



Non-Dairy Probiotic Drinks: An Underutilized Alternative

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Abstract

Probiotics are live microbial dietary adjuvant that when consumed in adequate amount beneficially affects the host physiology by improving immunity and maintaining balance in the intestinal tract. Recently, awareness of the health benefits of consuming microorganisms as probiotics has increased. Probiotic foods are categorized as functional food, which are foods, or food ingredients that may provide a health benefit beyond their nutritional composition. The efficacy of probiotics largely depends on the amount consumed and the viability of the cells in the food matrix. To this effect, different types of foods were proposed as a carrier for probiotic cells, of all these foods, dairy products offer the most suitable environment for probiotic viability and growth. However, dairy-based probiotic drinks have one or two limitations in term of cost and being unsuitable for lactose intolerant individuals. Considering these limitations, other non-dairy-based food matrices such as malt-based beverages, fruit juices, cereals, vegetables, and some underutilized and under-exploited plants have been suggested as an alternative. One major challenge of using these non-dairy vehicles for delivery of probiotics is the stereotypic nature of people's thoughts, and taste regarding dairy-based probiotics which has influenced their acceptability and consumption. This review is therefore meant to provide more information on these non-dairy based underutilized alternatives and possible ways to improve its general acceptability.

Keywords: Probiotics; Dairy; Underutilized; Non-dairy; Alternative; Vehicle

Introduction

Probiotics are non-toxic, non-pathogenic "live microorganisms which when administered in adequate amounts; confer a health benefit on the host [1-3]. Initially, the word 'probiotic' comes from the Greek word 'pro bios' which means 'for life' as opposed to 'antibiotics' which means 'against life' [4]. An increase in consumer awareness of the impact of food on health has led to the rapid growth of the global probiotic food market, statistically, probiotic products now account for about 60% to 70% of the total functional food market [5-7]. Probiotics have been with us for as long as people have taken fermented milk, but their association

with health benefits was established at the turn of the last century [8-10]. They can present both intestinal and non-intestinal health effects on the host [11], and are known to support or improve health by; the regulation of intestinal and respiratory microbiota [12-14], maintaining mucosa integrity, stimulation and development of the immune system [15], stimulation of repair mechanism of cells [13,16,17], decreasing the apoptosis of epithelial cells [18], aiding the production of antimicrobial peptides like defensins, cathelicidins bacteriocins, microcins [16], synthesizing and enhancing the bioavailability of nutrients [10,19,20], improving gut digestion by producing some extracellular enzymes, improving fat or lipid metabolism [2], reducing

symptoms of lactose intolerance [10], synthesis of vitamins, predigestion of proteins and Degradation of undigested food products (cellulose or oligosaccharides) [10,20-22].

With all these proven health benefits of probiotics, they still need to be delivered to their active site to perform their functions [23,24]. Thus, it is important to know that the efficiency of probiotics largely depends on the amount consumed and the viability of the cells in the food matrix [5,23]. Moreover, probiotics are majorly prepared as food supplements and different types of food and food products have been proposed as their carrier. Among all these food, dairy products offer the most suitable environment for probiotic viability and growth [25]. Notwithstanding these advantages, dairy-based probiotic drinks have some limitations such as the inability to digest lactose in milk due to the absence of lactase enzymes in some individuals, the presence of high levels of unsaturated fat and cholesterol, allergic reaction to the milk protein in some individuals, unsuitability of the food for vegetarians and relatively high cost as compared to other product [26,27]. Considering these limitations, other non-dairy-based food matrices such as cereals (single or multiple), fruit juices, vegetables, and

some underutilized and under-exploited plants have been suggested as an alternative [23,24]. However, the stereotypic nature of people's thoughts and tastes regarding dairy-based probiotics has influenced the acceptability and consumption of their non-dairy counter parts. Therefore, this review will elucidate more about this underutilized alternative and possible ways to improve its general acceptability.

Probiotics

Probiotics are live microbial dietary adjuvants, and their functionality is generally strain-dependent [28], however, the most popular strains of probiotics used are represented by the following genera: *Lactobacillus*, *Streptococcus*, and *Bifidobacteria*, but other organisms including *Bacillus* sp, *streptococcus* sp, *Enterococci* sp, *Escherichia coli* and yeasts have also been used as probiotics after detailed evaluation [29,30].

