demonstrated that *L. casei* LOCK 0900 also assisted proteolysis during fermentation and aging of meat products (Stadnik and Dolatowski 2014).

### Soy based probiotic food products

Soy has a high level of protein and contains polyunsaturated fats, fibre, minerals and vitamins. Although consumption of soy oligosaccharides such as stachyose and raffinose may cause bloating, cramping and flatulence, the fermentation of soybean extracts by probiotics can reduce these indigestible sugars (Champagne et al. 2010). For example, probiotic *L. acidophilus* LA-2 produced a high level of  $\alpha$ -galactosidase activity at 5.0 U/mg in soy protein bars throughout 14 weeks of storage at 4°C (Chen and Mustapha 2012). Soy protein is also considered a good protector for bacteria against bile and acid conditions in the gut (Shimakawa et al. 2003). For instance, a study of survival and sensory acceptability of five probiotic bacteria in non-fermented frozen soy dessert, *L. acidophilus* MJLA1, *L. rhamnosus* 100-C, *L. paracasei* spp. paracasei 01, *B. lactis* BBDB2 and *B. lactis* BB-12 demonstrated high viable numbers exceeding  $10^7$  CFU/g during 6-month storage, and desirable sensory acceptability (Heenan et al. 2004). *L. rhamnosus* GG and *L. johnsonii* La-1 were also successfully incorporated with yoghurt starters into fermented soy yoghurt, and generated an acceptable taste (Farnworth et al. 2007). *L. acidophilus* LA-5 in a fermented soy beverage exhibited good growth and viability of 8.73 – 9.11 log CFU/g after 21 days storage at 4°C (İçier et al. 2015).

Other factors such as drying techniques, storage temperature, packaging materials and the use of prebiotics are also important and influence the quality of probiotic soy foods. Wang, Yu, and Chou (2004) compared different conditions to produce probiotic soy milk, and suggested three elements; freeze-drying, storage temperature of 4°C and a laminated pouch to pack dehydrated soymilk, could ensure good probiotic survival. Response surface methodology was also used to study the formulation of synbiotic soy yoghurt by measuring effects of fructo-oligosaccharides concentration, inoculum size and fermentation temperature on fermentation time, hardness, whey separation and overall acceptability (Pandey and Mishra 2015). Authors found that the optimized product showed good nutritional, textual and sensory characteristics. An investigation on the addition of prebiotics to probiotic soymilk was conducted by Yeo and Liong (2010). They reported the viability of

six probiotics (*L. acidophilus* FTDC 2113, *L. acidophilus* FTDC 8033, *L. acidophilus* ATCC 4356, *L. casei* ATCC 393, *B. longum* FTDC 8943 and *B. longum* FTDC 8643) in a combination of FOS, inulin, mannitol, maltodextrin or pectin. All strains showed high viable counts in soymilk products after 24 h storage. In another study, higher production of peptides and amino acids by proteolysis of proteins was found in a soymilk fermented by *L. acidophilus* FTCC 0291 than other species. This may explain the viability of exceeding  $10^7$  CFU/g in soy cream cheese stored at 4°C and 25°C for 20 days (Liong et al. 2009).

## Technological challenges

The most studied technologies which were involved in fermentation, encapsulation, drying, rehydration, and storage have been developed and successfully applied to protect some

probiotics from environmental stresses associated with various non-dairy food matrices, but there are still many technological challenges (Table 2) in producing and preserving probiotic foods and these need to be resolved.

Undoubtedly, with probiotics as living microorganisms present in a food product, retention of sufficient viable bacteria is a significant component of quality. Simply using any bacterial species such as *Lactobacillus* and *Bifidobacterium* does not guarantee high viable content in fermented products after fermentation and during storage (Holko et al. 2013). Therefore, the choice of appropriate probiotic bacteria and their cultures as well as studying the relationship between bacteria and food matrices under different conditions are important. Also, safety of probiotic products must also be considered. Although a sharp decrease in pH could inhibit growth of *Enterobacteriaceae* during the fermentation of sausages, negative effects on

