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Review

Review on Non-Dairy Probiotics and Their Use in Non-Dairy Based Products

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Abstract: Consumer demands for foods promoting health while preventing diseases have led to development of functional foods that contain probiotic bacteria. Fermented dairy products are good substrates for probiotic delivery, but the large number of lactose intolerant people, their high fat and cholesterol content and also due to the growing vegetarianism the consumers are seeking for alternatives. Therefore, researches have been widely studied the feasibility of probiotic bacteria in non-dairy products such as fruits, vegetables, and cereals. This review describes the application of probiotic cultures in non-dairy food products.

Keywords: encapsulation; non-dairy probiotics; chocolate; meat; juices; cereal

1. Introduction

Foods are not only destined to fulfill hunger and to provide necessary nutrients for humans but also to prevent or reduce the development of nutrition-related diseases and to improve physical and mental well-being [1]. In this regard, functional foods play an outstanding role. The term functional food was first used in Japan, where the concept of food designed to be medically beneficial to the consumer evolved during the 1980s [2]. Functional food can be defined as food or dietary components that may provide a health benefit beyond the basic function of providing nutrients [3]. Probiotic foods are the fastest growing field of functional food production. Probiotic cultures are successfully applied in many types of food matrices. Several food products including dairy, meats, beverages, cereals, vegetables, and fruit have been utilized as delivery vehicles for probiotics. Classification of probiotic foods is shown in Figure 1. A number of health benefits have been linked to the consumption of probiotic products such as treatment of diarrhea, alleviation of symptoms of lactose intolerance, reduction of blood cholesterol, treatment of irritable bowel syndrome, and inflammatory bowel disease, anti-carcinogenic properties, synthesis of vitamins, and enhancing immunity [4].

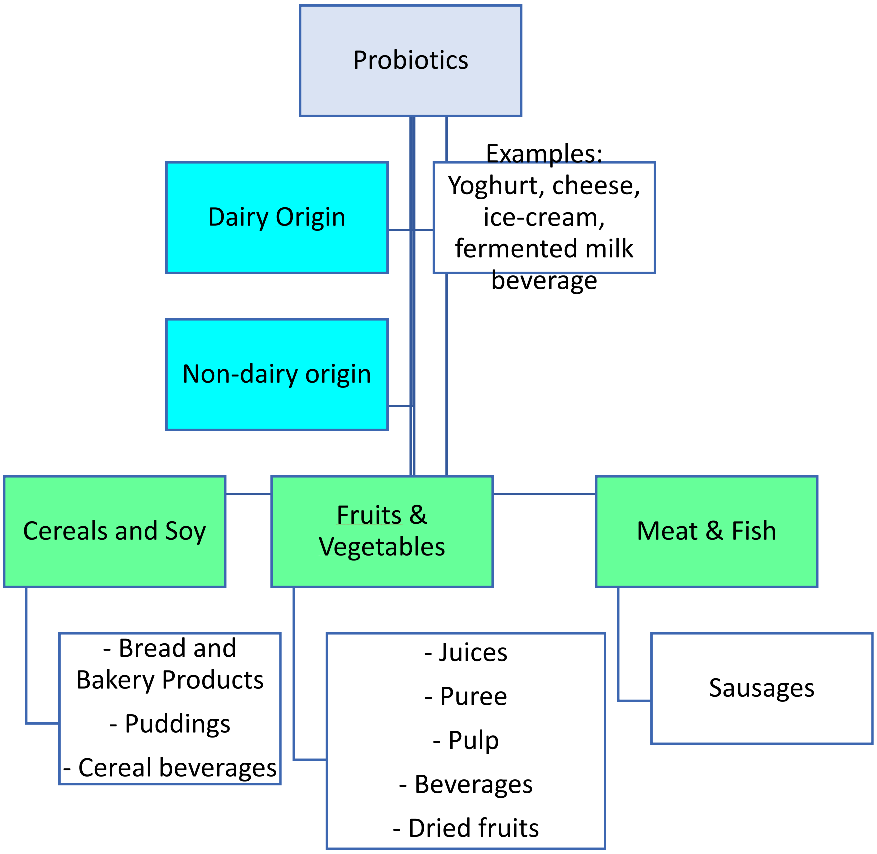

Figure 1. Classification of probiotic foods.

Based on its Greek etymology, probiotic is the combination of the words "pro bios" that means "for life" [5]. Specifically, probiotics are living microorganisms which contribute to the overall health of the host when they are provided in adequate amounts (FAO/WHO, 2001). Two additional terms that are significant are prebiotics and synbiotics. Prebiotics are defined as the indigestible food ingredients that promote the growth and activity of beneficial bacteria in the intestine, thereby benefiting the host, while could be providing textural attributes to the foods, while synbiotics are combinations of probiotics and prebiotics that are designed to improve the survival and the colonization of the ingested microorganisms to the intestinal tract Prebiotics are the indigestible food ingredients that stimulate the growth and activity of favorable bacteria in the intestine, thus being of advantage to the host, while also potentially adding textural attributes to the foods [6].

Traditionally, probiotics were commonly recognized to be derived from yoghurt and other dairy-based products. However, in recent years alternative non-dairy foods have been used for the isolation of potential probiotic isolates and to determine whether they are suitable for the production of novel nondairy probiotic products. This chapter reviews the present knowledge regarding various non-dairy-based probiotics available worldwide based on fruits, vegetables, cereals, chocolate-based products and meat products in order to give an insight to the subject and to show a way forward for the future.

2. Why Do We Need Non-Dairy Probiotics?

As mentioned above, dairy products have been traditionally considered as the best carriers for probiotic bacteria. In recent years, non-dairy probiotic foods have been attracting more attention due to consumer demands [7]. Therefore, in order to alleviate the drawbacks of dairy based probiotics, the development of non-dairy probiotic products, including food matrices from fruit, vegetables, and cereals has a promising future [8].

