Book Chapter

Wine and Non-Dairy Fermented Drinks: An Innovative Source of Probiotics and Prebiotics

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Abstract

Probiotics and prebiotics act as tools for managing microbiota to enhance human health, primarily by promoting healthy gut microbiota and bowel function. Probiotics primarily target the gut through the gastrointestinal tract. However, studies have explored their direct application to other areas of the body, such as the vaginal tract, oral cavity, and skin. While fermented dairy products have traditionally been a significant source of probiotics, there is a current demand for innovative, non-dairy alternatives. This demand arises from the increasing number of lactose-intolerant individuals globally, concerns about the adverse effects of cholesterol in fermented dairy foods, and the growing population of strict vegetarians. In this review, we explore the impact on the human gut of potential microorganisms isolated from wine, including *Saccharomyces* and non-*Saccharomyces* yeasts, bacteria, and other microorganisms present in non-dairy fermented beverages. These microorganisms can be grown and consumed as recommended probiotics. Additionally, wine and other drinks may serve as sources of prebiotics, such as polyphenols.

Keywords

Wine-Yeasts; Lactic Acid Bacteria; Prebiotics; Probiotics; Human Health

Introduction

While people commonly perceive bacteria and other microorganisms as harmful "germs," many play beneficial roles. Some bacteria help digest food, eliminate disease-causing cells, or contribute to vitamin production. The microorganisms found in probiotic products are the same as or like microorganisms that naturally live in our bodies. Probiotics are a component of the broader context involving microbes and our body—the microbiome. This microbiome encompasses a complex mixture of bacteria, fungi (including yeasts), viruses, and protozoa. Each person's microbiome is unique, and no two individuals have the same microbial cell composition [1].

The primary site associated with beneficial microbes is our gut, predominantly the large intestine. The human gut contains over 100–1000 microbial species, numbering approximately 10^{11}–10^{12} CFU/g [2], influencing the host's internal environment and playing a crucial role in overall health. Specifically, these organisms contribute significantly to defense mechanisms, effective digestion (both catabolism and anabolism), and impact brain-gut responses [3]. Therefore, the microbiota's primary functions can be categorized into metabolic, protective, and trophic functions [4]. Additionally, several locations in and on our body harbor beneficial microbes beyond the human gut. These areas, in contact with the "outside world," include the mouth, vagina, urinary tract, skin, and lungs.

The colonization of bacteria in the human gut undergoes evolution and transformation throughout a lifetime. The process initiates at birth when newborns encounter a non-sterile environment [3], leading to a shift in the gut microbiome towards a predominance of anaerobes within a few weeks of life [5]. This composition continues to change over time, influenced by dynamic interactions between diet, genetics, lifestyle, and the composition and quantity of drugs used, including antibiotics. Moreover, it can vary with age, particularly as the immune system declines [3,6].

In adults, the human gut microbiota exhibits a prominent phylogenetic core [7]. Utilizing the 16S rDNA sequencing technique, it was possible to identify Gram-negative Bacteroidetes, Gram-positive Firmicutes, and Gram-positive Actinobacteria as the dominant phyla [8]. Arumugam et al. [9], employing direct metagenomic sequencing methods, identified and defined three clusters or enterotypes, each characterized by a specific set of interconnected bacterial genera—*Bacteroides*, *Prevotella*, and *Ruminococcus* (Figure 1). This research underscores the global occurrence of this phenomenon. However, as mentioned earlier, each person's microbiota profile is unique; all humans share a typical pattern of gut microbiota. Typically, the composition of the basic intestinal microflora remains stable during adulthood [1,10], at least until the elderly age (those over 60 years). With advancing age, dietary factors primarily influence microbial diversity and composition, and the microbiota may significantly impact the inflammatory tone and immune function, leading to a decline. This decline is characterized by an increase in the number of facultative anaerobes, a shift in the ratio of *Bacteroides* to Firmicutes species, and a marked decrease in bifidobacteria [11,12].

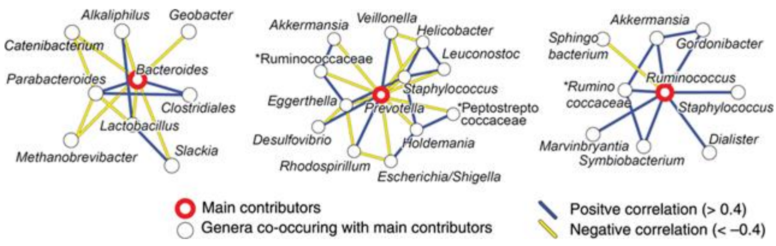


Figure 1: The main enterotypes of the human gut microbiota. Co-occurrence networks illustrate the three enterotypes. Genera that are unclassified under a higher rank are marked by asterisks [1,9]. Adapted from Arumugam et al. [9].

"Colonization resistance," or the beneficial utilization of intestinal microflora, is considered the "barrier effect"—a mechanism employed by autochthonous gut bacteria to sustain their existence and provide protection against ingested microorganisms, including pathogens [13]. Manipulating the gut microflora by increasing the relative number of "helpful bacteria" can positively influence immune function, digestion, metabolism, and brain-gut communication [14]. Alterations in gut microbiota diversity may lead to various disorders and diseases, which are not always effective, economically, or conveniently treated with drugs for everyday use. Therefore, finding a simple, low-cost, user-friendly, and natural way to enhance host health has become critical. In this perspective, probiotics serve as a bio-supplement to the host microflora, protecting against various enteric pathogens [15].

Probiotics, a term from the modern era derived from the meaning "for life," refers to live microorganisms intended to provide health benefits when consumed or applied to the human body [16]. They naturally occur in yogurt and other fermented foods and beverages. The microbiota of fermented products constitutes a complex microbial community composed of indigenous microorganisms inherently linked to the raw materials and/or those present on the equipment and surfaces of processing sites where selected microorganisms may serve as starter cultures [17].

