Non-Dairy Plant-Based Probiotic

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Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Traditionally, dairy products are the major and most popular probiotic carriers. At present, there is a growing demand for non-dairy probiotic products. Both fermented and non-fermented non-dairy plant-based food products are becoming highly appealing to both dairy and non-dairy consumers worldwide. Non-dairy plant-based food matrices such as fruits, vegetables, plant-based milk, cereals, and legumes have been used successfully in producing probiotic products with the minimum recommended viable probiotic numbers at the time of consumption.

Keywords: probiotics ; fruit and vegetable based probiotic products ; soymilk ; lactic acid bacteria ; Lactobacillus ; Bifidobacterium ; prebiotics

1. Cereal-Based Products

1.1. Oat-Based Products

Oat is a rich source of dietary fiber, both insoluble and soluble, good quality fat, and phytochemicals important for human health. Among different non-digestible dietary fibers, oat β-glucan has been reported to have beneficial effects on insulin resistance, dyslipidemia, hypertension, obesity, enhanced immune response to bacterial infection, and for their applications in cancer treatment and prevention [1]. In the human digestive tract, oat β-glucans act as prebiotics that selectively fermented by butyrate-producing microorganisms [2]. In addition to the benefits of fiber, oat is also a good source of selenium, which works with vitamin E in various antioxidant systems throughout the body. These antioxidative actions reported to have beneficial effects against asthma, heart disease and certain types of cancer [2][3].

Exopolysaccharides (EPS) producing *Pediococcus parvulus* 2.6 has successfully been employed to improve the viscosity, texture, and mouthfeel of fermented oat-based products [4]. Human trials of the oat-based products fermented by *P. parvulus* 2.6 showed decreased serum cholesterol levels and increased counts of faecal *Bifidobacterium* spp. [5]. Moreover, EPS obtained from *P. parvulus* 2.6 seems to enhance some probiotic properties of LAB strains in vitro. For example, probiotics combined with β-glucan reported enhancing the anti-inflammatory properties of probiotics [6]. Thus, EPS produced by LAB are considered promising molecules in the functional food area as well as prebiotic fermentable substrates able to modulate the intestinal microbiota [7].

In another study, a symbiotic functional drink from oats was manufactured by combining the health benefits of a probiotic culture, *Lb. plantarum* B28, with oat prebiotic beta-glucan. The levels of starter culture concentration (5%), oat flour (5.5%) and sucrose content (1.5%) were established for completing a controlled fermentation for 8 h. The addition of sweeteners aspartame, sodium cyclamate, saccharine, and Haxol (12% cyclamate and 1.2% saccharine) did not affect either fermentation dynamics or probiotic survivability during 21 days of refrigerated storage. The viable probiotic counts were maintained well above the minimum therapeutic threshold level throughout the storage [8].

1.2. Malt-Based Products

Malt can be recognized as an excellent matrix for probiotics to maintain their viability throughout cold storage. The higher viability of the probiotics may be attributed to the presence of sugars in malt substrate such as fructose, glucose, sucrose, maltose, maltotriose, and maltotetraose [9]. The concentration of monosaccharides and disaccharides in malt was reported to be approximately 3 and 12 g/L, respectively [10][11]. Malt-based beverages seem to favor the growth of *Bifidobacterium* spp. since these food matrices provide ample amounts of metabolizable sugars (3–4 g/L) that stimulate the growth of bifidobacteria. One study revealed that moltotriose and glucose were more preferably utilized by *B. adolescentis*, *B. breve* and *B. longum* whereas fructose consumed all by *B. infantis*. The authors also demonstrated that high concentrations of growth promoters (yeast extract and peptone; 10 g/L) enhanced the buffering capacity of medium and thereby resulted in higher growth of bifidobacteria. Out of the two growth promoters, yeast extract showed promising effects as it contains substantial levels of vitamins and specific amino acids. However, a combination of both growth promoters had inhibitory effects on bifidobacteria growth [9].