3. Market Potential for Non-Dairy Probiotics

The probiotic foods market is growing, globally, very rapidly due to increased consumer awareness about the impact of food on health. Today, probiotic products comprise between 60% and 70% of the total functional food market [9]. The global market for probiotic foods and drinks was around 24.8 billion euro in 2011, over 31.1 billion euro in 2015 and is predicted to reach around 43 billion euro by 2020 [10].

Many non-dairy probiotic drinks are already present in the market. The first non-dairy probiotic product came from a Swedish company Skane Dairy in 1994 with brand name ProViva [11]. The base substrate is oatmeal gruel being fermented by *Lactobacillus plantarum* 299v and malted barley was added to improve liquefaction of the product. Fermented oatmeal gruel was mixed at a concentration of 5% with different fruit juices like rose hip, black currant, blueberry, strawberry, or tropical fruits. The final product contains ~5×10^{10} cfu/l of *Lactobacillus plantarum* 299 v/L [12]. A similar product GoodBelly, prepared from oatmeal and *Lactobacillus plantarum* 299v was the first nondairy probiotic drink in US market in 2006 [13].

In addition, several cereal probiotic foods have showed up recently, such as CornyActiv® cereal bars in Germany, Muesli® probiotic flakes in Portugal, Weetaflakes® whole wheat breakfast cereals in France, wholegrain porridge in UK, and Goodness® snack bar in UK, Whole Grain Probiotic LiquidR (Grainfields, Australia) [14]. Table 1 presents some examples of non-dairy probiotic products that are available in the market.

Table 1. Commercially available non-dairy based probiotic products.

Probiotic Product	Type	Probiotic Microorganisms	Company
Avenly velle	Oat based drink	*Lactobacillus* and *Bifidobacterium*	Avenly Oy Ltd., Finland
Biola	Fruit Juice	*Lacctobacillus rhamnosus* GG	Tine BA, Norway
Bioprofit	Fruit Juice	*Lactobacillus rhamnosus* GG, *Probionibacterium freudenreichii*, *Shermanii JS*	Valio Ltd., Finland
Bravo Friscus	Fruit Juice	*Lactobacillus plantarum* HEAL9, *Lactobacillus paracasei* 8700:2	Skanemajerier, Sweden
Gefilus	Fruit Juice	*Lactobacillus rhamnosus* GG	Valio Ltd., Finland
GoodBelly drink	Fruit Juice	*Lactobacillus plantarum* 299v	NextFoods, Colorado
Grainfields wholegrain liquid	Grains, beans and seeds	*Lactobacillus acidophilus*, *Lactobacillus delbreukki*, *Saccharomyces boulardii*, *Saccharomyces cerevisiae*	AGM Foods Pvt. Ltd., Australia
Healthy life probiotics	Fruit Juice	*Lactobacillus paracasei* 8700:2, *Lactobacillus plantarum* Hea19	Golden circle, Australia
Kefir Soy	Soya beans	*Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Lactobacillus brevis*, *Lactobacillus. kefir*, *Leuconostoc mesenteroides*, *Lactobacillus helveticus*	Life way, Greek
KeVita	Sparkling lemon and ginger drink	*Bacillus coagulans* GBI-306086, *Lactobacillus paracasei* 8700:2, *Lactobacillus plantarum* HEAL 9	H-E-B, USA
Mucilon	Oat and Rice	*Bifidus* BL	Nestle
Proviva	Fermented fruit drink with oatmeal	*Lactobacillus plantarum* 299v	Skane Dairy, Sweden
Rela	Fruit Juice	*Lactobacillus reuteri* MM53	Biogaia, Sweden

4. Probiotic Preparation and Viability

The health benefits of probiotic strains are mainly dependent on their ability to survive the passage through the upper GT, colonize, and multiply in the host intestine [15]. If not an adequate number of viable probiotic bacteria enter the target site, the probiotic product would not be useful. Numerous reviews on probiotics highlight the loss of probiotic survival during processing, storage and after digestion [16]. Therefore, the main challenge for the effectiveness of a probiotic food product is to maintain the viability of the probiotic strain which is a prerequisite for achieving health benefits since the health benefits of probiotic food products depends upon the number of viable cells present at the moment of consumption [17].

The above fact is highlighted by WHO/FAO (2002), which has established the criterion that any food claimed to have a probiotic effect must contain at least 10^6 to 10^7 cfu/mL of viable probiotic bacteria. There are many stages that can affect the viability and survival of probiotic bacteria. The main stages that affect the probiotic viability and survival is processing, storage, handling, transport and shelf-life of the probiotic food. Finally, following ingestion, probiotics must be able to survive the acidic conditions of the stomach as well as the bile salts in the small intestine, before they reach the lower gastrointestinal tract where they will provide beneficial effects.

The main factors that affect the viability and activity of probiotic cultures include environmental, food and processing parameters such as pH, titratable acidity, water activity, incubation temperature, presence of salt, sugar, and chemicals like hydrogen peroxide, molecular oxygen, bacteriocins, artificial flavorin, coloring agents, heat treatment, rate and proportion of inoculation, strain species, packaging materials and conditions, storage methods, and conditions [18]. Moreover, apart from the production and storage factors, probiotic survival can also be affected by the acidity of the stomach, bile salts, enzymes such as lysozyme present in the intestine, toxic metabolites including phenols produced during digestion, bacteriophage, antibiotics, and anaerobic conditions [19].

Several attempts have been made to improve the growth and the viability of probiotics in different food products during their production and shelf life [20]. Strategies to improve viability of probiotic organisms include the suitable selection of acid and bile resistant strains, use of oxygen impermeable containers, two-step fermentation, microencapsulation and incorporation of micronutrients such as peptides and amino acids [21].

Microencapsulation is the most investigated method in order to enhance the ability of probiotics to survive under adverse conditions such as low pH, oxygen, extreme temperatures during production, storage, and gastrointestinal transit (Figure 2). In addition, microencapsulation may be an interesting alternative to reduce the negative attributes that the probiotic cultures may cause in food products [22]. There are many techniques that can be used to microencapsulated probiotic bacteria such as extrusion, emulsion, spray drying and freeze drying [23]. A detailed review on microencapsulation techniques and their effects is recently published by [23,24]. Table 2 gives a summary of recent applications of encapsulation technologies on probiotic bacteria used for the production of probiotic non-dairy beverages.