**Table 2.** Technological challenges associated with survival of probiotics during processing and storage.

Bacterial survival circumstance	Technique	Existing challenge	Strategy
Fermentation	One step fermentation;	Temperature	Control of fermentation temperature/ time and pH;
	Continuous fermentation; Membrane bioreactors; Immobilized cell fermentation	Oxygen content; Acidification; Undesirable change of sensory	Addition of antioxidant
Encapsulation	Extrusion	Production capacity; Particle size	Alginate; Alginate with starch/ chitosan/ calcium chloride/ poly-amino acids;
	Emulsion	Water-soluble capsuled particle; Choice of solvents	Whey protein; Proteins derived from legumes /milk; Pectin; Milk; $\kappa$ -carrageenan; Sodium carboxymethyl cellulose (NaCMC); Cellulose acetate phthalate (CAP); Cryo-, lyoprotectants (skim milk, whey protein, glucose, maltodextrin, trehalose); Freeze drying temperature/ rate; Pre-treatment to sub-lethal stress; Fermentation condition
Drying	Freeze drying Freeze-vacuum drying	Crystal formation; Dry cake; Osmotic stress; Mechanical stress	Protectants (disaccharides); Outlet/inlet temperature; Feed rate; Moisture content in the powder; Pre-treatment to sub-lethal stress
	Spray drying Two-step spray drying Spray freeze drying Spray chilling	Heat stress; Shear stress; Osmotic stress; Oxidative stress	Protectants (disaccharides); Outlet/inlet temperature; Feed rate; Moisture content in the powder; Pre-treatment to sub-lethal stress
	Fluidized-bed drying	Low yields of probiotic cells	Protectants; Process parameters; Pre-treatment to sub-lethal stress
	Other drying	High cost	Alginate combined with other coating materials
	(hybridization; impinging aerosol technology; Electrospinning)	Probiotic survival is species dependent	
Rehydration	Dissolving	Rehydration media (osmotic stress, pH, composition and volume)	Increase buffering capacity Supply nutrients Optimize pH Proper cell density
Storage	Frozen; Chilling; Room temperature	Food ingredients Oxygen content Water activity	Optimal formulation of probiotics; Addition of antioxidant; Control of storage temperature and humidity;
		Storage temperature pH and titratable acidity	Package

Adapted from Broeckx et al. 2016; Lacroix and Yildirim 2007; Martín et al. 2015; Sohail et al. 2012; Tripathi and Giri 2014.



the survival of probiotic bacteria and the sensory characteristics of fermented meat products have been noted (Rubio et al. 2013). Decarboxylation of free amino acids or amination and transamination of aldehydes and ketones in ageing fermented meat products are undesirable since these biogenic amines have a negative effect on health (Kołożyn-Krajewska and Dolatowski 2012). Furthermore, these authors found oxidation of lipid and protein caused loss of nutritional values, colour and other characteristics of sensory. For example, raw-fermented sausages with *L. casei* LOCK 0900 showed bitter taste and other undesirable flavours, acrid odour, fatty flavour and “visible” fat present in the final products (Trzaskowska et al. 2014).

Other common technological challenges such as processing, storage temperature and time, oxygen content, pH and external stresses may impact the application of probiotics in food products (Vasudha and Mishra 2013). These challenges have been discussed in the previous sections of non-dairy origin in this review. Besides, water activity as a critical parameter can impact the viability of bacteria in these food products. Many food ingredients such as salts and sugar can bind water to create ‘dry’ and low water activity environments, resulting in an improved survival of microorganisms (Holck et al. 2011). In contrast, excess water activity (e.g. in fruit juice) also can reduce the viability of bacteria during storage (Vasudha and Mishra 2013).

### Technological advancements

To produce high viability and yields of probiotic bacterial cells and nutritionally active biomass, fermentation process has been extensively improved. Many probiotic bacteria have shown good survival with viable cells of  $10^8 - 10^9$  CFU/ml in fermented juices (Bialonska et al. 2010; Chaikham et al. 2013; Sharma and Mishra 2013; Valerio et al. 2011). However, some drawbacks of applying these techniques to produce probiotic non-dairy foods still need to be addressed on a case by case basis.