Supplementation with probiotics, prebiotics, and symbiotics has demonstrated promising results against various enteric pathogens. This is attributed to their unique ability to compete with pathogenic microbiota for adhesion sites, alienate pathogens, or stimulate, modulate, and regulate the host's immune response by activating specific genes within and outside the host intestinal tract. Probiotics have also been shown to regulate fat storage and stimulate intestinal angiogenesis [3].

Probiotics naturally comprise beneficial live bacteria and/or yeasts in our bodies [15]. For a microbe to be labeled as a probiotic, it must possess several characteristics. These include (i) the ability to survive in our intestine after ingestion, (ii) proven health benefits, and (iii) safe consumption. Probiotics can also enhance gut barrier function by competing with pathogenic microbiota for adhesion to the gut and improving their colonization [15].

Probiotics may consist of various microorganisms, with the most common being bacteria belonging to lactobacilli, lactococci, and bifidobacteria [18]. Other bacteria, including *Pediococcus* and *Streptococcus* [19,20], as well as yeasts of the *Saccharomyces* genus, such as *Saccharomyces boulardii* [21], may also be used as probiotics (Figure 2).

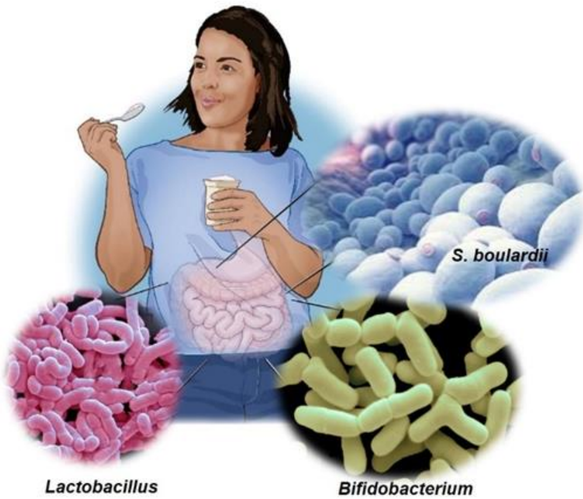


Figure 2: Probiotics consist of both bacteria and yeast. Common probiotic bacteria include *Lactobacillus* and *Bifidobacterium*. The most common yeast found in probiotics is *Saccharomyces boulardii* [22].

In the vast realm of biotics, alongside probiotics, the literature introduces the terms "postbiotics," "prebiotics," and "symbiotics." Postbiotics are the newest addition to the biotics family, denoting bioactive compounds generated during a fermentation process by food-grade microorganisms. Postbiotics encompass microbial cells, cell constituents, and metabolites [23], including short-chain fatty acids (SCFAs), functional proteins, secreted polysaccharides, extracellular polysaccharides (EPS), cell lysates, teichoic acid, peptidoglycan-derived muropeptides, and pili-type structures [24,25]. These compounds resist hydrolysis by mammalian enzymes [3] and are suggested to have health effects akin to adding probiotics. Postbiotics can be categorized into paraprobiotics (ghost or inactivated probiotics), defined as "non-viable microbial cells, intact or broken, or crude cell extracts that, when administered in sufficient amounts, confer benefits on the human consumer." Examples include *S. boulardii*, which enhances barrier function against species and improves angiogenesis in vitro and in vivo in epithelial cells by activating α2β1 integrin collagen receptors [26]. Another category is probioceuticals/probiotaceuticals, describing probiotic-derived factors such as reuterin from *Lactobacillus reuteri* [27]. The term

FIFs (fortified infant foods) is also used to designate "infant or follow-on formula" fermented with lactic acid-producing or other bacteria, typically devoid of viable bacteria [28].

Prebiotics are nutrients that modify the gut microbial flora, altering microbiota composition by stimulating the growth of specific species and promoting health benefits in the host [23,29]. Not easily digested by humans, they have a selective function in stimulating the growth or activity of beneficial bacteria in the gut [30]. Well-known prebiotics include inulin, oligofructose, fructooligosaccharides (FOS), galactose-containing, and xylose-containing oligosaccharides [3,31]. Derived from sources like vegetables, fruits, and grains, prebiotics serve as an energy source and offer health benefits, acting as anti-inflammatory agents, reducing symptoms associated with intestinal bowel disorders, and preventing colon cancer [32]. Symbiotics, a fusion of probiotics and prebiotics, enhance the survival and implantation of live microbial dietary supplements in the gut [33]. They significantly contribute to human health. Commercial interest in functional foods containing symbiotics has increased, leading to research on developing new health-promoting foods and drinks [3].

Probiotics in Non-Dairy Fermented Drinks

The primary source of probiotics traditionally comes from fermented dairy products. However, a 2014 study published in Food Microbiology [34] focused on isolating bacteria from red wine and evaluating their probiotic characteristics. Eleven Lactic Acid Bacteria (LAB) strains belonging to *Pediococcus pentosaceus*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Oenococcus oeni* were isolated and tested. A recent study by Le Roy et al. [35] supported the earlier findings, suggesting that occasional red wine could benefit gut health. This research revealed the impact of alcoholic beverages, including beer, cider, spirits, and red and white wine, on gut microbiomes. The diverse array of alcoholic, low-alcoholic, and non-alcoholic fermented beverages is a significant part of the food culture in many European countries. These beverages are produced from various substrates, including cereals, fruits, and vegetables. In the subsequent sections of this article, we will delve into recent discoveries regarding probiotics, prebiotics, and symbiotics derived from fermented non-dairy beverages, such as wine, beer, and kombucha, among others.

Yeasts as Probiotics in Alcoholic Beverages

For a considerable period, the only yeast recognized as a probiotic has been *Saccharomyces cerevisiae* var. *boulardii* [36]. This yeast, *S. boulardii*, exhibits exceptional physiological properties, including tolerance to variations in pH, temperature, and ambient stresses [37]. Clinical studies support *S. boulardii* as an extraordinary organism with inhibitory effects on pathogens [38,39]. The mechanism for eliminating pathogenic bacteria is primarily attributed to the adhesion proteins of *S. boulardii*, which bind to bacteria and impede their adhesion to the mucous intestinal membrane [40]. Additionally, other beneficial actions include the degradation of toxins from pathogens within the intestinal lumen, modulation of normal microbiota, preservation of normal intestinal physiology, restoration of the normal balance of short-chain fatty acids (SCFA), and an increase in secretory IgA (sIgA) levels. *S. boulardii* also acts as an immune regulator by influencing cytokine levels [41], as illustrated in Figure 3.