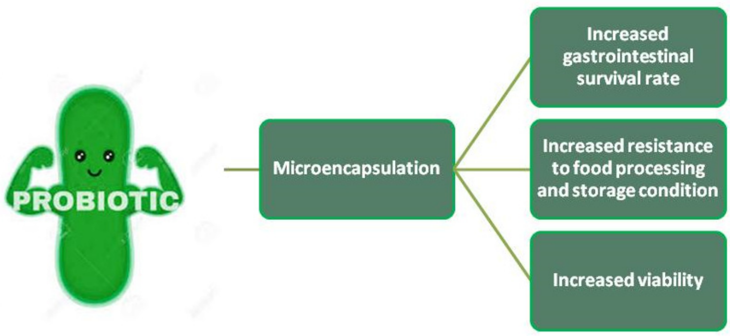

Figure 2. Role of microencapsulation on probiotic microorganisms.

Table 2. Applications of encapsulation technologies on probiotic bacteria used for non-dairy beverages.

Probiotic Beverage	Probiotic Bacteria	Encapsulation Material	Encapsulation Technique	Reference
Mandarin Juice	*Lactobacillus* salivarius spp. *salivarius* CECT 4063	Alginate	Emulsion	[25]
Pineapple, raspberry and orange juice	*Lactobacillus casei* (DSM 20011)	Alginate	Vibration technology	[26]
Apple Juice	*Lactobacillus rhamnosus* GG	Chitosan-alginate with/without inulin	Extrusion	[27]
	Lactobacillus rhamnosus GG	Alginate-Silica	Freeze drying	[28]
Passion Fruit Juice	*Bifidobacterium animalis* ssp. *lactis* BB-12	Maltodextrin and/or inulin	Spay drying	[29]
Grape Juice	*Lactobacillus acidophilus* and *Bifidobacterium bifidum*	Alginate	Internal gelation	[30]

5. Challenges of Probiotics in Non-Dairy Products

Application of probiotic cultures in non-dairy products represents a great challenge. As mentioned above, the choice of food matrix is important for the viability of probiotics during both processing and storage. The survival and stability of probiotics during production and storage of fruit, vegetables, and cereal probiotic foods has been shown to not only depend on the food matrix, water activity, and pH of the final products, but also on the selection of the probiotic strains. Although fruit and vegetable juices contain some essential nutrients, there are factors that may limit probiotic viability such as low pH which is associated with the high levels of organic acids and dissolved oxygen [16]. In addition, dairy products are generally stored at temperatures close to 5 °C, so probiotic cell viability is probably guaranteed during product shelf life. Storage at room temperature, which is common for many types of nondairy products can create a great challenge for probiotic viability [31]. Figure 3 summarizes the main advantages and disadvantages of using probiotics in fruit, vegetables, and cereals.

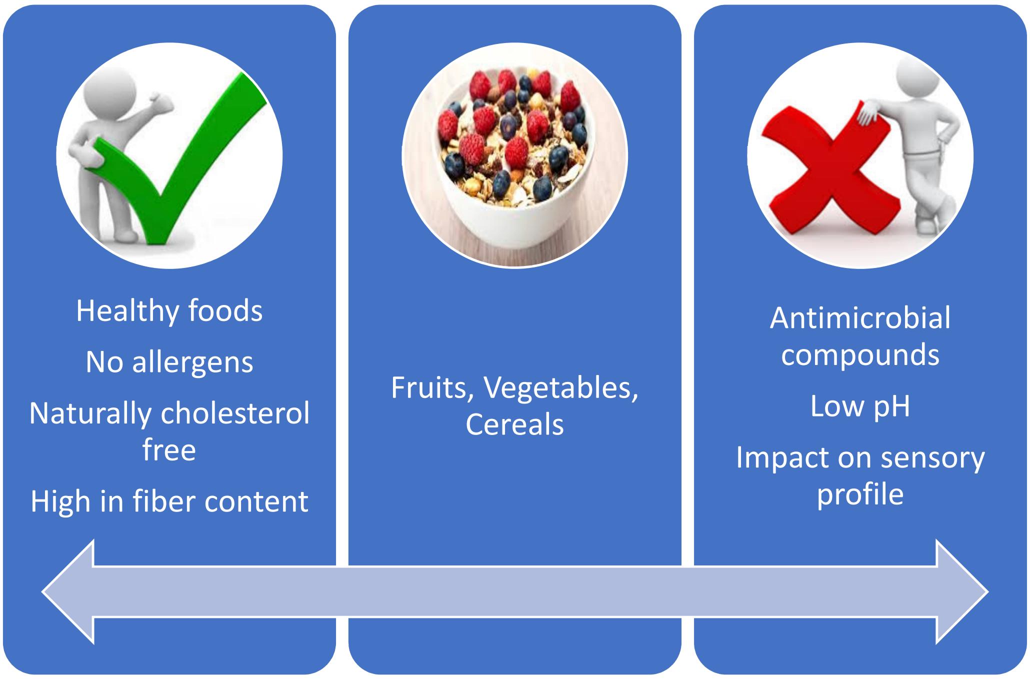

Figure 3. Advantages and disadvantages of using probiotics, fruits, vegetables, and cereals.

6. Sensory and Overall Acceptance of Non-Dairy Probiotics

In addition to previous challenges, sensory traits and overall acceptance of non-dairy probiotics are also showing some limitations. Therefore, the sensory evaluation of probiotic microorganisms in non-dairy products has vital commercial importance.

It is important to consider the sensory acceptance by the consumers during the development of non-dairy probiotic products with respect to appearance, aroma, texture or taste, with the goal of conveying the direction for the optimal production and formulation of these products [32]. The sensory properties of non-dairy probiotic foods may be influenced by interactions between different probiotics strains and food substrates, where textures, taste, flavor, aroma, and color might be improved or aggravated by the production of different metabolic compounds such as lactic acid and other metabolites in living cells by different species during processing and storage [13]. Therefore, it is important to review not only the good probiotic survival, but also the sensory acceptance during production and storage of probiotic non-dairy foods.