1.3. Wheat-Based Products

Flour from emmer, (*Triticum dicoccon*; hulled wheat; an ancient wheat and an ancestor of modern durum), was successfully utilized to produce probiotic emmer beverages. Coda and colleagues (2011) manufactured a series of probiotic emmer beverages from emmer flour, emmer gelatinized flour, and emmer malt at percentages ranging 5–30% (*w*/*w*) using *Lb. plantarum* 6E as the starter [12]. The results showed that the combination of EPS-producing strain *Lb. rhamnosus* SP1 with *Lb. plantarum* 6E during the manufacture of emmer beverage containing 30% of gelatinized flour increased the viability of *Lb. plantarum* 6E than it is alone. *Lb. rhamnosus* SP1 with *Lb. plantarum* 6E showed viability of 8.9 log cfu/mL and 8.1 log cfu/ ml, respectively, during a month-long storage period at 4 °C. *Saccharomyces cerevisiae* var *boulardii* has been suggested as a suitable candidate for potential probiotic wheat beer development. The probiotic yeast showed equivalent metabolic efficiency on sugar wort and growth as that of the brewer's yeast. More importantly, it remained alive during processing, storage (for over 60 days) and GI transit (>10^6 cfu/mL) [13].

The gastric tolerance of the probiotics delivered in cereal-based products including wheat has already been assayed. In a study conducted by Charalampopouls et al. (2003), the gastric tolerance of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactobacillus reuteri* delivered in wheat, malt, and barley extracts were assayed [14]. All strains showed a significant reduction in their cell concentrations in the absence of cereal extracts. In contrast, the viability of *Lb. plantarum* has been increased by approximately 4 $\log_{10}$ cycles in the presence of malt, and by 3 $\log_{10}$ cycles in the presence of either wheat or barley. The viability of *Lb. acidophilus* and *Lb. reuteri* has been increased by more than 1.5 and 0.7 $\log_{10}$ cycles. The results suggest that malt, wheat and barley extracts demonstrate a significant protective effect on the viability of the above probiotics. Moreover, Tian et al. (2021) reported that *Lb. rhamnosus* GG releases additional phenolic acids including trans-ferulic acid from digestive residues during colonic fermentation of whole wheat products (e.g., bread, cookie and pasta) leading to overall colon health. The incorporation of *Lb. rhamnosus* GG into wheat-based products would, therefore, provide additional health benefits for health-conscious customers [15].

1.4. Rice-Based Products

Rice has given rise to various rice-based fermented beverages and foods in the Asia-Pacific region. Rice beer is one such beverage popular among the ethnic communites in different parts of India [16]. In most cases, probiotics are the predominant strains found in these traditional beverages. *Bacillus velezensis* strain DU14 and *Lactobacillus fermentum* KKL1 are two such probiotic candidates isolated from the traditional rice beers Apong and Haria, respectively [16][17]. These probiotics do not only aid in fermentation, but also possess multi functionalities. For instance, *Lb. fermentum* KKL1 found to improve the accumulation of functional compositions (e.g., minerals), digestibility (due to α-amylase, glucoamylase, and phytase activities) and therapeutic potentials (e.g., antioxidative properties) [16]. Fermented sour rice is another traditional food of the Indian subcontinent which is believed to have therapeutic and prophylactic applications against various disorders. The probiotic candidate, *Weissella confusa* strain GCC 19R1 was found to be the predominant fermentative bacteria in this product [18]. Interestingly, a probiotic-fermented rice tablet has also been tested recently. The starter composed of *Brettanomyces custersii* ZSM-001 and *Lactobacillus plantarum* ZSM-002, and the sensory properties of the resulting tablet was similar to the tablet prepared using commercial starters. The viable bacterial counts for *Lb. plantarum* ZSM-002 remained >8 log cfu/g after simulated gastric and intestinal digestion [19].