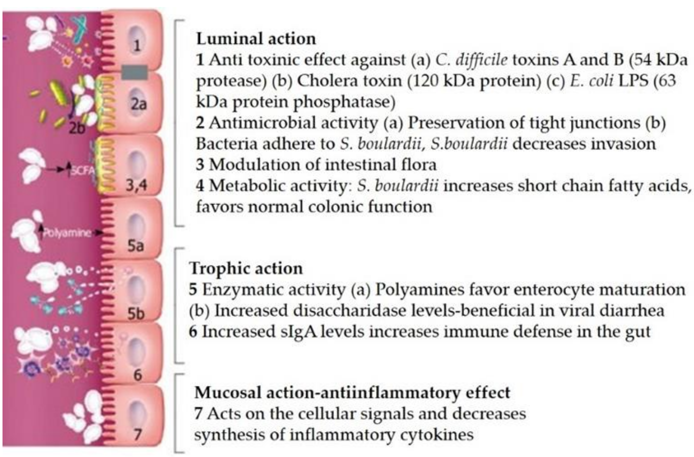
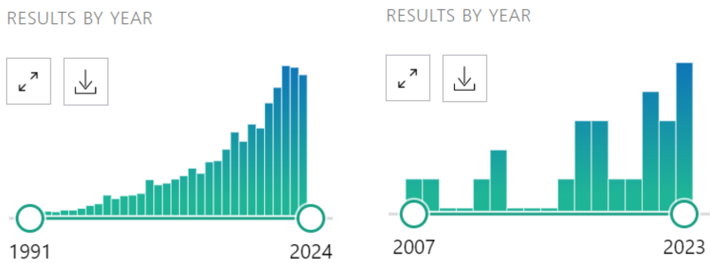


Figure 3: Schematic of the intestinal tract, illustrating various potential mechanisms of action of *Saccharomyces boulardii*. Adapted from McFarland [41].

So, there is a growing interest in the probiotic potential of yeasts, as evidenced by the increasing number of articles on PubMed using the keywords "probiotic" and "yeast." In 2022 alone, 253 articles were published, with 240 in 2023 from January to November, contributing to a total of 2,344 results from 1991 to the present (Figure 4A).



(A) **(B)**

(B)

Figure 4: (A) — Number of articles on PubMed using the keywords "probiotic" and "yeast" from 1995 to 2020. Data retrieved from https://pubmed.ncbi.nlm.nih.gov/?term=probiotic+and+yeast&filter=years.1991-2023 (B) — Number of articles on PubMed using the keywords "probiotic," "yeast," and "wine." Data retrieved from https://pubmed.ncbi.nlm.nih.gov/?term=probiotic+yeast+wine, accessed in November 2023.

If the search terms included wine (probiotic wine yeast), only 23 results were found, published between 2007 and 2023, with the distribution shown in Figure 4B. The first article that appears was written by Fleet in 2007 [42], and it includes a topic about "Probiotics and other health benefits." The article already mentions *S. cerevisiae* var *boulardii* as a possible candidate for use as a probiotic. Besides stating its advantages, it also notes its negative aspects: "(...) When incorporated into some products, it caused gassy, ethanolic spoilage, and off-flavors (...)" and also reports the occurrence of fungemia infections caused by *S. boulardii* [42,43].

In 2008, MacKenzie et al. [44] studied ten medically necessary *S. cerevisiae* strains, consisting of six clinical isolates of *S. cerevisiae* and four probiotic strains of *S. boulardii*. These yeast strains were characterized at the genetic and metabolic levels, and comparisons were made with non-medical commercial yeast strains used in baking and winemaking. One of the strains investigated was *S. cerevisiae* CECT 1482, a wine strain isolated in Spain. Based on ITS1 sequence differences, it was evident that the probiotic strains showed less divergence than the clinical isolates. However, preserving *S. boulardii* ITS1 sequences resembling those from other *S. cerevisiae* strains further reinforces the conspecificity of *S. boulardii* with *S. cerevisiae*, including that isolated from wine.

Supporting subsequent findings, Sen and Mansell [45] indicate that *S. boulardii* and *S. cerevisiae* share genetic similarities, each possessing 16 chromosomes with greater than 99% relatedness [46]. Notable differences include variations in the genes expressing specific flocculation proteins, contributing to a distinct adhesion profile of *S. boulardii* compared to *S. cerevisiae* [47]. Additionally, there are intriguing studies on the search for probiotic yeasts in beer. Capece et al. [48] examined a probiotic strain of *S. cerevisiae* var. *boulardii* in mixed cultures with selected *S. cerevisiae* strains during wort fermentation. Among various considerations, they evaluated the potential of *S. boulardii* to survive until the end of the fermentation process, aiming to select a starter culture for producing unfiltered and unpasteurized beer with potential probiotic activity. Pulque, a traditional Mexican alcoholic beverage made by fermenting fresh sap called "aguamiel," extracted from certain agave plant species [49], can also contain probiotic yeasts — Páez-Lerma et al. [50] isolated yeast species from Agave duranguesis fermentation in different regions of Mexico. The predominant species included *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Pichia fermentans*, and *Candida diversa*. Among these, *T. delbrueckii*, also isolated from grapes and fermented grape must [51], exhibited probiotic characteristics such as resistance to gastric and intestinal conditions and growth at 37°C [48].