Acidification can be a major problem in the fermentation, sonication was carried out to reduce acidification by probiotics without affecting their viability (Racioppo et al. 2017). They reported acidification by four probiotic bacteria treated with ultrasound were significantly lower than controls after 14 days at 15°C. However, the authors did not observe differences of viability between ultrasound treated and untreated bacteria. Calcium lactate to buffer the pH within 4.0 to 5.0 in fermented soy food that was inoculated with *L. rhamnosus* LR32 and a mixture of *L. acidophilus* LAC4, *L. paracasei* LBC81 had viability of 9 – 11 logs CFU/mL or inoculated with *B. longum* BL04 had viability of 8 – 9 logs CFU/mL after storage of 30 days at 4°C (Mondragón-Bernal et al. 2017). Four strains of *L. plantarum* showed a good tolerance to acidic conditions in fermented oat foods, and high viability of 8 logs CFU/g for all strains after storage at 4°C, but viscosity reduced due to degradation of oat  $\beta$ -glucan and production of microbial EPS (Russo et al. 2016). Yeast was found to have high antioxidant activity in fermentation with cassava and rice, and hence reduced oxidative stress effects on probiotic viability during fermentation (Freire et al. 2017).

Microencapsulation was given great attention to achieve high viability of probiotic cells in fruit and vegetable bases (Antunes et al. 2013; Chaikham et al. 2013; Khan et al. 2013). The addition of protectants to the culture medium can improve viable counts of probiotics in the final product. Different lyo-protectants have also been used to increase the survival of *B. infantis* UV16PR after freezing drying (Basholli-Salih et al. 2014). Authors found cellobiose, lactose, sucrose and trehalose could enhance viability of *B. infantis* UV16PR, and cellobiose exhibited the best performance maintaining the viability by stabilizing cell membranes and preventing intracellular ice-formation. A combination of air drying and radiant energy vacuum drying to dehydrate apple slices supplemented with *L. rhamnosus* ATCC 7469 showed viable counts of  $10^5$  CFU/g during storage at 25°C for 60 days (Mestry, Mujumdar, and Thorat 2011). Another study conducted by Borges et al. (2016) found that the drying method and formulation were highly related to the survival of probiotics in the fruit powders during storage at 4°C and at room temperatures.

Table 3 includes some investigations of microencapsulated probiotics and protective capacity of coating materials used alone or in a combination with other materials to form single or multilayer(s) to maintain probiotic bacteria viability during storage and passage through the GIT. Furthermore, addition of protectants in the formulation of microencapsulated probiotics also showed the possibility of high viability of probiotics after drying.

Alginate as a core coating material consistently receives extensive attention, especially in combination with other novel materials. Alginate-gelatin encapsulated *L. salivarius* Li01 lost 1.7 logs CFU after 5 weeks storage at 4°C compared to reduced viability of 2.4 logs CFU of alginate alone and 3.5 logs CFU for free cells after as little as 4 weeks. However, alginate-gelatin microgels eroded or swelled under simulated small intestine conditions (Yao et al. 2017). Muhammad et al. (2017) reported the viability of *L. plantarum* KLDS 1.0344 encapsulated with potato resistant starch or potassium alginate were 5.7 logs CFU and 4.5 logs CFU, respectively, during storage at 25°C for 42 days, compared with viability loss of 3.1 logs CFU for free cells. They suggested the amorphous glassy state and low water content of potato resistant starch coated bacteria attributed to high bacterial survivals. However, they also found potassium alginate encapsulated cells less ideal for bacterial survival under simulated gastric conditions. To reduce erosion of alginate in simulated gastric juice, the cell wall of yeast (*S. cerevisiae*) was used to coat alginate particles of *L. acidophilus* or *B. bifidum* under another layer of alginate coating (Mokhtari et al. 2017). Although the tolerance of multilayer encapsulated *L. acidophilus* to gastric juice increased significantly compared to free and single layer of alginate encapsulated cells, the size of the multi-layer microcapsules (103  $\mu$ m) was two times bigger than the single layer preparation. Moreover, there was no significant difference in survival of encapsulated *B. bifidum* in simulated intestinal juice between single layer and multi layers coating. Attempts to reduce swelling of alginate microcapsules at pH 1.5 and 5 solutions by incorporation of cellulose nanocrystal (CNC)