In the preparation of probiotic food, the probiotic bacteria ferment the carbohydrates present in fruits, vegetables, cereal, and legumes and release gases and alcohol [13]. It has been reported that probiotification of fruit juice can result in flavors described as "dairy," "medicinal," "acidic," "salty," "bitter," "astringent," "artificial," or "earthy" [33]. Depending on the kind of fruit, probiotic organism, the temperature in which they are stored and the supplementation of prebiotics and protectants, it can affect the sensorial properties of the probiotic juice [34]. Several studied have demonstrated that probiotics did not affect the overall acceptance of fruit juices. For instance Perricone et al. [18] showed that there are no negative effects on the flavor of pineapple juice containing *Lactobacillus reuteri*. A possible solution to reduce the sensations of unpleasant odours and flavors in non-dairy probiotic foods is masking, such as adding a pleasant aroma and volatile compounds which can disguise ("mask") the existence of probiotics. Specifically, the incorporation of tropical fruit juices like pineapple, mango or passion fruit (10%, *v*/*v*) has been reported by Luckow et al. [35] that it can be instrumental in the final product's aroma and flavor.

7. Examples of Non-Dairy Probiotics

7.1. Fruits and Vegetables

Fruits and vegetables are considered healthy foods and are an ideal medium for the functional ingredients as they contain several beneficial nutrients such as various phytochemicals, antioxidants, minerals, vitamins, and dietary fibers [36]. They have a high nutrient and sugar content that is important for probiotic growth and in combination with the fast passage through the harsh acidic conditions of stomach result in high probiotic cell viability [37]. In contrast to dairy products, fruits and vegetables lack allergens, lactose, and cholesterol, that adversely affect certain groups of the population. Fruits are healthy, refreshing, and have an appealing taste and flavor for all the population groups [13]. All these characteristics have drawn some researchers' interest in developing fruit juice based probiotic beverages.

Pineapple, cranberry, strawberry, sweet lime, mango, grapes, cashew apple, olive, carrot, beetroot, and oranges are some examples of fruit juices used as food substrates for probiotic bacteria delivery. A variety of types of probiotic fruit and vegetable products have been developed and commercialised, including juices of fruits and vegetables, dried fruits, fermented vegetables, and deserts for vegetarians. Even though, there are many studies showing the feasibility of incorporating probiotic bacteria into fruit and vegetables, their viability and stability in these foods have been found to be highly strain dependent [38]. Wide range of probiotic strains, mainly species of *Lactobacillus* and *Bifidobacteria*, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum*, *L actobacillus fermentum*, and *Bifidobacterium bifidum* have been widely used in the development of many fruit and vegetable probiotic products. Some examples of fruit and vegetables probiotic products are summarised in Table 3.

Table 3. Examples of fruit and vegetables probiotic products.

Product	Probiotic Microorganism	Reference
Apple	*Lactobacillus casei*	[39]
	Lactobacillus plantarum ATCC14917	[40]
Apricot Juice	*Bifidobacterium lactis* Bb-12, *Bifidobacterium longum* Bb-46, *Lactobacillus casei* 01 *and Lactobacillus acidophilus* La-5	[41]
Banana puree	*Lactobacillus acidophilus*	[42]
Beet Juice	*Lactobacillus plantarum, Lactobacillus casei, Lactobacillus delbrueckii*	[43]
Beverage with juçara fruit	*Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5	[44]
Blended orange, carrot, apple and Chinese jujube juice	*Lactobacillus plantarum* CICC20265, *Bifidobacterium breve* CICC6184, *and Streptococcus thermophilus* CICC6220	[45]
Cabbage Juice	*Lactobacillus plantarum* C3, *Lactobacillus delbrueckii* D7	[46]
Carrot and orange juice	*Lactobacillus plantarum*	[47]
Cashew apple juice	*Lactobacillus plantarum*	[48]
	Lactobacillus casei	[49]
Cantaloupe juice	*Lactobacillus casei* NRRL B-442	[50]
Cherry juice	*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus*	[51]
Cornelian cherry juice	*Lactobacillus plantarum* ATCC 14917	[52]
Fruit smoothies	*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis spp. lactis* BB-12	[53]
Honeydew melon juice	*Lactobacillus casei* NCIMB 4114	[54]
Litchi juice	*Lactobacillus casei*	[55]
Mango and guava juice	*Lactobacillus casei*, *Streptococcus thermophillus*, *Lactobacilluc bulgaricus*	[56]
Moringa leaves and beetroot beverage	*Lactobacillus plantarum, Enterococcus hirae*	[57]
Orange, grapefruit, black currant, pineapple, pomegranate, cranberry and lemon juice	*Lactobacillus plantarum*	[58]
Orange, pineapple and cranberry juice	*Lactobacillus casei* DN 114001, *Lactobacillus rhamnosus* GG, *Lactobacillus paracasei* NFBC 43338, *Bifidobacterium lactis* BB-12	[59]
Passion fruit juice	*Bifidobacterium animalis subsp. lactis* BB-12	[29]
Pear Juice	*Lactobacillus acidophilus*	[60]
Pineapple Juice	*Lactobacillus plantarum* 299V, *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb-12	[61]
Plum Juice	*Lactobacillus kefiranofaciens*, *Candida kefir*, *Saccharomyces boluradii*	[62]
Pomegranate juice	*Lactobacillus plantarum* ATCC 14917	[63]
	Lactobacillus plantarum, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*	[64]
Pumpkin juice	*Lactobacillus reuteri*	[65]
Sohiong juice	*Lactobacillus plantarum* MCC 2974	[66]
Star fruit juice	*Lactobacillus helveticus* L10, *Lactobacillus paracasei* L26, and *Lactobacillus rhamnosus* HN001	[67]
Table olives	*Lactobacillus* GG, *Lactobacillus paracasei*	[68]
	Lactobacillus plantarum	[69]
Tomato Juice	*Lactobacillus plantarum* and *Lactobacillus casei*	[70]
	Lactobacillus acidophilus LA39, *Lactobacillus plantarum* C3, *Lactobacillus casei* A4, *Lactobacillus delbrueckii* D7	[71]
Vegetable probiotic beverage (beetroot, carrot, celery, honey)	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Saccharomyces boulardii*	[72]
Vegetable-fruit beverage	*Lactobacillus plantarum*	[73]
Yam	*Lactobacillus acidophilus*	[74]