1.5. Maize-Based Products

Maize is a widely used raw material in indigenous beverage production [20]. The available literature suggests that the maize matrix has been successfully utilized in the production of fermented probiotic beverages using yeast-lactic fermentation. For example, a novel, functional fermented beverage has been developed using potentially probiotic yeasts (*Saccharomyces cerevisiae* CCMA 0732, *S. cerevisiae* CCMA 0731, and *Pichia kluyveri* CCMA 0615) in combination with commercial probiotic strain *Lactobacillus paracasei* LBC-81. During fermentation and storage, except *P. kluyveri*, all tested strains showed viabilities >6 log cfu/mL. Interestingly, the beverages lacked a sweet taste and had no flavoring additive effect [20]. In another study, a series of novel fermented beverages from a blend of maize and rice were developed using *Lactobacillus plantarum* CCMA 0743, *Torulaspora delbrueckii* CCMA 0235, and the commercial probiotic *Lb. acidophilus* LACA 4 in mixed culture. These beverages were supplemented with the prebiotic, fructooligosaccharide (FOS). The results showed that FOS favored the growth of *Lb. acidophilus* and the yeast *T. delbrueckii*. The viable probiotic counts were maintained ≥T^7 cfu/mL during fermentation and refrigerated storage for 28 d. A sensory analysis showed that >50% of the panellists liked the beverages slightly or extremely [21].

Apart from this, a fermented probiotic maize porridge has been prepared using maize flour and barley. The porridge was fermented with either *Lactobacillus reuteri*, *Lb. acidophilus* LA5, *Lb. acidophilus* 1748, or *Lb. rhamnosus* GG. Most strains reported to reach maximum cell counts (7.2–8.2 log cfu/g) after 12-h fermentation [22].

1.6. Millet-Based Products

The pearl millet substrate is rich in proteins, macro and micro minerals, resistant starch, soluble and insoluble dietary fibers, and dietary antioxidants (e.g., C-glycosylflavones, ferulic acid, β-carotene, etc.) [23]. Millet-based traditional food products are rich sources of potential probiotic microorganisms with various functionalities. For example, Palaniswamy and Govindasamy (2016) isolated five ferulolyl esterase-producing *Lactobacillus* strains that belonged to *Lb. fermentum* and *Lb. delbrueckii* from a traditional pearl millet porridge (*kambu koozh*) and characterized their probiotic potential. Out of these five strains, *Lb. fermentum* CFR5 found to be a promising probiotic candidate with the abilities to produce β-galactosidase and glutamate decarboxylase enzymes and demonstrated cholesterol-lowering effects in vitro [23]. Further, five probiotic strains of *Lactobacillus* (*Lb. pentosus* SW02; *Lb. plantarum* subsp. *plantarum* SW03, SW06 and SW07; *Lb. sakei* subsp. *sakei* SW04), and two strains of *Pediococcus* (*P. pentosaceus* SW01 and *P. acidilactici* SW05) were isolated from *Omegisool*, a traditionally-fermented alcoholic beverage in South Korea, and possessed antioxidative properties [24]. Previous studies showed that probiotic LAB populations were as high as 10^8 cfu/mL after fermentation [25].

2. Legume-Based Products

2.1. Soya-Based Products

Soybean (*Glycine max*) provides high-quality protein, fats, and carbohydrates and contains no cholesterol or lactose. It is a good source of nutrients for lactose-intolerant individuals, vegetarians, and those with milk allergy [26]. The production of soy products has been emerging as an interesting alternative to dairy products and their incorporation into human diets is increasing due to their nutritional and functional properties [27].

Several studies have shown that soy products, especially soy yoghurt, is a good vehicle for probiotic delivery [28][29][30][31]. Due to the presence of raffinose and stachyose, soymilk is a good medium for *Bifidobacterium* spp., as most of the strains belong to this genus can ferment these sugars. Strains of *Lb. acidophilus* has also been reported to metabolize oligosaccharides present in soymilk during fermentation [27][29][30]. Many probiotic strains possess α-galactosidase activity that allows their growth in soymilk [27][32]. These probiotic strains seem to have no negative effects when they incorporated with traditional yoghurt starter cultures. Farnworth et al. (2007) reported that the presence of probiotic bacteria [*Lb. johnsonii* NCC533 (La-1), *Lb. rhamnosus* ATCC 53103 (GG), and human-derived bifidobacteria] did not affect the growth of the yoghurt strains [30]. Approximately 2 log increases in both *Lb. rhamnosus* GG and *Lb. johnsonii* La-1 were observed when each was added with yoghurt strains in the soy beverage.