Selection Criteria for Probiotics

In the search for probiotic candidates, Table 1 gives a summary of some of the selection criteria to look out for.

Sr. No	Selection criteria	References
1	The probiotic strain itself should not compete with or hinder the growth of desirable microbes.	[28,29]
2	The probiotic strain and its fermentation products, or its cell components should be tolerated by the immune system.	[15,31]
3	The probiotic strain should be tolerant to low pH and high concentrations of both conjugated and deconjugated bile acids and must not deconjugate bile salts.	[10,32,33]
4	The probiotic strain should not carry transmissible antibiotic-resistance genes.	[28]
5	The probiotic strain, its fermentation products, or its cell components should be non-pathogenic, allergic, carcinogenic, or mutagenic to the host, it should have a 'generally regarded as safe' (GRAS) status, having a low risk of inducing any disease.	[10,11,17]
6	The probiotic strain should be able to adhere, survive, proliferate within the host, and be indigenous to the environment where it is presumed to be active.	[4,10,17,21,34]
7	The probiotic strain must be capable of being prepared on a large scale and should be able to multiply rapidly, with good viability and stability in the product during storage.	[23,30,35]
8	The strains must not produce off flavors or textures once incorporated into foods.	[26,36,37]
9	The probiotic strain itself should not compete with or hinder the growth of desirable microbes.	[28,29]
10	The probiotic strain and its fermentation products, or its cell components should be tolerated by the immune system.	[15,31]
11	The probiotic strain should be tolerant to low pH and high concentrations of both conjugated and deconjugated bile acids and must not deconjugate bile salts.	[10,32,33]

Table 1: Summary of Some Criteria Used in Selecting Probiotics.

Probiotic Foods

Probiotic foods are categorized as functional food, which are foods or food products that may provide a health benefit

beyond their nutritional composition [23]. Food materials are known to play a specific role in the growth, functionality, viability, and survival of probiotics. Features of raw materials such as the presence of natural antimicrobial compounds,

acidity, diacetyl, and hydrogen peroxide can cause the loss of viability of probiotic microorganisms [38]. Therefore, strains should be carefully selected for each type of food product [39]. Generally, most probiotic products are dairy-based, according to statistics; there are over 380 types of probiotic products in the world, of which 80% are from dairy sources [5-7]. This has limited the benefits of probiotic foods to only a specific group of people as nondairy probiotic products with cereals, legumes, fruit, and vegetable origins are very rare [40]. However, the demand for new flavors and tastes and the emerging evidence of the health benefits that can be acquired from the interactions between non-dairy food components, probiotics, and gut commensals has led to the exploration of nondairy-based products [41,42].

Non-Dairy Probiotic Foods

Non-dairy products have been suggested as an alternative probiotic food carrier to dairy milk due to their low cost, availability, suitability for vegetarians, health benefits, and good impact on the environment (their production results in the reduction of greenhouse gas emissions compared to dairy probiotics) [12,43,44]. In 2019, a global survey conducted showed that 40% of the surveyed consumers try to limit their consumption of animal proteins, which resulted from concerns about climate change [45-47]. The global dairy alternatives market size was valued at USD 26.01 billion in 2022 and is expected to expand at a compound annual growth rate (CAGR) of 12.6% from 2023 to 2030 [48]. The market is gaining momentum and witnessing a high demand owing to the shift in consumer eating patterns and changes in diet trends. Furthermore, the increasing occurrences of cases of milk allergies and lactose intolerances are expected to drive the demand further in the forecast period [48]. Dairy alternative products such as soy, hemp, cashew, coconut, and almond milk are gaining popularity around the globe for their unique taste and health benefits. A variety of other raw materials include millet, legumes, fruits, vegetables, and nuts [49-51]. Nondairy probiotic products can either be developed by fermentation or non-fermentation processes [42,52].