Therefore, yeasts isolated from alcoholic drinks may serve as a viable alternative to probiotic bacteria, as they are resistant to antibiotic effects, mitigating the risk of antibiotic-associated intestinal diseases and limiting the development of antibiotic resistance [51]. These considerations, among others, mentioned earlier, have prompted scientists to shift their focus to studying various yeast species beyond *S. boulardii*, each with potential health benefits. Numerous yeasts from the *Debaryomyces*, *Kluyveromyces*, *Yarrowia*, and *Torulaspora* genera, isolated from diverse environments, have been proposed as microorganisms with potential health benefits [52–57]. This includes species from the *Candida*, *Pichia*, *Hanseniaspora*, and *Metschnikowia* genera, as well as other non-*Saccharomyces* species like *Lachancea thermotolerans* (isolated from moss on oak) and *Metschnikowia ziziphicola* (isolated from beech tree bark) [50]. Moreover, *Debaryomyces hansenii* and *Kluyveromyces lactic*, among others, has recently gained approval from the European Food Safety Authority (EFSA) and are included in the list of "Qualified Presumption of Safety" (QPS) microorganisms deemed safe [58]. However, as of now, only *S. boulardii* is officially recognized as a probiotic yeast. Other yeast species considered for probiotic use require further in vitro characterization before proceeding to human trials [51].

Yeasts as Probiotics/Symbiotics in Low/Non-Alcoholic Beverages

Kombucha tea, derived from *Medusomyces gisevii* Lindau, is a fermented sweetened tea believed to have originated in Manchuria, China, and is known for its probiotic characteristics. The microorganisms in this fermented tea, called "tea fungus" or SCOBY (Symbiotic Culture of Bacteria and Yeast), convert added sugar into organic acids and ethanol during tea fermentation. This symbiotic community is resilient to other microorganisms due to ethanol and the low pH resulting from organic acid formation [58,59]. The yeast present in this community may include species from *Brettanomyces/Dekkera*, *Pichia*, *Schizosaccharomyces*, *Torulaspora*, and *Zygosaccharomyces* genera. In contrast, the bacterial community comprises *Acetobacter nitrogenifigens* sp. nov, *A. intermedius* sp. nov**,** *Gluconacetobacter xylinus*, and *G. kombuchae* sp. nov. [60-63].

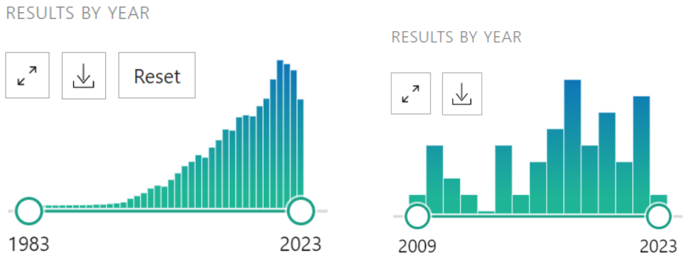
Water kefir, or "tepache de tibicos," is another slightly alcoholic fermented beverage with probiotic and symbiotic attributes. Made with sugar cane and fruits like figs or lemons, it involves using starter cultures in grains containing water, polysaccharides, and various microorganisms. Martínez-Torres et al. [64] identified *S. cerevisiae*, *Candida californica*, and *Pichia membranefaciens* during the early stages of the fermentation process.

Boza, a cereal-based fermented beverage, is recognized for its probiotic properties. Originating around 8000–9000 years ago, it is made from wheat, rice semolina, or a combination of rye, oat, barley, and millet flour. Boza undergoes lactic acid fermentation by LAB and alcohol fermentation by yeasts. The microbiota of Boza consists mainly of LAB (*Lactobacillus plantarum*, *Lb. acidophilus*, *Lb. fermentum*, *Lb. coprophilus*, *Lb. brevis*, *Leuconostoc raffinolactis* and *Ln. mesenteroides*), yeasts (*S. cerevisiae*, *Candida tropicalis*, *Candida glabrata*), and fungi (*Geotrichum penicillatum* and *G. candidum*) [65,66].

Kvass, a popular beverage in the former Soviet Union, especially Russia, is rich in carbohydrates, proteins, amino acids, lactic acid and acetic acid, ethanol, minerals, and vitamins. The microbiota of kvass fermentation includes LAB (*Lb. casei*, *Ln. mesenteroides*) and yeasts (*S. cerevisiae*) [66].

Lactic Acid Bacteria (LAB) as Probiotics

Beyond food probiotics such as yogurt, acidophilus milk, kefir, sourdough bread, soft cheese, miso soup, sauerkraut, sour pickles, and tempeh, among many other fermented foods by LAB [67–70], there are numerous probiotic formulations available worldwide as supplements. These are primarily marketed as capsules, tablets, powder, and liquid forms [71,72]. These formulations typically consist of strains of LAB, ranging in number from one to 13 strains within each trademark. Examples of LAB probiotics available in the market include Protexin Balance, Protexin Protect, Protexin Restore, Protexin Vitality, Protexin Biokult from Prebiotech Healthcare [73], Ultrabiotique Equilibre 30 jours from Nutrisanté laboratories [74], Zumub Advanced Probiotic are from Zumub [75], Digest Ultra Probiotics from Eladiet [76], among others. Notably, the expression of other bacterial groups as probiotics is limited, with only one strain of *Bacillus subtilis* appears in a formulation. Some formulations also include yeasts, particularly *Saccharomyces boulardii* strains, which can also be sold independently, as their significance has been previously presented in this article. The LAB strains found in the aforementioned probiotic formulations belong to various species, including *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus* *acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* ssp**,** *Lactobacillus helveticus*, *Lactococcus lactis* ssp. *lactis*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Enterococcus faecium*, and *Streptococcus thermophilus*. An analysis of PubMed articles with keywords "probiotics" and "lactic acid bacteria" revealed a high number of publications, with 1,027 articles published in 2023 (January to November) alone, totaling 14,829 results from 1983 to the present year (Figure 5A). In contrast, a search including "wine" as an additional keyword yielded only 43 results published between 2009 and 2023 (Figure 5B). The first article in this subset, authored by Eytan Wine and colleagues in 2009 [77], explored the strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells. The authors concluded that the ability of selected probiotics to prevent *Campylobacter jejuni*-mediated disease pathogenesis depended on the pathogen strain, probiotic strain, and epithelial cell type chosen. The data supported the probiotic strain selectivity concept, which depends on the setting in which it is being evaluated and tested.