The amount of necessary peptides and free amino acids in juices is insufficient for the metabolism of probiotic culture. Additional factors that influence the stability of probiotics in fruit matrices are the microorganism strain and its inoculum, pH, acidity, water activity, oxygen concentration, the presence of antimicrobial compounds, artificial and natural dyes and flavors, preservatives and last but not least the production processes and subsequent handling (pasteurization, storage temperature and packaging material used) [18]. Among these factors, pH is the most important influencing the probiotic survival. Although fruits are a good substrate for the growth of probiotic bacteria, it is more complicated for these microorganisms to survive in such an arrangement than it is in dairy products due to the acid conditions of the fruit from which the probiotic bacteria need to be protected [75]. Generally, fruits have a low pH and high levels of organic acids which increases the concentration of undissociated form. Therefore, the viability of probiotic bacteria is determined by the high acidic environment and the intrinsic antimicrobial activity of accumulated organic acids. Lactobacilli can resist and survive better than bifidobacteria in fruit juices with pH of 3.7 to 4.3 [76].

However, in some cases fruit juices may contain ingredients that sustain the viability of probiotics like ascorbic acid, that decreases redox potential, saccharides or organic acids that can be used as a carbon source or cellulose that can protect probiotics during processing and storage [8]. Probiotic juices can be produced either with direct addition of the probiotic strain into the juice or by the fermentation of the juice with the probiotic bacteria. The latter is more advantageous than the direct addition due to the fact that the microbial strain growth into the juice conduces to a low-sugar product and a more adapted microbial strain, which could advance its survival rates [75]. Also, during the fermentation of the juice with the probiotic bacteria, microbial metabolites such as bacteriocins can be produced that can help to improve the quality of the product and increase their shelf-life during storage.

Numerous studies have been published on how to improve the survival of probiotic bacteria in fruit juices. The most attractive and successful methods are fortification by prebiotics such as dietary fiber, cellulose or with certain ingredients capable of exerting a protective effect in fruit juice, refrigerated storage in atmosphere enriched carbon dioxide, addition of antioxidants or the probiotics encapsulation [77]. In a study carried out by [78] demonstrated the enrichment of beetroot juice and carrot juice with brewer's yeast autolysate before fermentation with *Lactobacillus acidophilus* enriched the juices with pigments, vitamins and minerals, decreased the fermentation time and affects positively the viability of probiotic bacteria.

Also, in another study by [79] demonstrated that fortification of apple juice with oat flour, containing 20% of β-glucan, could protect *Lactobacillus rhamnosus* during refrigerated storage.

To develop novel probiotic fruit products, many studies have been carried out. Yoon et al. determined the suitability of tomato [71], beet juice [43], and cabbage juice [46] using four strains of probiotic LAB; *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus delbrueckii*. The viable cell counts of the four lactic acid bacteria in all fermented products raged from 10^5 to 10^8 cfu/mL after 4 weeks of cold storage at 4 °C. In another study, [59] showed that *Lactobacillus casei* DN-114 001, *Lactobacillus rhamnosus* GG, and *Lactobacillus paracasei* NFBC43338 strains could survive in orange and pineapple juice for at least 12 weeks of storage under refrigeration with cell counts above 10^7 cfu/mL and 10^6 cfu/mL respectively. Reference [80] demonstrated that carrot juice can support the growth of *Bifidobacterium lactis* Bb-12, Bifidobacterium *bifidum* B7 and *Bifidobacterium bifidum* B 3.2. All strains showed high initial viable cell counts of 10^{10} cfu/mL. Wang et al. [81] studied whether noni juice as a raw material to produce probiotics was suitable and discovered that *Bifidobacterium longum* and *Lactobacillus plantarum* have the ability to be optimal probiotics for fermented noni juice. Study [50] showed that the probiotic strain *Lactobacillus casei* B-442 can be successfully used for the fermentation of cantaloupe melon juice. They found that an initial pH of 6.1 resulted in good cell viability (10^8 cfu/mL) and this level was kept over the 42 days of refrigerated storage. The authors of [49] studied the ability of *Lactobacillus casei* NRRL B442 to ferment and survive in cashew apple juice and a high viability of *Lactobacillus casei* was found during the 42 days refrigerated storage that had viable cell counts higher than 10^8 cfu/mL. By using the two Lactobacillus strains (*Lactobacillus* *plantarum* and *Lactobacillus acidophilus*) to produce probiotic orange juice and probiotic grape juice, it was shown that, despite their high acidity, both cultures maintained good viability [82]. In a study by [52], a Cornelian cherry juice produced with the application of probiotic *Lactobacillus plantarum* ATCC 14917. Results of the study showed that *Lactobacillus plantarum* ATCC 14917 was remained viable during the whole cold storage period and no significant sensory differences were observed among the fermented and the non-fermented samples. Also in another study by the same group [63], showed that the same probiotic strain can be used for the fermentation of pomegranate juice. Reference [41] used as a substrate apricot juice for the production of a novel non-dairy probiotic beverage. In this study, mono- and mixed cultures was investigated. All tested strains were used for the fermentation of the juice. Fermentation of juiced carried out using probiotic bacteria individually showed cell yields of, 7.2, 7.25, 7.06, and 7.16 log (cfu/mL h) *Bifidobacterium lactis* Bb-12, *Bifidobacterium longum* Bb-46, *Lactobacillus casei* 01, and *Lactobacillus acidophilus* La-5 strains, respectively, while juice fermented by a mixed bacteria culture a higher cell yields was observed. Study [67] evaluated the ability of star fruit which is popular in Southeast Asia to support the growth and viability of three commercial probiotic strains, *Lactobacillus helveticus* L10, *Lactobacillus paracasei* L26, and *Lactobacillus rhamnosus* HN001, showing that all strains grew well with the final cell counts of around 10^8 cfu/mL. A recent study by [61] showed that pineapple juice can be used for the production of probiotic beverage with probiotic bacteria Lactobacillus and Bifidobacterium. Both strains exhibited good growth properties on pineapple juice during the specified storage period. In another study by [40], *showed that Lactobacillus plantarum* ATCC 14917 were able to ferment the apple juice demonstration that *Lactobacillus plantarum* ATCC 14917 can modified positively the phenolic composition of apple juice and enhanced its overall antioxidant capacity. It was demonstrated by [66] that it is possible to use Sohiong (*Prunus nepalensis*) juice to develop a possible probiotic product. The juice was fermented using *Lactobacillus plantarum* subsp. *plantarum*. Results showed that probiotic strains survived at the end of fermentation during storage period at 4 ± 1 °C in the Sohiong juice with final viable cell count above the recommended minimum value of 10^6 cfu/mL.