Mounting evidence suggests that soymilk matrix may also provide adequate protection to the probiotics during gastrointestinal transit. For instance, *Lb. acidophilus* and *Bifidobacterium* spp. showed resistance to simulated gastrointestinal conditions when delivered through a fermented drink made of a mixed extract of soy and rice by-products with added waxy cornstarch [33]. In another study, fermented soy matrix protected *Lb. acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 against gastrointestinal juices, where the Bb-12 showed higher resistance to artificial gastrointestinal juices compared to La-5 [27]. Moreover, *Lb. casei Zhang* has also shown good tolerance to simulated gastric transit and intestinal juice in the fermented soymilk and maintained high viability (>10^8 cfu/g) during cold storage (at 4 °C for 28 days) [31].

Soy oligosaccharides, mainly α-galactosides, are prevalently present in soy protein products and can result in unfavorable digestive effects when consumed. Certain probiotic strains are capable of decreasing α-galactoside content due to their high level of α-galactosidase activity while maintaining acceptable viability counts. *Lb. acidophilus* LA-2 showed greater α-galactosidase activity when induced by raffinose and was able to retain viability over 14 weeks of cold storage (4 °C) when microencapsulated and freeze-dried [34].

Soymilk is an excellent source of bioactive peptides and fermentation is an effective way of generating bioactive peptides. The β-glucosidase-producing probiotic *Lb. rhamnosus* CRL981 allowed for obtaining a soy beverage with enhanced antioxidant capacity. The higher antioxidant activity was due to increased isoflavone aglycone contents during fermentation because of β-glucosidase activity towards isoflavone glucosides [35]. *Lb. plantarum* C2 was excellent in terms of growth and peptide generation in soymilk, which showed excellent log count increases, protein hydrolysis, and α-galactosidase activities. Seventeen biofunctional soy peptides showing both antioxidant and ACE-inhibitory activities have been identified from the fermented soymilk produced using *Lb. plantarum* C2 [36]. ACE inhibitory activity in vitro has also been reported in fermented soy whey produced by using *Lb. acidophilus* FTCC 0291 in the optimized soy-whey medium [32]. Accordingly, soymilk consumption could improve some oxidative stress factors among patients with diabetic kidney disease [37]. In addition, a regular intake of a soy-based probiotic drink (*Enterococcus faecium* CRL 183 and *Bifidobacterium longum* ATCC 15707) was reported to modulate the microbiota and reduce body weight gain in diet-induced obesity in mice [38]. These pieces of evidence suggest that certain probiotics strains can be used for the preparation of soy-based functional fermented foods and bioactive food supplements.

Probiotics in the soy matrix could alter the sensory attributes of the products as well. A synbiotic soy yoghurt prepared using optimized FOS concentration (8.1% *w*/*v*) resulted in well-set products with very less whey separation (1.14%). The developed product showed good nutritional, textual, and sensory characteristics [39]. Norouzi et al. (2019) compared the survival rate of *Lb. paracasei* in fermented and non-fermented frozen soy dessert and their sensory properties over 180 days of storage at −24 °C. Results showed that the colour, mouthfeel and overall acceptability were significantly improved in probiotic products compared to frozen dessert without probiotics. Further, both fermented and non-fermented products reported maintaining viable *Lb. paracasei* counts well over 10^6 cfu/mL [40].