(**A**) (**B**)

Figure 5: (A)—Number of articles on PubMed using the keywords "probiotic" and "lactic acid bacteria" from 1983 to 2023. Data retrieved from https://pubmed.ncbi.nlm.nih.gov/?term=probiotics+lactic+acid+bacteria&filter=years.1983-2023. (B)—Number of articles on PubMed: using the keywords "probiotics," "lactic acid bacteria," and "wine," data retrieved from https://pubmed.ncbi.nlm.nih.gov/?term=probiotics+lactic+acid+bacteria+wine. Accessed on November 20, 2023

From traditional non-dairy fermented beverages like Boza, Pozol, Bushera, Mahewu, and Togwa, among others, researchers have identified them as good sources of probiotics and prebiotics

[78,79]. In the case of Boza, a Turkish traditional fermented drink made by fermenting various grains such as maize and wheat, Kivanc et al. [80] isolated numerous strains of *Lactobacillus plantarum* that exhibited antagonistic activity against pathogenic bacteria such as *Listeria monocytogenes*, *Bacillus cereus*, *B. subtilis*, *Yersinia enterocolitica*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica Serovar Typhimurium*, and *Klebsiella pneumoniae*. Oluwajoba et al. [81] isolated probiotic LAB strains belonging to *Lactobacillus*, *Pediococcus*, and *Lactococcus* species with antimicrobial activity against the reference strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* from Kunuzaki, a Nigerian traditional fermented drink made from non-germinated sorghum and millet cereal grains. In the case of Hardaliye, a Turkish non-alcoholic fermented beverage produced from a mixture of red grapes (*Vitis vinifera*), crushed mustard seeds (*Sinapis alba*), and dried sour cherry leaves (*Prunus cerasus*), Amoutzopoulos et al. [82] observed a LAB population constituted exclusively by *Lactobacillus* species. Arici and Coskun [83] isolated and identified 15 strains as *Lactobacillus casei* subsp. *pseudoplantarum*, six as *Lactobacillus pontis*, three as *Lactobacillus brevis*, three as *Lactobacillus acetotolerans*, three as *Lactobacillus sanfransisco*, and three as *Lactobacillus vaccinostercus*. Considering water kefir, a sour, alcoholic, and fruity fermented beverage, the most crucial LAB species present and identified using molecular approaches were *Lactobacillus casei/paracasei*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum/crudilactis* [84], *Lactobacillus nagelii*, *Lactobacillus* species similar to *Lactobacillus hordei/mali*, *Bifidobacterium aquikefiri*, and a novel *Oenococcus* species related to *Oenococcus oeni* and *Oenococcus kitaharae* under the name of *Candidatus Oenococcus aquikefiri* [85].

In another beverage named Makgeolli, a traditional Korean alcoholic beverage, Park et al. [86] isolated 17 different LAB strains identified as *Pediococcus acidilactici* (8), *P. pentosaceus* (6), *Lactobacillus curvatus* (2), and *L. curstorum* (1). Most strains presented immunomodulatory effects; some exhibited high and good bile acid tolerance properties. One strain of *P. acidilactici*

(PA5) also revealed high cell adhesion, making it a promising candidate as a probiotic for further food industry applications.

Palm wine is a traditional beverage made using sap collected from different palm tree species according to their geographic origin, being consumed in other parts of the world (Borneo, India, Mexico, Cameroon, and Côte d'Ivoire), known with local and different designations [87–89]. Using a molecular approach by next-generation sequencing of the V3–V4 regions of the 16S rRNA gene to analyze bacterial diversity and population dynamics during the fermentation process of Tuba (palm wine) samples from laboratory-controlled conditions and commercial samples, Astudillo-Melgar et al. [88] showed that *Fructobacillus* was the main genus in all the samples, followed by *Leuconostoc*, inside the LAB group. Also, molecular techniques, namely 16S rRNA gene sequencing and typification by (GTG) 5-PCR fingerprinting [89], identified their LAB isolates as *Fructobacillus durionis* (40.33%) and *Leuconostoc mesenteroides* (45.66%), with *Leuconostoc pseudomesenteroides*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Weissella cibaria*, *Enterococcus casseliflavus*, and *Lactococcus lactis* occurring occasionally. Fossi et al. [87] isolated three bacteriocinogenic LAB strains identified as *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus brevis* that showed high antimicrobial activity against *Escherichia coli*, *Salmonella enterica*, *Salmonella enterica* subsp. enterica Serovar Typhimurium, *Staphylococcus aureus*, and *Listeria monocytogenes.*

Malolactic Bacteria as a Source of LAB Probiotics

In wine, LAB performs a second fermentation consisting of decarboxylation of L-malic acid to L-lactic acid, designated by malolactic fermentation (MLF). In a recent review work, Inês and Falco [90] collected a panoply of bibliographic references that bring together, to date, the different described species responsible for MLF. Thus, it is possible to find the following species of LAB: (i) Facultative Heterofermentative *Bacilli* (*Lactobacillus casei*, *Lb. coryniformis*, *Lb. curvatus*, *Lb. homohoichii*, *Lb. paracasei*, *Lb. pentosus*, *Lb. plantarum*, *Lb. sakei*, *Lb. zeae*, *Lb. nagelli*, *Lb. diolivorans*); (ii) Heterofermentative *Bacilli* (*Lactobacillus brevis*, *Lb. buchnerii*, *Lb. collinoides*, *Lb. fermentum*, *Lb.*

fructivorans, *Lb. hilgardii*, *Lb. kunkeei*, *Lb. sanfrancisensis*, *Lactobacillus* spp**,** *Lb. vacinostercus*); (iii) Homofermentative *Bacilli* (*Lactobacillus delbrueckii*, *Lb. jensenii*, *Lb. mali*, *Lb. vini*); (iv) Homofermentative *Cocci* (*Pediococcus acidilactici*, *Pc. damnosus*, *Pc. dextrinicus*, *Pc. inopinatus*, *Pc. parvulus*, *Pc. pentosaceus*, *Pediococcus* spp**,** *Lactococcus lactis*, *Lactococcus* spp**,** *Enterococcus* spp.) and Heterofermentative *Cocci* (*Leuconostoc citrovorum*, *Leuc. mesenteroides* subsp. *dextranicum*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuconostoc* spp**,** *Weissella confusa*, W. *paramesenteroides*, *Weissella* spp**,** *W. uvarum*, *Oenococcus oeni*).