7.2. Cereals

One of the staple foods that are consumed daily all over the world are cereals like wheat, maize, oat, barley, and other grains, which have complex nutrient composition [83]. Cereals offer a substantial amount of vitamins, minerals, proteins, carbohydrates, fiber, and oligosaccharides. These constituents enable cereals to be a suitable way to grow probiotic bacteria, they are consequently considered healthy non-dairy carriers for the preparation of probiotic foods [84].

One of the oldest processing methods still in practice is the fermentation of cereals, done in countries such as Asia and African to produce beverages, gruels and porridge [83]. To this end, different types of cereal grains like maize, sorghum, millet, oats, barley, wheat, and rye have being used. An advantage of fermented cereals is that the fermentation process can increase the availability of minerals such as phosphorous, calcium, and iron, because of either the action of microbial enzymes like phytases, or because of the organic acids produced [84]. Another advantage of the consumption of cereal food products is due to the availability of dietary fiber and the presence of non-digestible carbohydrates such as oligosaccharides that can act as a prebiotics and can stimulate the growth of probiotic LAB present in the colon [83]. It is therefore concluded that by fermenting cereals can be an inexpensive way to obtain a rich substrate which supports the probiotic bacteria's growth [85].

Even though non-dairy fermented cereal food products have been created and used for human nutrition throughout the years, it is only lately that scientists have evaluated the probiotic potential of microorganisms which are involved in cereals fermentation. Some examples of non-dairy cereal-based fermented beverages created historically are, Boza, Bushera, Pozol, Mahewu, and Togwa. Some examples of traditional nonalcoholic beverages manufactured with cereals are described below.

To begin with, Boza is a traditional cold beverage consumed in many countries such as Bulgaria, Albania, Turkey, and Romania. It is obtained from the spontaneous fermentation of cereals like wheat, rye, millet, maize, and other that are blended with sugar [84]. Boza shows a large diversity of LAB and yeasts which include *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus coprophilus*, *Leuconostoc reffinolactis*, *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum*, and *Geotrichum candidum* [86]. Bushera is another traditional beverage of Ugandan made with shorgum or millet flour. *Lactobacillus*, mainly *Lactobacillus brevis*, but also *Lactococcus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus* were LAB isolated from Bushera [87]. In South Africa, a probiotic product consumed is Mahewu which is a sour cereal based. It is made by a multi-grain mix which can includes maize, sorghum, millet, malt and wheat flour [13]. The spontaneous fermentation process is performed by the malt's natural microflora at room temperature. The main microorganism isolated from Mahewu is *Lactococcus lactis* subsp. *lactis* [88]. A probiotic product that evolved from Japan and China is Togwa. It is produced by fermenting multi-grains like maize, sorghum, finger millet flour with probiotic bacteria like *Lactobacillus plantarum* and *Streptococcus* [13].

Several studies have been carried out to develop cereal probiotic products and to evaluate the suitability of different cereal grains to enhance probiotic bacteria growth and maintain their viability. Some examples of cereal probiotic products are summarised in Table 4.

Table 4. Examples of cereal probiotic products.

Cereal Probiotic Product	Probiotic Strains	Viable Cell Counts	Reference
Barley malt fermented beverage	*Weissella cibaria*	10^7 cfu/mL	[89]
Beverage from rice, barley, oats, wheat, soy flour and red grape juice	*Lactobacillus plantarum* 6E and M6	10^8 cfu/mL	[90]
Breadfruit Flour beverage	*Lactobacillus plantarum* DPC 206, *Lactobacillus acidophilus* "de Winkel", *Lactobacillus casei* Shirota	10^7–10^8 cfu/mL	[91]
Cashew juice	*Lactobacillus casei* NRRL B 442	10^8 cfu/mL	[49]
Fermented beverage from maize and rice	*Lactobacillus plantarum*, *Torulaspora delbrueckii*, *Lactobacillus acidophilus*	10^7 cfu/mL	[92]
Fermented oat flour	*Streptococcus thermophilus* TH-4, *Lactobacillus acidophilus* LA-5	10^6 cfu/g	[93]
Fermented oat flour beverage	*Lactobacillus plantarum*	10^{14} cfu/mL	[94]
Legume sprouts	*Lactobacillllus plantarum* 299V	10^9 cfu/mL	[95]
maize-based substrate	*Lactobacillus paracasei* LBC-81, *Saccharomyces cerevisiae* CCMA 0731, *Saccharomyces cerevisiae* CCMA 0732 *and Pichia kluyveri* CCMA 0615	10^6 cfu/mL	[96]
Malt beverage	*Lactobacillus plantarum* NCIMB 8826, *Lactobacillus acidophilus* NCIMB 8821	10^8 cfu/mL	[97]
Millet-Based Probiotic Fermented Food	*Lactobacillus rhamnosus* GR-1 and *Streptococcus thermophilus* C106	10^6 cfu/g	[98]
Oat based symbiotic drink	*Rhizopus oryzae*, *L. acidophilus*	10^8 cfu/mL	[99]
Oat-based probiotic drink	*Lactobacillus plantarum* B28; *Lactobacillus reuteri* ATCC 55730	10^8–10^9 cfu/mL	[100]
Peanut milk	*Bifidobacterium pseudocatenulatum* G4	10^8 cfu/mL	[101]
Soymilk	*Lactobacillus acidophilus*	10^8 cfu/mL	[102]
Soymilk with apple juice	*Lactobacillus acidophilus*	10^9 cfu/mL	[103]
Wheat based probiotic beverage	*Lactobacillus acidophilus* NCDC-14, *Lactobacillus acidophilus* NCDC-16	10^8–10^{10} cfu/mL	[104]
Wheat/rice cereal infant products	*Bifidobacterium animalis* subsp. *lactis* BB-12®	10^6 cfu/g	[105]