**Table 3.** Examples of microencapsulation technology affecting viability and stability of probiotics.

Material	Technology	Bacterial Strain	Survival Rate (log CFU ml <sup>-1</sup> or g <sup>-1</sup> )	References
Resistant rice starch	Emulsification	<i>L. brevis</i> MTCC01 <i>L. casei</i> MTCC297 <i>L. plantarum</i> MTCC021	> 7 log at 55°C, > 3 log at 65°C and > 2 log at 75°C for 10 min, while > 7 log at 4°C for 60 days.	Ashwar et al. 2018
Alginate or alginate-gelatin	Extrusion	<i>L. salivarius</i> Li01	2 times higher of viability loss of free cells than encapsulated cells with alginate-gelatin after 5 weeks storage at 4°C. Free cells were not detectable after 15 min heating at 63°C but reduction in viability of alginate-gelatin coated cells was < 3 log.	Yao et al. 2017
Three cultivars of Thai rice	Homogenization + package in capsules	<i>L. amylovorus</i> TISTR1110	>6.4 log at 4°C for 60 days.	Chumphon, Sriprasertsak, and Promsai 2016
Alginate, soya oil	Modified emulsification + freeze drying	<i>Lactobacillus</i> strain (strain name not given)	100% of survival of microencapsulated bacteria after 150 days at -20, 4 and 25°C.	Sánchez et al. 2017
Holy basil essential oil	Emulsification + Spray drying	<i>L. reuteri</i> KUB-AC5	Survival of 9 log corresponding to 97% survival using 6 mg/ml of HBEO at the inlet air temperature of 130°C.	Rodklongtan and Chitprasert 2017
Potassium alginate, pectin and potato resistant starch with maltodextrin, whey protein and mannose	Homogenization + spray drying	<i>L. plantarum</i> KLDS 1.0344	Viability loss of encapsulated bacteria < 0.35 log at 25°C for 42 days.	Muhammad et al. 2017
Yeast cell wall and calcium alginate	Emulsification	<i>L. acidophilus</i> PTCC 1643 <i>B. bifidum</i> PTCC 1644	Viability of double-layer alginate encapsulated <i>L. acidophilus</i> was 8.6 log and 6.1 log in simulated gastric juice and intestinal juice, respectively, and viability of encapsulated <i>B. bifidum</i> was 7.3 log and 6.2 log.	Mokhtari et al. 2017
Yacon root Trehalose	Homogenization	<i>L. casei</i> LC1	Viability of <i>L. casei</i> loaded on yacon flakes without trehalose was 6.5 log after 56 days at 25 °C.	Leone et al. 2017
Sodium alginate, sodium caseinate, soy protein isolate	Emulsification + spray drying	<i>L. zeae</i> LB1	Inactivation rate of encapsulated <i>L. zeae</i> with NaCas-AG was 0.4 log and 0.7 log with SPI-AG at a <sub>w</sub> of 0.76, compared to 0.01 log at a <sub>w</sub> of 0.11 during a 16-week storage at 25°C.	Liu et al. 2017
Alginate, nanocrystal lecithin	Emulsification + freeze drying	<i>L. rhamnosus</i> ATCC9595	Reduction of 1.2 log and 1.1 log at 25°C and 4°C for 42 days. 8 log of encapsulated <i>L. rhamnosus</i> with CNC and lecithin was after SGF.	Huq et al. 2017

or lecithin or starch has been reported (Huq et al. 2017). They found that the addition of CNC and lecithin in alginate microbeads decreased the gastric fluid absorption but increased the dissolution time compared to alginate microbeads.

Other coating materials such as resistant rice starch were used to encapsulate three *Lactobacillus* strains by emulsification and these showed high viability of more than 7 logs CFU/g for 60 days at 4°C or 120 min under simulated gastrointestinal conditions (Ashwar et al. 2018). However, low encapsulation efficiency of 43% – 48% and moderate size of 45.5 – 49.3 µm diameter of microcapsules were also found, which may increase the difficulty of introducing this encapsulated probiotic to food products.

Encapsulation technology may appear to be an all-encompassing means to maintain viability of probiotics in the encapsulated particles, because examples such as those discussed above showed stable viabilities of probiotics during storage and/or under simulated gastrointestinal juice. However, the production and size of encapsulated probiotics are not always satisfactory, and the survival of encapsulated probiotic incorporated into different non-dairy foods is also strain-dependent. In other words,

a successful formulation encapsulating probiotic A may be unsuitable for probiotic B in terms of developing new probiotic foods.