Albuquerque et al. (2019) evaluated the effect of passion fruit by-product (PFBP) and fructooligosaccharides (FOS) on the viability of *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG in folate bio-enriched fermented soy products, and on probiotic survival and folate bio-accessibility under in vitro simulated gastrointestinal conditions during storage (at 4 °C for 28 days) [41]. Only *Lb. rhamnosus* LGG retained the desired viability (>8 log cfu/mL) during storage, whereas *St. thermophilus* TH-4 populations decreased to 5.5 log cfu/mL by day 28. Therefore, the bio-enriched probiotic fermented soy products present great potential as innovative functional food by delivering probiotic microorganisms and providing 14% of the recommended daily folate intake.

2.2. Chick-Pea-Based Products

Chick-peas (*Cicer arietinum* L.) are an excellent source of essential amino acids, raffinose-family oligosaccharides, resistant starches and fibers, and possess prebiotic effects on the growth and survival of the probiotic microorganisms [42]. A beverage produced with chick-pea and coconut extract at the 9:1 ratio found to be a viable matrix to deliver *Lb. paracasei* LBC 81, maintaining viable counts of $>10^8$ cfu/mL during 10 days of refrigerated storage [43]. Interestingly, roasted chick-peas containing *Lb. plantarum* 299v and *Lb. rhamnosus* GG (produced by immersing in probiotic dispersions followed by drying at 42 °C) was also suggested as a viable probiotic carrier matrix. The viability of *Lb. plantarum* 299v in roasted-chick peas was $>10^9$ cfu/g at 4 °C, and $>10^7$ cfu/g at 25 °C after a 3-month long storage period [44].

2.3. Miscellaneous Legume-Based Products

Recently, there is an increasing trend of utilizing legume sprouts as probiotic carrier foods. A probiotic drink produced from sprouted green gram showed viable *Lb. acidophilus* NCDC14 counts of 10^{10}–10^{11} cfu/mL after 8 h of fermentation [45]. Swieca et al. (2019) investigated the effectiveness of lentil and adzuki bean sprouts as carriers for the probiotic yeast *S. cerevisiae* var. *boulardii* and found that the sprouts obtained from seeds soaked in the inoculum and further cultivated at 30 °C for 4 days gave the highest probiotic counts ($>10^7$ cfu/g). More importantly, the two matrixes effectively protected the probiotic yeasts during digestion in vitro. Further, the sprouts enriched with *S. boulardii* were characterized by lower mold counts and coliform counts [46]. Chick-pea sprouts fermented with *Lactobacillus casei* 0979 hinder the growth of pathogenic microorganisms and result in products with safety. *Lb. plantarum* 299v reported increasing starch digestibility in the lentil and mung bean sprouts [47]. These results suggest that legume sprout-based food matrices are effective probiotic carrier foods and probiotics can be utilized to improve the microbial safety, nutritional composition, and nutrient digestibility of these products.

Lb. plantarum B1-6 has been successfully utilized to produce a probiotic food using the mung bean (*Vigna radiata*) as a probiotic carrier. Probiotic fermentation resulted in viable counts of $>10^8$ cfu/mL and significantly higher ACE inhibitory activity at the end of fermentation [48]. In another study, Romero-Espinoza et al. (2020) successfully used the combination of yeasts *S. cerevisiae* and *S. boulardii* and a mix of commercial probiotic bacteria composed of *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus*, *Lb. plantarum*, and *Bifidobacterium infantis* to ferment whole meal lupin (*Lupinus mutabilis* var. bola L.). The probiotic fermentation resulted in significant degradation of oligosaccharides (27.3–82.3%), phytic acid (61.9–67%), and alkaloids (25.5–36.7%) which are the antinutritional factors that limit the consumption of lupin [49].

3. Fruit-Based Probiotic Products

Fruit juices may represent an alternative means of delivering probiotics to consumers as they could be considered as healthy and refreshing beverages consumed regularly by people of all ages. They are rich in sugars and bioactive compounds (minerals, vitamins, fiber, and antioxidants) that can be utilized by probiotics. More importantly, these fruit-based probiotic products do not contain starter cultures as those in dairy products, which compete with probiotics for nutrients [50][51].

3.1. Apple-Based Products

Due to the high porosity that makes it easy for the incorporation of probiotics, the apple food matrix has been suggested as an excellent matrix for the delivery of probiotics. Furthermore, cellulose in apple is not digestible, and therefore serve as a protective matrix for probiotics during the gastrointestinal transit [52].