As seen from the diversity of species responsible for MLF and already isolated, wine is an excellent source of lactic bacteria for probiotics. Furthermore, many of these species are already used in the formulations of the probiotics previously presented. There is, therefore, a need to investigate strains of other species that may have high probiotic potential that have not yet been explored. Also, according to some authors, as wine is a hostile environment (acid, ethanol, and phenolic compounds), these strains present natural tolerance towards these conditions, thus being additional features that increase their potential to be live probiotics [91].

In the study carried out by García-Ruiz et al. [34], probiotic properties of 11 oenological LAB strains belonging to *Lactobacillus* and *Pediococcus* genera and *Oenococcus oeni* and two probiotic reference strains (*Lactobacillus plantarum* CLC 17 and *Lactobacillus fermentum* CECT5716) were evaluated. The authors investigated many probiotic features, including saliva and acid resistance, bile tolerance, and exopolysaccharides production. They found that the *Lactobacillus* and *Pediococcus* strains showed high resistance to lysozyme (conditions simulating the in vivo dilution by saliva) and were capable of surviving at low pH values of 1.8 and bile salts (simulating stomach and small intestine, respectively), suggesting good adaptation of the wine strains to gastrointestinal conditions.

The same authors [34] also evaluated the ability of the wine strains to adhere to the intestinal mucosa and inhibit the adhesion of *Escherichia coli* to human intestinal cells using Caco-2 cells. They found a *Pediococcus pentosaceus* strain that showed a higher percentage of adhesion to intestinal cells and high anti-adhesion activity against *E. coli,* even higher than that offered by the probiotic reference strains, supporting this wine LAB strain as a potential probiotic.

In another work, with strains of *O. oeni*, beyond their excellent adaptation to gastrointestinal conditions and high bile resistance abilities, inferred by exhibited good survival abilities at low pH values (pH 1.8) and bile salts (1%), Su et al. [91] also verified the effectiveness of *O. oeni* strains in the defense against in vitro oxidative stress, potentially serving as defensive agents in the intestinal microbial ecosystem to overcome exogenous and endogenous oxidative stress.

Foligné et al. [77] suggest that the biochemical and physiological characteristics mentioned earlier and those identified by different researchers could provide additional advantages in various applications. Furthermore, bacteria deemed either "beneficial" or "unsuitable" for winemaking might harbor health-promoting potential. These features include (i) high resistance of wine LAB to acidic conditions, alcohol, and sulfur dioxide stress; (ii) the ability of some wine LAB to produce EPS (responsible for colitis attenuation by probiotic strains, contributing to resistance against bile salts and low pH, promoting adhesion to intestinal epithelial cells, and reducing the biofilm formation of pathogens); (iii) adherence of some wine LAB to epithelial cells, a characteristic believed to hold relevance in probiotic functionality; (iv) secretion of bacteriocins by specific wine LAB, crucial not only as food preservatives but also with potential health benefits by exhibiting activities against *Listeria monocytogenes* and *Enterococci*; (v) modulation of cytokine production by monocytes and lymphocytes by wine LAB, similar to some other strains of *lactobacilli* and bifidobacteria. The intake of these strains in sufficient quantities may redirect the immune system toward a regulatory or tolerance mode, potentially helpful in preventing or treating atopic or inflammatory bowel diseases. It is emphasized that oenological strains as probiotics may primarily exhibit probiotic potential outside their natural habitat and might be more suitable as supplements than in functional food [77].

Prebiotics Derived from Wine and Other Fermented Beverages

Consumers worldwide are increasingly aware of the relationship between nutrition and health. The central segment of this market includes foods designed to improve gut health, such as prebiotics. Prebiotics were defined in 1995 by Gibson and Roberfroid [92] as "non-digestible food constituents that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon." Later, Gibson et al. [93] defined prebiotics as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits upon host well-being and health.".

The International Scientific Association for Prebiotics and Probiotics (ISAPP) in 2010 extended the definition to include the functionality of the prebiotics: "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [94]. Prebiotic dietary fibers become carbon sources for primary and secondary fermentation pathways in the colon and maintain digestive health in several ways. Prebiotics are generally low or non-digestible oligosaccharides, particularly fructooligosaccharides, and inulin, which are usually agreed-upon prebiotics, Table 1. Other prebiotic dietary fibers are beta-glucans [95], isomaltooligosaccharides, guar gum, maltodextrin, xylooligosaccharides (XOS), and arabinooligosaccharides. Resistant starch can also promote health by producing a high level of butyrate, so it has been suggested to be classified as a prebiotic [96]. They are classified as prebiotics since they are not hydrolyzed or absorbed in the upper part of the gastrointestinal tract. They beneficially affect the selective stimulation of the growth of bacteria in the colon and may inhibit pathogens; therefore, they can have health benefits [92,97]. Prebiotics of several types occur naturally in asparagus, leeks, onions, soybeans, wheat, oats, Jerusalem artichokes, garlic, barley, bananas, rye flour, and chicory [98,99]. In most of these foods, the prebiotics concentrations range between 0.3% and 6% of fresh weight; for chicory, these values are between 5% and 10%, while in Jerusalem artichoke, they can reach up to 20%.

Table 1: Individual prebiotics and their fruit or vegetable sources. Adapted from Shigwedha et al. [100].