Fruits and Vegetables: Fruits and vegetables are considered healthy foods, as they contain several beneficial nutrients, such as minerals, vitamins, dietary fibers, and antioxidants [50,53,54]. In addition, unlike dairy products, fruits and vegetables lack allergens, lactose, and cholesterol. Several types of probiotic fruit and vegetable products such as fruits and vegetable juices, dried fruits, fermented vegetables, and vegetarian desserts with probiotics viability ranging from 10^6 - 10^{10} CFU/mL or CFU/g have been developed and marketed [34,50,55,56]. Owing to their pleasant taste, flavour, and acceptability by all age and economic groups, fruit, and vegetable juices became one of the most studied, developed,

and consumed probiotic fruit and vegetable products [57]. Several studies have demonstrated the feasibility of fruits and vegetables as suitable probiotic food matrices. Zhu, et al. [58], determine the functional efficacy of probiotics in some fruits and found tomato juice to be a good food matrix that maintains the viability of *L. sanfranciscensis* even after 4 weeks of storage at 4°C. Besides this, the juice was also found suitable for *L. casei* and *L. delbrueki*. Similarly, beetroot was found to be a suitable food matrix for *L. plantarum*, as the viability of the cells was retained even after 21 days of storage at 4°C [59]. Almada-Erix, et al. [60], found orange juice suitable for the delivery of *L. rhamnosus* with cell viability maintained at $6 \log$ CFU/mL after 28 days of storage at 4°C. However, the juice was found unsuitable for *Bacillus coagulans*. Similarly, Wang, et al. [61], confirmed kiwi fruit as a befitting food matrix for *L. helveticus*. Furthermore, Wu, et al. [62], also found blueberry and blackberry drinks to be a favourable matrix for *Bifidobacterium bifidum* with viability in the range of 7.3 log-8.2 log CFU/mL. Other fruits and fruits product that has been found to support the growth and delivery of probiotics include the peel of pineapple and pear [63], and banana powder [64]. Moreover, probiotic strains usually found in vegetable materials include; *L. plantarum*, *L. casei*, and *L. delbrueckii* [65].

Cereals: Cereal grains are still considered one of the most important food sources of protein, carbohydrates, vitamins, minerals, fiber, and phenolic acids for large segments of people all over the world [52,66]. They are good sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects also act as prebiotics that selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* species in the colon [35,67]. This is because Strains of *Lactobacillus* have been recognized as complex microorganisms that require fermentable carbohydrates, amino acids, B vitamins, nucleic acids, and minerals to grow [28]. Fermentation of cereals is known to increase the bioavailability of vitamin B group and minerals such as calcium, phosphorous, iron, and zinc, due to the action of microbial enzymes such as phytases or the organic acids produced [67]. Additionally, fermentation also results in the decrease of non-digestible carbohydrates (poly- and oligosaccharides) and improves the quality and level of lysine [68]. Traditionally, cereals and whole grains have been used in the production of some indigenous probiotic drinks such as boza, bushera, mahewu, pozol, togwa, ogi [55,69]. Many of these beverages are non-alcoholic manufactured with cereals as the principal raw material. Furthermore, oat-based substrates have proved promissory for the growth of *L. reuteri*, *L. acidophilus* and *B. bifidum*. Additionally, malt, wheat, and barley extracts have been demonstrated to have a good influence in increasing bile tolerance and viability of *L. acidophilus*, *L. reuteri* and *L. plantarum* [67].

Legumes: legumes are a rich source of carbohydrates (30–60% of total content), dietary fiber (9–25%), and protein (19–36%) containing the necessary amino acids such as lysine, leucine, and arginine [70]. They are a source of bioactive ingredients (including, e. g., polyphenols and phytosterols) [51,71]. Usually, legumes are low in fat and contain no cholesterol, with a favorable fatty acid profile dominated by unsaturated fatty acids. They are also a good source of iron, calcium, zinc, selenium, magnesium, phosphorus, copper, potassium, and B-group vitamins. These intrinsic properties of legumes have been found to be key determinants of their suitability as a food matrix for probiotics and fermentation processes [72]. The development of legume-based probiotic beverages has expanded and enriched the range of milk alternatives available [73]. The most popular alternatives to cow's milk are beverages made from soybeans, which are a type of legume [26,74,75]. Other legumes such as beans, peas, peanuts, and chickpeas are highly suitable to produce plant-based beverages [76]. Additionally, legumes like soybeans have been used in the fortification of cereal-based weaning food [77].