Reference [106] described that maize porridge prepared using maize flour and barley malt and fermented with four probiotic strains *Lactobacillus reuteri*, *Lb. acidophilus* (LA5 and 1748) and *Lb. rhamnosus* GG can be a suitable substrate for the delivery of probiotics and exhibit high cell viability (10^7–10^8 cfu/g) after fermentation for 12 h at 37 °C. [100] tested the ability of a whole-grain oat substrate to support the growth of probiotic bacteria in order to obtain *a* drink, *combining the health benefits of a* probiotic *culture with the* oat prebiotic β-glucan. They have found that viable cell counts at the end of the process were about 7.5×10^{10} cfu/mL. [107] investigated ability of probiotic bacteria to ferment the following oat samples: whole oat flour, PeriTec white flour, and bran. All samples demonstrated good probiotic growth with values above the minimum required in a probiotic product. The highest value was found in white flour (10^9 cfu/mL) and the lowest in the bran sample (10^8 cfu/mL).

A different study showed that *L. plantarum* was able to grow and survive in fermented products made of extracts of malt, barley and wheat even under refrigerated conditions [108]. The viable cell counts in all fresh cereals extracts (pH 2.9–3.4) ranging between 9.5 and 10.3 log cfu/g after 24 h fermentation. The authors of [109] have reported that both *Lactobacillus casei* and *Lactobacillus plantarum* B2 can grow effectively in fermented rice bran with viable cell counts of 1.07×10^9 cfu/g and 1.25×10^9 cfu/g respectively after 12 h fermentation. A study by [104] was carried out to evaluate the potential of two *Lactobacillus acidophilus* (NCDC-14 and NCDC-16) for development of wheat based probiotic beverage. The authors of [110] have also developed a novel multi-cereal-based fermented beverage using *Lactobacillus helveticus* KLDS1.9204. Study [95] inoculated legume sprouts with *Lactobacilllus plantarum* 299v in order to develop a new functional food. The sprouts that have been enriched with the probiotic culture, a lower mesophilic bacteria flora (especially lactic acid bacteria) was observed compared to the control sprouts (without probiotic). The *Lactobacillus plantarum* population was also stable during the cold storage. In a work by [96], the commercial probiotic, *Lactobacillus paracasei* LBC-81, was used alone and in combination with potential probiotic yeasts, *Saccharomyces cerevisiae* CCMA 0731, *S. cerevisiae* CCMA 0732, and *Pichia kluyveri* CCMA 0615, to ferment a maize-based substrate. Three out of the four probiotic strains showed good viability with counts higher than 10^6 cfu/mL, which is the recommended for food probiotic products, except for the yeast *Pichia kluyveri* which decreased during fermentation and storage time. In a recent study by [105], three novel dehydrated wheat/rice cereal functional products with the addition of probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12® were developed for the infants older than 4 month. BB-12 strain showed the high viability during the storage period of 106 weeks showing the potential to be used for the production of a novel functional cereal product. In another study by [91], a fermented beverage was developed using breadfruit flour as a substrate and a mixture pf probiotic *L. acidophilus* and *L. plantarum* DPC 206 strains. The beverage formulated was found to have acceptable sensory characteristic and good cell viability.

7.3. Meat Products

Meat has been also shown to be an excellent vehicle for the delivery of probiotic bacteria. Fermented sausages (dry sausages) are promising target meat products with probiotic bacteria, as such products are processed without heat treatment and probiotic bacteria can survive in the final product [111].

However, the question still is, if those types of meat products can be called functional foods and consumed daily [112]. The addition of probiotic bacteria to meat products is more difficult, due to the complexity of the meat and the probiotic sensitivity to the processing conditions and the additives using during their production [113]. Some of the main technological factors that can affect their growth and viability include the pH, acidity, presence of other microorganisms (native microflora of meat), water activity, processing and storage temperature, concentration of additives (nitrite, and nitrate) and salt, composition of the protein matrix and the low content of natural sugars. Another important aspect to consider in the preparation of probiotic fermented sausages is their impact on the texture and sensory properties of the final product since the presence of probiotics could change the physicochemical properties such as pH and a_w compared to traditional sausages [114].

In spite of facing these hurdles, fermented sausages are considered good vehicles for the growth and survival of probiotic bacteria because of the protection of the bacterial cells to low pH and bile salts which are exerted from the fat molecules in the passage through the gastrointestinal tract and the stimulation of probiotic growth by the presence of the prebiotic fibers [113]. The most commonly used species of probiotic microorganisms in fermented meat products are: *Lactobaccillus casei*, *Lactobaccillus paracasei*, *Lactobaccillus plantarum*, *Lactobaccillus rhamnosus*, *Lactobaccillus sakei*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*.

The incorporation of the probiotic bacteria can be achieved by replacing the traditional starter culture or by using the traditional culture in consortium with the probiotic strain [113]. Several studies demonstrate the successful application of probiotic strains into different fermented meat products such as *fuet* (low-acid sausage), Italian salami, Swiss salami, mutton fermented sausage, sturgeon fermented sausage, Norwegian salami, Hungarian salami, Longaniza de Pascua, Salchichón (Iberian dry-fermented sausage), and dry cured pork loins (Table 5).