Probiotics incorporated into apple juice and other apple-based products resulted in satisfactory viable counts over the refrigerated storage suggesting that apple is an ideal vehicle for the delivery of probiotics. Pimentel et al. (2015) evaluated the effect of the supplementation of clarified apple juice with probiotic *Lb. paracasei* subsp. *paracasei* and/or oligofructose on the physiochemical characteristics, probiotic viability, and acceptability during refrigerated storage (4 °C) either in plastic or glass packages [50]. The results showed that apple juice is a suitable medium for incorporating the probiotic strain, resulting in products with similar chemical composition, density, acceptability and purchase intention compared to pure juice. However, probiotic products had higher acidity, turbidity, and red color. The addition of oligofructose did not change either the physiochemical characteristics, acceptability, purchase intention, or storage stability of the products; however, it enhanced the probiotic survival during storage. The glass package was more efficient in maintaining the viability of probiotics than the plastic package.

Drying brings several advantages to foods such as increased shelf life, no requirement of refrigeration, and reduction of storage, packaging, and transporting costs [53]. Different drying methods have been tested on a variety of apple-based probiotic products. Out of four common drying methods (air drying, freeze-drying, freeze-drying followed by microwave vacuum drying, and air drying followed by explosion puffing drying), freeze-drying followed by microwave vacuum drying was most suitable drying method in order to develop probiotic enriched apple snack with anticipated quality. The probiotic viability (*Lactobacillus plantarum* SICC) in this product remained above 10^6 cfu/g over 120 days of storage at 25 °C. Another study revealed that the viability of the probiotic in the apple snack was similar to that of the commercial probiotic dairy products when the apples were dried 60 °C or when ultrasound-assisted air-drying was applied [53]. Viable counts of apple slices impregnated with *Lactobacillus paracasei* LL13, dried with either conventional or vacuum drying at 45 °C, and stored at 4 °C for 28 days were maintained above 7 and 6 log cfu/g, respectively over the cold storage. Vacuum dried apple snacks were more pleasing to consumers in terms of sensory evaluation [54]. In another study, free freeze-dried *Lactobacillus rhamnosus* GG was able to maintain high viability (>10^6 cfu/mL) in apple juice for an entire week at 4 °C [55]. When *Lactobacillus plantarum* 299v was incorporated (10^7–10^8 cfu/g) into osmotically dehydrated apple cubes (24 h at 37 °C) using sucrose and sorbitol as osmotic agents, the probiotic survived over a period of 6 days at 4 °C maintaining viability counts of >10^7 cfu/g. Moreover, the viability did not decrease during a simulated gastro-intestinal passage of 2 h [52].

The fermentation of traditional food and beverages by Selenium (Se)-enriched probiotics provides an easy and appropriate alternative to increase daily consumption of both vegetables and fruits as well as selenium. In a recent study, the effect of adding Se-enriched probiotics and the Se-enrichment of probiotic-fermented blended juices were evaluated. Among the probiotics tested (*Streptococcus thermophilus*, *Bifidobacterium breve*, and *Lactobacillus plantarum*), *S. thermophilus* showed the best Se-tolerance ability, which was then used to produce a Se-enriched probiotic. Addition of 1% Se-enriched *S. thermophilus* starters resulted in a 13-fold increase in Se content of the fermentation juice. The optimum processing parameters were found to be: liquid ratio of apple juice, orange juice, carrot juice, Chinese jujube juice of 25:35:30:10, a ratio of strains of *B.breve*, *Lb. plantarum*, Se-enriched *S. thermophilus* of 1:1:2, inoculum size of 2%, and a fermentation time of 18 h [56].

3.2. Pineapple-Based Products

Pineapple (*Ananas cosmosus* L. Merril) is a tropical fruit with a good balance between acidity and sugar that makes it one of the most popular fruits in producing regions and in importing countries. Pineapple is widely used to produce juice, jams and wine [57].