It is interesting to note that the number of studies using encapsulation to develop formulations of probiotic cells far outnumber the studies of encapsulated probiotic cells in a real food system. The observation that studies on encapsulated probiotics in food are rare and has been reported elsewhere. Some reasons for there being not many commercial products containing encapsulated probiotic cells may due to insufficient consideration of structural effects of encapsulating materials, improper encapsulating methods, issues in evaluation methods and risk assessments for applications (Chen et al. 2017). Among these reasons, microcapsule material is a critical factor for affecting the effectiveness of microencapsulation (Dianawati, Mishra, and Shah 2016).

With regard to the review of Probiotication of Foods, microencapsulated probiotics for foods has failed to deliver its promise (De Prisco and Mauriello 2016). The authors emphasised the importance of directing this technology towards practical production of desirable probiotics in food and suggested new

polymers for probiotics worthy of focus. Huq et al. (2013) also reviewed the effects of biopolymeric system on encapsulation of probiotics and pointed out that when applying encapsulation of probiotics to new foods, some factors including appropriate encapsulation techniques, safe and effective encapsulating materials and potent strains of probiotics as well as increasing layers of biopolymers needs to be further investigated. However, the authors did not take food systems into account for retention of viable cells during storage.

The development of probiotic microcapsules and their application in food may indicate the food system is a complex environment and unsuitable for bacterial survival. Microencapsulation technology applied to simply make probiotic capsules cannot ensure probiotics are viable and stable in a food product. Work to explore different coating materials and protectants along with optimization of drying process may not solve these technological hurdles to improve survival of probiotics in foods, if the interactions of probiotics and food substrates are not investigated. Further research of probiotic applications in foods should be directed towards the study an intrinsic relationship between free probiotic cells and food matrix during processing, storage and in the host, which agrees with the findings of Flach et al. (2017). They highlighted the importance of studying relationship between carrier matrices and the quality of probiotic products. Besides, studying biofilm formation of pure or a mixture of probiotic species under certain conditions may offer valuable information and understanding of bacterial behaviour under various stress conditions.

### Functional advancements and sensory acceptance

Functionality improvements of non-dairy probiotic food products are dependent on subtle action of bioactive food components on human health. For example, development of soy-based probiotic products has received considerable attention from researchers due to many functional properties such as good amino acid profile and compounds (isoflavones) with strong antioxidant activity (Wang, Yu, and Chou 2006). Probiotic products containing a combination of soy and fruit juices have successfully showed good numbers of probiotic, and functional and sensory properties (Shimakawa et al. 2003). In some probiotic beverages, probiotic bacteria were directly added to the finished products and achieved high viable cell counts and functionality (Acosta et al. 2008).

Probiotic dairy foods have successfully pioneered the introduction of probiotics to consumers, and indeed, consumers are willing to improve their health and wellness through the consumption of dairy or non-dairy food products supplemented with probiotics. Apart from microbiological quality, sensory properties and consumer acceptance are also essential for the development of innovative probiotic food products. Sensory properties of probiotic non-dairy foods can be affected by interactions of different species of probiotics and food matrices, where textures, taste, aroma, and colour for example might be improved or worsened by production of different metabolic compounds. Therefore, it is important to review not only good survival of probiotics but also sensory acceptance of probiotic non-dairy foods during production and storage.

Some examples of the effects of probiotics incorporated into non-dairy foods on sensory characteristics and consumers' acceptance along with bacterial viability and stability are presented in Table 4. Fruit juice and cereal beverages received much interest as potential non-dairy food carriers to deliver probiotics, but some undesirable sensory characteristics such as off-flavour, acidification of taste and after taste are observed mostly in this type of food. Regarding semi and solid probiotic non-dairy foods, the mean scores of texture, taste and odour are lower than of non-dairy foods without probiotics. On the other hand, bacterial survival for most probiotics in the foods listed in the Table 4 was over 8.0 logs CFU/g or mL after processing and/or at 4°C storage, whereas their viabilities could decrease below 1.0 log CFU /g or mL when the storage temperature increased to 20 to 30°C.