Table 5. Probiotics applied in fermented meat products.

Meat Probiotic Product	Probiotic Strains	Reference
Beef sausage	*Lactobacillus plantarum* TN8 *and Pediococcus acidilactici* MA 18/5M	[115]
Bovine Salami	*Lactobacillus plantarum* 299v	[116]
Dry fermented sausage	*Lactobacillus paracasei* LPC02	[117]
	Lactobacillus plantarum L125	[118]
	Lactobacillus casei ATCC 393	[119]
Dry-fermented pork neck and sausage	*Lactobacillus acidophilus* Bauer, *Bifidobacterium animalis* BB-12 and *Lactobacillus rhamnosus* LOCK900	[120]
Fermented sausage	*Lactobacillus gasseri* JCM1131T, *Lactobacillus fermentum* CTC1693	[121]
	Lactobacillus paracasei and *Lactobacillus rhamnosus GG*	[122]
	Bifidobacterium longum KACC 91563	[123]
Fuet (fermented sausage)	*Lactobacillus rhamnosus* CTC1679	[124]
Italian salami sausage	*Lactobacillus acidophilus*, *Bifidobacterium lactis*	[125]
Pork fermented sausage	*Lactobacillus rhamnosus* FERMP-15120, *Lactobacillus paracasei* subsp. *paracasei* FERMP-15121	[126]
Spanish *Salchichón*	*Lactobacillus paracasei, Lactobacillus rhmanosus* GG	[127]

The authors of [128] studied the ability of *Lactobacillus acidophilus* Bauer and *Lactobacillus casei* Bif3/IV to be used in the production of fermented pork loins. The findings showed a good ability of the probiotic strains to survive under the processing conditions, with the potential to replace the starter culture without altering the sensory and technological characteristics of the final product. In another study by [129] showed that the probiotic strains *Lactobacillus fermentum* HL57and *Pediococcus acidilactici* SP979 can be used for the manufacture of Iberian dry-fermented sausages. However, the inoculation with *L. fermentum* HL 57 increased the amount of acetic acid and lipid degradation products such as malonaldehyde in Iberian dry-fermented sausages, affecting the color and taste negatively. In a study by [124] showed that *Lactobacillus rhamnosus* CTC1679 can be used for the production of a low acid fermented sausage (Fuet) with reduced salt and fat content. *Lactobacillus rhamnosus* CTC1679 was able to grow and reach counts of 10^8 cfu/g without affecting the characteristic sensory properties of the product. Study [120] investigated the ability of *Lactobacillus acidophilus* Bauer, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus rhamnosus* LOCK900 to be used in dry-fermented pork sausage with regards to oxidative and microbiological stability and color changes occurring during 200 days of ripening. The results strongly suggested that *Lactobacillus acidophilus* Bauer preserved the quality of dry-fermented pork neck and sausage better than the *Bifidobacterium animalis* and *Lactobacillus rhamnosus* LOCK0900. Study [115] evaluated the effects on the microbiological and physico-chemical characteristics of fermented beef sausages using a mixed culture of probiotic bacteria (*Lactobacillus plantarum* TN8 and *Pediococcus acidilactici* MA 18/5M) with and dietary fiber. Results showed that these probiotic strains could be used as bioprotective strains to prolong sausage shelf-life and also improving cooking yield and texture. Study [118] evaluated the potential of *Lactobacillus plantarum* L125 to be used as adjunct culture for the production of a probiotic dry-fermented pork sausage. Results showed that the probiotic strain could survive during the specified storage period without affecting the technological and the sensory characteristics of the final product.

7.4. Chocolate

Chocolate is one of the most well-known and extensively consumed product in the world, because of its delicious taste and flavor, high nutritious energy, fast metabolism, and good digestibility. Currently, one of the most important trends in chocolate production has arisen from consumer demand for functional chocolate, such as a chocolate that not only do not adversely affects health, but also prevents diseases such as heart disease, osteoporosis, cancer, diabetes. Cocoa is a functional food because is rich in polyphenolic antioxidants and flavonoids, minerals, proteins, and carbohydrates.

Numerous authors have suggested that chocolate is a good substrate for probiotic bacteria as the lipid fraction of cocoa butter provides protection to probiotics during storage and during upper gastro-intestinal tract passage [130].

Several studies have generated promising results regarding the viability and stability of probiotics in chocolate substrates during storage. Study [131] demonstrated that freeze-dried *Lactobacillus casei* and *Lactobacillus paracasei* probiotic bacteria supplemented in dark chocolate can remain viable during 12 months of storage either at 4 °C or 18 °C. Study [132] showed that chocolate mousse is an excellent vehicle for the incorporation of *Lactobacillus paracasei subsp. paracasei* LBC 82 alone or in combination with the prebiotic ingredient inulin. Study [133] showed that *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* HN019 incorporated into chocolate (dark and milk chocolate) remained viable during storage and also during the passage of the upper gastrointestinal track, without affecting the sensory quality of the chocolates. Study [134] also evaluated the survival of *Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HN019) in milk chocolate masses. Results showed that after 6 months of storage, the survival of all three probiotic strains was above 90%, with viable cell count of about 10^8 cfu/g. In a recent study by [135] encapsulated probiotic *Lactobacillus plantarum* 564 and commercial probiotic *Lactobacillus plantarum* 299v were added in dark chocolate. The results showed very good survival of both probiotic strains after production and during storage, reaching 10^8 cfu/g in the first 60 days and over 10^6 cfu/g up to 180 days.

8. Conclusions

In conclusion, the development of non-dairy probiotic food products is possible, allowing the consumption of these beneficial microorganisms by people who do not like dairy products or with intolerance or allergy to milk components. Probiotic and prebiotic non-dairy products have a great marketing future, since recent studies have shown the application of strains that adapt well in alternative matrices. There are two main challenges during manufacture; the maintenance of the probiotic viability during the shelf life of the products and after ingestion to the gastrointestinal tract and the maintenance of the physicochemical and sensory characteristics of the conventional products. Despite the challenges, the future of non-dairy probiotic products is promising.

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