Prebiotics	Primary Source
Cellulose/hemicellulose/lignin/waxes	Plant foods
Guar gum	Guar bean (legume)
Inulin/oligofructose/Fructooligosaccharides (FOS)	Chicory root, wheat, Jerusalem artichoke, banana, onions, leeks, garlic
Chitooligosaccharides (COS)	Derivative of chitin
Xylooligosaccharides (XOS)	Corn cobs, rice hulls, straws, bagasse, malt cakes, and bran
Soybean oligosaccharides	Soybean
β-Glucan and oat bran	Oats and barley
Pectin, gums	Plant foods
Resistant dextrin	Corn and wheat
Resistant starch	Plant foods
Soluble corn fiber	Corn

Fructooligosaccharides are mainly present in asparagus, Jerusalem artichoke, chicory, sugar beet, onion, garlic, barley, wheat, honey, banana, tomato, and rye, with concentrations ranging from 0.15% to 0.75% of FOS in natural food [100–102]. Chemically, fructooligosaccharides are short- and medium-length chains of β-D-fructans in which fructose units are bound by β-2-1 osidic linkage. The human gastrointestinal tract does not digest fructooligosaccharides. Obtained from plant sources, when they reach the colon, they beneficially stimulate the growth and strengthening of specific bacteria in the intestine [103].

Inulin is a heterogeneous blend of fructose polymers found in nature as plant storage polysaccharides (degree of polymerization, DP < 10) [104], such as in wheat (1.0–3.8 g/100 g), raw onion pulp (1.1–7.5 g/100 g), garlic (9.0–16.0 g/100 g), Jerusalem artichoke tuber (16.0–20.0 g/100 g), chicory root (35.7–47.6 g/100 g), asparagus raw (2.0–3.0 g/100 g), and barley (0.5–1.0 g/100 g) [105].

Beta-glucans (β-glucans) are linear polysaccharides formed by D-glucopyranosyl units with a mixture of β-(1,3) and β-(1,4) glycosidic linkages. β-glucans are chemical, non-starch polysaccharides with repeating glucose residues in linear chains or multiply branched structures, with the glucose units being branched in diverse ways depending on the source of origin. Cereal β-glucan chains are linear, composed of consecutive linked (via β-1–3 linkages) cellulosic oligomers, i.e., segments of β-1–4 linked glucose residues [106,107]. For microbial β-glucans, the β-D-glucopyranose units are linked together through β-(1,3) linkages to form a long backbone, whereas side chains mainly arise through β-(1,6) linkages [108]. β-glucans are soluble fibers in the endosperm cell walls of cereal grains', such as in oats and barley (up to 7%) or in mushrooms, algae, and other marine plants [95,109,110]; they can also occur in rye and rice [111].

Isomaltulose is a naturally occurring disaccharide composed of α-1,6-linked glucose and fructose, and it is considered a prebiotic [112]. It occurs naturally in honey, sugarcane juice, and derived products [113].

Xylooligosaccharides are a prebiotic with health benefits [114]. They comprise sugar oligomers of xylose units and are found naturally in fruits, honey, vegetables, and bamboo shoots [115]. Phenolic compounds, naturally occurring secondary metabolites of plants, comprise several compounds classified mainly based on structure [116]. Plant-based beverages, including fruits, vegetables, and wine, are rich sources of dietary polyphenols. Preclinical and clinical studies suggest polyphenols can express prebiotic properties and exert antimicrobial activities against pathogenic gut microflora [117–120].

Parkar et al. [121] showed the inhibitory effect of citrus polyphenols, such as hesperetin, naringenin, poncirin, and diosmetin, on the growth of human gut bacteria. Wine phenolic compounds have also been suggested to modulate gut microbiota, inducing prebiotic-like effects by promoting the growth of beneficial bacteria and inhibiting pathogenic bacteria [122–124]. For example, during wine consumption, oligomeric procyanidins arrive in the colon [125]. Red wine phenolic compounds may modify the gut microbial composition through their antimicrobial properties, inhibiting non-beneficial bacteria and promoting the growth of probiotic bacteria like bifidobacteria. This, in turn, can affect their functional relations with the host [126]. Intestinal bacteria metabolize wine polyphenols into specific bioavailable metabolites, and the beneficial actions attributed to wine are recognized as these phenolic microbial-derived metabolites rather than the initial precursors present in wine [126,127].

Non-dairy probiotic fermented beverages, using various substrates such as cereals, soy milk, fruit, and vegetable juices, are known for their health-promoting qualities. These fermented beverages are appealing due to their absence of dairy allergens such as lactose, low cholesterol content, and vegan-friendliness [128].

Cereal-based beverages have natural prebiotics due to the presence of indigestible fibers. Cereals like oats, wheat, maize, rye, millet, sorghum, barley, or rice produce cereal-based fermented beverages. Oats, a notable source of β-glucans that can reduce LDL cholesterol, are known to act as a prebiotic by boosting bifidobacteria numbers in the gut. Processing conditions, such as fermentation, could support the development of a cereal beverage with high beta-glucan values [129]. Oat-fermented beverages with high β-glucans and bananas have been studied as alternatives for individuals avoiding milk proteins or lactose intolerance [109]. Barley and malt have also been used as beverage substrates [130].

Numerous traditional cereal-based fermented beverages exist, both alcoholic and non-alcoholic, and some examples are listed in Table 2 [131–133].

Table 2: Cereal fermented drinks. Adapted from Blandino et al. [131].