Scientists employed addition of sweetness and flavour masks, optimization of fermentation and encapsulation by using single or multiple layers of bacterial capsules, along with air drying, freeze drying or vacuum drying to produce probiotic non-dairy foods. However, sensory acceptance and bacterial survival can be intrinsically related to the strain of probiotic, and to a less extent to a type of bacterial cells (free or encapsulated) inoculated onto foods. A good example to illustrate this statement was a comprehensive study of viable cells, volatile compounds, free amino nitrogen and total reducing sugars after fermentation of oat, barley or malt drink with *L. acidophilus* NCIMB 8821, *L. plantarum* NCIMB 8826 or *L. reuteri* NCIMB 11951 (Salmerón, Thomas, and Pandiella 2014). The authors found the highest amounts of acetaldehyde and diacetyl or lactic acid in malt drink fermented by *L. plantarum* or *L. acidophilus*, indicating the different combination of probiotic bacteria and cereal foods resulted in different flavour of the fermented cereal-based drinks.

### Future directions

It is important to understand that delivering live microorganisms using food vehicles is a challenging task. As discussed in this article, various food matrices are being used to deliver probiotics. However, each food matrix not only has unique properties and advantages but may also impose technological barriers for successful delivery of probiotics. Development of novel, economical, and technologically feasible non-dairy products that satisfy consumer demands should be the focus of future research efforts. Some important areas of future research may include:

- Developing a range of probiotics products to meet the demand from consumers with certain dietary restrictions like vegetarianism, milk allergies, low cholesterol or fat content and lactose intolerance.
- Innovative non-dairy probiotics products for specialized market segments such as children, chronically ill patients and the elderly.
- Addressing the technological barriers to use of non-dairy food matrices.
- Stability and viability of probiotics strains under specific environmental stress factors associated with non-dairy food substances.
- Impact of combining different non-dairy food matrices on probiotic, sensory and functional characteristics of a product.