Limitations and Challenges in the Production and Utilization of Non-Dairy Probiotic Drinks

Non-dairy probiotic drinks have been greatly underutilized, generally unacceptable by all, and less consumed compared to their dairy counterpart [36,37]. The reasons for this are mainly due to the stereotypic nature of people's thoughts and taste preference for dairy-based probiotics [78]. This has caused a setback in research related to the development, production, and preservation of non-dairy probiotic drinks compared to dairy probiotic drinks. However, recently the awareness of the numerous health benefits associated with non-dairy probiotic beverages has turned the tide in its favour [23,48]. The production of non-dairy probiotics has experienced some limitations and challenges which have been minimized by the discoveries and findings of some research and the advent of modern technologies [78,79].

Intrinsic and extrinsic physiochemical and biological properties of food matrix such as pH, oxygen residues, product composition, storage temperature, antagonistic activity among probiotic strains, and varying tolerances of different probiotic strains to stresses have been found to influence the viability of most probiotic strains [30,80]. Additionally, the presence of antimicrobials and inhibitors in many non-dairy food matrices has been found to influence their viability [28,33,43]. Among the antimicrobial compounds and inhibitors present in food, flavonoids, glycosides, saponins, tannins, alkaloids and organic acids top the list.

In plants, phenolic compounds, such as terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids are known to possess pharmacological properties [81]. The bactericidal effect of Phenolic compounds is mainly through the disruption of the cytoplasmic membrane, binding or modification of proteins anchored on the cellular wall and lipid bilayer, or even changing the fluidity and permeability of the membrane [81].

Technologies involved in fermentation, encapsulation, drying, rehydration, and storage of probiotics have been developed and successfully applied to protect some of them from environmental stresses associated with various on-dairy food matrices, but there are still many technological challenges [37,78]. Fruits juices are characterized by low pH and the incorporation of probiotics in fruit juices require protection against acid conditions [78,82]. This has been extensively studied and probiotics are protected by microencapsulation technologies, which allow the entrapment of cells into matrices with a protective coating [78,79]. However, there is a need to resolve some technical issues when applying this technology to produce new probiotics foods [37]. Things to consider include the appropriate encapsulation technique to select, safe and effective encapsulating material to choose from, and the most suitable bacterial strain to use. These choices have been found to affect the efficiency of encapsulation or result in a decrease in bacterial viability [82,83]. Currently, only a few microencapsulated probiotics have been developed as food products [84,85]. However, regardless of the promising benefits of this technique, some microencapsulated cells do not always show better survival than free cells. For instance, Roy, et al. [86] reported that 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 of storage at 4°C or 8°C.

Probiotic bacteria are oxygen sensitive; oxygen residues in any probiotic product have an inhibition effect on probiotic bacteria viability. However, supplementations with ascorbic acid, and oxygen scavenger have been found to improve probiotic viability. Also, the incorporation of prebiotics (indigestible carbohydrates, such as fructo oligosaccharides and inulin) and nutraceuticals combination (isoflavones, phytosterols, and Omega-3-fatty acids) have been found to stimulate the viability and activity of probiotic bacteria [30].

Generally, the presence of probiotics in any food product does not infer or imply beneficial activities, retention of sufficient viable bacteria is a significant component of their quality and activities [87]. Strain-dependent properties of probiotics have increased the complexity of research into the interactions between probiotics and foods [23,28]. 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 [23,33]. For instance, in some cases, an undesirable effect of probiotics on the aesthetic and sensory parameters such as textures, taste, aroma, and color of non-dairy drinks occurs [37]. This is mostly due to the production of different metabolic compounds during fermentation. This also has limited the general acceptability of these products by consumers. However, methods such as deodorizing, and the addition of sweetener, edible colour pigment, and flavour have been developed to reduce this limitation and improve the properties of these drinks and make them more appealing [26].

Conclusion

Dairy probiotic products, especially yogurts, have been dominating the market and the heart of men since time immemorial. However, there is a growing demand for nondairy probiotics and the market is gaining momentum and witnessing a high demand, owing to the shift in consumer eating patterns, awareness of its health benefits, and changes in diet trends coupled with the increasing occurrences of cases of milk allergies and lactose intolerance. However, due to the uniqueness of each non-dairy food matrix, the delivery of probiotics in adequate quantity to elicit beneficial effects has been a challenging task. This has led to underutilization and a reduction in the acceptability of these alternatives. Regardless of these limitations, technological advancement in the modification or supplementation of food matrices and probiotics has proven helpful in tackling and eliminating some of the roadblocks in the production, packaging, and storage of nondairy drinks and hence, improving their utilization and acceptance as a dairy substitute.

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