Mixed pineapple and jussara (*Euterpe edulis* Martius) juices have been suggested as an excellent carrier matrix for *Lactobacillus rhamnosus* GG (LGG). The LGG viability in probiotic juice was maintained well above 7.2 log cfu/mL throughout 28 days of storage at 8 °C. More importantly, LGG in mixed pineapple and jussara juice showed greater resistance to gastrointestinal conditions in vitro and in vivo. A blood analysis of Wistar rats fed the probiotic juice for 10 weeks (1 mL/day) showed that the probiotic juice did not induce hepato- and/or nephrotoxicity, and was capable of regulating the cholesterolemic index [58].

Pineapple juice is also a suitable substrate for the incorporation of probiotic yeast. Two strains of *Meyerozyma caribbica* (9 C and 9 D) have been shown desirable in vitro probiotic properties similar or superior to the reference probiotic yeast strain, *S. cerevisiae* var. *boulardii*. Out of the two strains, *M. caribbica* 9 D was able to result in a fermented pineapple beverage with good sensorial characteristics. *M. caribbica* population remained stable during refrigerated storage with cell counts greater than 7 log cfu/g for over 21 days [57].

Costa et al. (2013) analyzed sonication as a pre-treatment for cultivating the probiotic strain *Lactobacillus casei* NRRL B-442, which was able to ferment sonicated pineapple juice without any nutrient supplementation [51]. Greater viable cells counts were obtained within a shorter fermentation time (12 h) and probiotic viability was maintained above the acceptable range for at least 21 days under cold storage (4 °C).

3.3. Orange-Based Products

Orange (*Citrus sinensis* L. Osbeck) is a fruit with a high water content and is high in protein, sugars, fiber, minerals, and vitamins such as vitamin C (57 mg per 100 mL) and carotene (120 mg per 100 mL). If probiotics are incorporated, the nutrient content in juice can enhance the survivability of the added microorganisms [59]. Orange juice is the most commonly consumed juice worldwide, mainly due to its pleasant taste [60].

Probiotic survivability is probably the most important parameter in probiotic orange juice powder. Barbosa et al. (2015) evaluated the effect of three drying methods (spray-drying, freeze-drying, and convective drying) on the survivability of *Lb. plantarum* 299v and *Pediococcus acidilactici* HA-6111-2 [59]. Both probiotic strains reported good survival rates after spray drying and freeze-drying processes (>9 log cfu/g) compared to convective drying (~6 log cfu/g). Furthermore, after 180 days of cold storage at 4 °C, greater probiotic survivability was observed in the products manufactured by spray drying and freeze-drying (10^8 cfu/g) compared to those manufactured by convective drying (10^4 cfu/g) [59].

Prebiotics, which are known as nondigestible food ingredients may lead to improve the survival of probiotics in fruit juices [61]. The combined use of nettle (*Urtica dioica* L.) and probiotic lead to an increase in total phenolic content of the juice samples and slowed down the decline of antioxidative capacity during storage [61]. Research evidence suggest that it is possible to develop symbiotic orange juice beverages by using prebiotics and probiotic cultures without altering physiochemical and sensory attributes of pure juice. For example, a symbiotic orange juice with probiotic culture, ascorbic acid, and/or oligofructose (prebiotic) supplementation showed similar physiochemical and sensory attributes as those of pure juice. Oligofructose or ascorbic acid did not exert a protective effect on probiotic during storage, but the juices showed probiotic viability greater than 10^6 cfu/mL [33].

It is considered that the orange juice flavanones undergo limited absorption in the upper gastrointestinal tract leaving them to reach the colon where they are transformed and get absorbed. Pereira-Caro et al. (2018) investigated the ability of *Bifidobacterium longum* R0175 and *Lactobacillus rhamnosus* subsp. *rhamnosus* NCTC 10302 to catabolize orange juice flavanones. Results found that both strains were able to transform hesperetin and naringenin suggesting involvement in the colonic catabolism of orange juice flavanones [62].

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