**Table 4.** Selected publications on sensory evaluation of and bacterial survival in probiotic non-dairy foods.

Probiotic Bacteria	Type of Food	Sensory Evaluation	Viability (log CFU per ml or g)	References
<i>L. casei</i> NRRL B-442	Fermented cupuassu beverage	Lower acceptance of texture, flavour, sweetness and overall in the probiotic samples	Viability of 9.3 log after 18 h of fermentation	Pereira et al. 2017
<i>L. plantarum</i> ATCC 20174, <i>L. casei</i> ATCC 393, <i>L. rhamnosus</i> ATCC 7469, <i>L. casei</i> T4, <i>L. casei</i> TD4	Cornelian cherry juice	No significant difference of odour, taste and overall acceptance between probiotic samples and control samples but <i>L. casei</i> TD4 produced pungent odour and astringent taste	<i>L. plantarum</i> (2.5 log), <i>L. casei</i> (5.1 log), <i>L. rhamnosus</i> (< 1 log), <i>L. casei</i> T4 (8.5 log), <i>L. casei</i> TD4 (5.0 log) after 28 days storage at 4°C	Nematollahi et al. 2016
<i>L. paracasei</i> NFBC 43338	Orange juice	“Medicinal” flavour of probiotic orange juice could be masked by adding 10% (v/v) of tropical fruit juice.	Initial viability of 8.1 log in orange juice	Luckow et al. 2006
<i>L. plantarum</i> DW12	Fermented coconut water	Moderate acceptance of fermented coconut water, presenting sour flavour and fermented odour after 48 h of fermentation	Viability of 8.5 log after 48 h of fermentation	Kantachote et al. 2017
<i>L. casei</i> NRRL B-442	Fermented pineapple juice	Sweetened juice received a higher preference than un-sweetened juice due to Post-acidification of fermented pineapple juice.	Viability of 8.6 log in the pineapple juice after 24 h of fermentation, and 6.0 log in non-sweetened juice and 4.8 in sweetened juice after 42 days storage at 4°C	Costa et al. 2013
<i>L. plantarum</i> B2, <i>L. fermentum</i> PBCC11.5	Fresh-cut cantaloupe	Addition of <i>L. plantarum</i> onto cantaloupe produced off-odor and off-flavor, but not negative effects were found in cantaloupe inoculated with <i>L. fermentum</i> during storage	<i>L. plantarum</i> (8.1 log) and <i>L. fermentum</i> (7.8 log) after 11 days storage at 4°C	Russo et al. 2015
<i>L. rhamnosus</i> GG	Fresh cut apple slices	Apple slices inoculated with <i>L. rhamnosus</i> on day 0 were accepted but a softer texture and lactic acid odour were found	Viability of 8.74 log in unwashed apple slices after 10 days storage at 4°C	Rößle et al. 2010
<i>L. rhamnosus</i> ATCC7469	Dried apple slices	Air dried slices showed a lower acceptance of texture than that of the sliced dried by other two methods on day 0.  Acceptance of air dried slices for taste, flavour, texture, colour and overall was below the acceptance level after 30 days at 25°C and 180 days at 4°C compared with the sliced dried by other two methods.	Viability of 1.0 – 3.0 log in the slices dried by freezing and a combination of air drying and vacuum drying after 120 days storage at 25°C but a higher viability of 9.3 – 7.8 log was found at 4°C for 180 days.  Viability of < 1.0 log and 7.8 log in the air-dried slices was found after 120 days at 25°C and 180 days at 4°C.	Noorbakhsh, Yaghmaee, and Durance 2013
<i>L. pentosus</i> B281, <i>L. plantarum</i> B282	Fermented olive	Mean scores of bitter, acid, hardness, crunchiness in olives fermented with both species were similar. The highest score of overall acceptance was found in <i>L. pentosus</i> fermented olive and the lowest score of 5.15 in mixed species.	Viability of 6.5 – 7.5 log in the olive fermented with <i>L. pentosus</i> , and 5.7 – 7.2 log with <i>L. plantarum</i> , but lower viability of 4.8 – 5.8 log of a mix of both species, after 110 days fermentation at 20 – 22°C.	Argyri et al. 2014
<i>L. pentosus</i> B281, <i>L. plantarum</i> B282	Fermented olives	Bitterness of olives fermented by <i>L. plantarum</i> and stored at both temperatures; olives fermented by <i>L. pentosus</i> and stored at 4°C was the most accepted.	<i>L. pentosus</i> (3.4 log and 4.6 log at 4 and 20°C) and <i>L. plantarum</i> (3.5 log and 4.4 at 4 and 20°C) after 357 days storage	Blana et al. 2016
<i>L. plantarum</i> 33, <i>L. casei</i> Shirota	Olive paste	Paste inoculated with free and encapsulated species induced off-flavour, grainy texture and a bitter taste when compared with conventional olive paste. Mean scores of overall acceptances of all samples were 5.7 – 6.2 in a 9-hedonic scale study.	Viability of 6.0 – 9.0 log in the paste inoculated with free and encapsulated <i>L. plantarum</i> and <i>L. casei</i> at 4°C, and of 4.0 – 5.0 log at 22°C after 30 days storage, furthermore encapsulated cells showed 1.0 – 2.0 log higher survival than free cells.	Alves et al. 2015
<i>L. plantarum</i> 6E, <i>L. rhamnosus</i> SP1	Fermented emmer beverage	Emmer beverage fermented with both species showed more acidic taste and after-taste with more intense flavour than control samples.	<i>L. plantarum</i> (8.1 log) and <i>L. rhamnosus</i> (8.9 log) after 30 days storage at 4°C	Coda et al. (2011)

(Continued on next page)

Table 4. (Continued)