Product	Subtract	Nature of Use	Country
Bagni	Millet	Liquid drink	Caucasus
Bouza	Wheat	Thick, acidic, yellow, alcoholic beverage	Egypt
Boza	Wheat, millet, maize, and other cereals	Thick, sweet, slightly sour beverage	Albania, Turkey, Bulgaria, Romania
Braga	Millet	Liquid drink	Romania
Brembali	Rice	Dark brown alcoholic drink	Indonesia
Burukutu	Sorghum	Alcoholic beverage of vinegar-like flavor	Nigeria, Benin, Ghana

Busa	Rice or millet	Liquid drink	Syria, Egypt, Turkestan
Busaa	Maise	Alcoholic beverage	Nigeria, Ghana
Chikokivana	Maize and millet	Alcoholic beverage	Zimbabwe
Chongju	Rice	Alcoholic clear drink	Korea
Darassum	Millet	Liquid drink	Mongolia
Doro	Finger millet malt	Colloidal thick alcoholic drink	Zimbabwe
Kachasu	Maize	Alcoholic beverage	Zimbabwe
Kaffir beer	Kaffir corn	Alcoholic drink	South Africa
Khaomak	Rice	Alcoholic sweet beverage	Thailand
Mangisi	Millet	Sweet-sour non-alcoholic drink	Zimbabwe
Merissa	Sorghum and millet	Alcoholic drink	Sudan
Otika	Sorghum	Alcoholic beverage	Nigeria
Pito	Maize, sorghum, maize, and sorghum	Alcoholic dark brown drink	Nigeria, Ghana
Sake	Rice	Alcoholic clear drink	Japan
Seketeh	Maize	Alcoholic beverage	Nigeria
Shaosinghjiu	Rice	Alcoholic clear beverage	China
Sorghum beer	Sorghum, maize	Liquid drink, acidic, weakly alcoholic	South Africa
Takju	Rice, wheat	Alcoholic turbid drink	Korea
Talla	Sorghum	Alcoholic drink	Ethiopia
Tapai pulut	Rice	Alcoholic dense drink	Malaysia
Tapuy	Rice	Sour, sweet alcoholic drink	Philippines
Tesgüino	Maise	Alcoholic beverage	Northern and North-Western Mexico

European barley beer is an example of a cereal-based fermented beverage [132]. In Africa, traditional beers differ from Western types; they are often less carbonated, sour, and lack hops. Examples include pito and burukutu, brewed by fermenting malted or germinated single cereal grains or a mixture [134]. Cereal fermentation in Africa and Asia mainly involves processing maize, rice, sorghum, and millet [135]. For instance, Zambia and the Democratic Republic of Congo produce a non-alcoholic spontaneously fermented cereal-based beverage called Munkoyo [136]. In Tanzania, a fermented cereal grains beverage with sorghum, maize, and millet called Togwa is produced [131]. West Africa has a fermented sorghum gruel named Ogi-baba [137]. In Africa and Asia, various opaque beers called Tchoukoutou are produced by malting red sorghum [138]. Water-soluble and insoluble arabinoxylans, β-glucan, oligosaccharides, and resistant starch are indigestible but fermentable dietary carbohydrates in cereals. These serve as fermentation substrates for probiotic lactic acid bacteria, realizing the beneficial effect of both probiotic organisms and imparting prebiotic effects [139].

Jovanovic-Malinovska et al. [140] profiled oligosaccharides in 32 fruits (total FOS ranged from nd-0.89 g/100 g fresh weight of the edible sample) and 41 vegetables (full FOS ranged from nd-3.32 g/100 g fresh weight of the edible sample), observed that fruits generally contained low amounts of total fructooligosaccharides. The content of total oligosaccharides in fruits, in descending order, was nectarine > watermelon > pear > raspberry > blueberry. It was scallion > onion > garlic > leek > spring garlic for vegetables. Nectarine had the highest fructooligosaccharide content (0.89 ± 0.031 g/100 g fresh weight). The vegetable with the highest fructooligosaccharide quantity was scallion (3.32 ± 0.108 g/100 g fresh weight). According to several authors, beverages formulated with fruit and vegetable juices are promising food matrices to serve as carriers of probiotic bacteria [141,142].

Various fermented beverages are produced with fruits from Asia, Africa, and Latin America. While grape/grape juice is perhaps the most economically fermented beverage (wine), other fruit-fermented drinks, such as pineapple in Latin America and jackfruit in Asia, exist [143]. Mexico produces fermented beverages from agave (Pulque), coconut palm (Taberna), or coyol palm (Tuba) [144–146].

Final Remarks

While bacterial colonization of the human gut undergoes evolution and transformations throughout a lifetime, it remains a crucial component of our microbiome. In the gut, these bacteria play a pivotal role in modulating the internal environment, influencing human health from defense functions to digestion improvement and brain-gut responses. Manipulating the gut microflora by increasing the relative number of "helpful bacteria" can beneficially influence our body functions. Consequently, a simple, low-cost, friendly, and natural way to enhance host health is to ingest probiotics and naturally add them to our food chain.

The microbiota of fermented products constitutes a complex microbial system, with fermented dairy products being a primary source of probiotics. However, alcoholic, low-alcoholic, and non-alcoholic fermented beverages are integral parts of the food culture in many countries worldwide. Various fermented beverages are produced using multiple substrates, including cereals, fruits, and vegetables. These beverages naturally contain probiotics, prebiotics, and symbiotics, serving as sources of health-promoting components.

Probiotics may consist of various microorganisms, with the most common belonging to groups such as *Lactobacillus*, *Lactococcus*, and *Bifidobacterium*. Yeasts like *Saccharomyces*, including *S. boulardii*, and other non-*Saccharomyces* yeasts, often termed "wine yeasts," are also widely recognized for their beneficial use. Wine, among other alcoholic drinks like Boza and Kvass, contains many *Lactobacillus* species, making it an essential source of probiotic bacteria. Interestingly, many of these species are already utilized in the formulations of pharmaceutical-origin probiotics.

In conjunction with probiotics, global consumers increasingly recognize the connection between nutrition, health, and prebiotics. Prebiotics are selectively fermented ingredients resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota, conferring benefits upon host health. Examples include low or non-digestible oligosaccharides, fructooligosaccharides, β-glucans, isomaltooligosaccharides, guar gum, maltodextrin, xylooligosaccharides, and arabinooligosaccharides, stimulating the growth of beneficial bacteria in the colon and inhibiting pathogens.

Plant-based beverages, such as wine, also serve as rich sources of dietary polyphenols expressing prebiotic properties and exerting antimicrobial activities against pathogenic gut microflora. They inhibit non-beneficial bacteria and promote the growth of probiotic bacteria, such as bifidobacteria.

In conclusion, including fermented drinks in our daily meals can significantly contribute to our health. These flavoured beverages offer potential health benefits due to their microbial composition.

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