Probiotic Bacteria	Type of Food	Sensory Evaluation	Viability (log CFU per ml or g)	References
<i>L. acidophilus</i> NCIMB8821, <i>L. plantarum</i> NCIMB8826, <i>L. reuteri</i> NCIMB11951	Fermented beverage made from oats, barley or malt	Mean scores between 2.71 and 5.33 of consumers' acceptance in a 9-hodonic scale study. Barley and malt beverage fermented with <i>L. plantarum</i> showed the highest scores than other fermented beverages.	Viability between 7.8 and 8.1 log of the three species in fermented beverage after 10 h of fermentation at 37°C.	Salmerón, Thomas, and Pandiella (2015)
<i>L. plantarum</i> 12, <i>L. casei</i> LC01, <i>B. animalis</i> BB12	Rice drink	higher sensory scores in attenuated bacteria samples	Storage stability was not studied. ultra-sound attenuated <i>L. plantarum</i> (8.0 log) 12 after 11 days storage 4°C	Bevilacqua et al. (2016)
<i>L. acidophilus</i> (strains not reported)	Bread	Crust appearance, colour, crispiness and hardness of bread coated with different layers of <i>L. acidophilus</i> were lower than the control bread.	Viability of 7.3 – 7.5 log in fresh bread coated with one, two or three layers of encapsulated cells, and their viability decreased to 6.1 – 6.2 log after 24 h storage at 25°C 61% RH. Viability of free cells was < 1.0 log.	Altamirano-Fortoul et al. (2012)
<i>L. acidophilus</i> MJLA1, <i>L. rhamnosus</i> 100-C, <i>L. paracasei</i> 01, <i>B. lactis</i> BDBB 2, <i>B. lactis</i> BB-12, <i>S. boulardii</i> 74012	Frozen soy dessert	Soy dessert containing <i>L. acidophilus</i> was not discriminated from the control, but containing <i>S. boulardii</i> was different from the control and <i>L. acidophilus</i> samples and developed off-flavour during the storage	>7.0 logs for all strains of lactobacilli and bifidobacteria, but <i>S. boulardii</i> was < 6.0 log after 6 months storage at –20°	Heenan et al. (2004)
<i>L. rhamnosus</i> GG, <i>L. paracasei</i> F19, <i>L. casei</i> DG, <i>L. reuteri</i> DSM17938	Dark chocolate (with 80% cocoa)	Mean scores of 7.85 – 8.02 on appearance, flavour, texture, colour and overall acceptance of dark chocolate inoculated by all species but these species pre-suspended in UHT milk had negative effects for all sensory characteristics.	<i>L. rhamnosus</i> (7.8 log), <i>L. paracasei</i> (8.1 log), <i>L. casei</i> (7.8 log), <i>L. reuteri</i> (5.3 log) compared with a pre-suspension of bacteria in UHT milk, <i>L. rhamnosus</i> (6.6 log), <i>L. paracasei</i> (6.2 log), <i>L. casei</i> (6.1 log), <i>L. reuteri</i> (4.3 log) after 90 days storage at 18°C	Succi et al. (2017)
<i>L. acidophilus</i> NCFM, <i>B. lactis</i> HN019	Dark chocolate	Lower mean scores of colour, gloss, form and surface of chocolate inoculated with <i>L. acidophilus</i> than that with <i>B. lactis</i> after 180 days storage at 20°C	<i>L. acidophilus</i> (8.5 log at 4°C and 7.8 at 20°C log), <i>B. lactis</i> (<1 log at both temperatures) after 180 days storage	Laličić-Petronijević et al. (2015)
<i>L. acidophilus</i> CCDM 476, <i>B. animalis</i> 241a	Fermented mutton sausages	Better texture and reduced typical smell of mutton	<i>L. acidophilus</i> (6.0 log) and <i>B. animalis</i> (<1.0 log) after 60 days storage (storage temperature not reported)	Hoobin et al. (2013)
<i>L. reuteri</i> ATCC 55730	Dry fermented salami	Mean scores of 7.04 – 7.34 for appearance, flavour, texture and overall acceptance. Addition of <i>L. reuteri</i> to the salami did not affect the scores significantly.	Free cells (4.5 log), micro-capsulated cells by extrusion (6.8 log) and by emulsion (6.6 log) after 27 days storage at 13°C and 75% RH	Muthukumarasamy and Holley (2006)

- Identification of suitable microencapsulation materials to enhance probiotic viability and survivability in non-dairy food matrices.
- Assurance of microbiological quality and safety of non-dairy probiotics products.
- Development of efficient methods to confirm the probiotic effects of a products so that product labelling will be highly useful in future.

## Conclusion

The potential applications of some probiotic bacteria incorporated into four groups of non-dairy foods (fruit and vegetables, cereals, meat and meat products, and soy) were discussed. A better understanding of physiological and technological properties of probiotic bacteria and food matrices as well as their combinations through scientific investigations are key elements to produce innovative probiotic food products. In terms of developing innovative probiotic non-dairy products, it is important to consider factors such as viability and stability of probiotic

bacteria, resistance to oxidation, pH, temperature and other stresses, appropriate levels of water content, utilization of carbohydrates and metabolites from non-dairy foods with or without fermentation. Most probiotic food market currently are dairy based, non-dairy food matrices have shown potential for delivering probiotics with high viability and could be an ideal alternative to provide benefits without milk proteins, lactose, saturated fat and cholesterol.

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