beverages

Review

Beer with Probiotics: Benefits and Challenges of Their Incorporation

Diana Santos [1], Luisa Barreiros [1,2], Ângelo Jesus [1,3], Ana Luísa Silva [1,4,5], João Paulo Martins [1,6], Ana Isabel Oliveira [1,3] and Cláudia Pinho [1,3,*]

1 ESS, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal; drs@ess.ipp.pt (D.S.); lsb@ess.ipp.pt (L.B.); acj@ess.ipp.pt (Â.J.); alu@ess.ipp.pt (A.L.S.); jom@ess.ipp.pt (J.P.M.); aio@ess.ipp.pt (A.I.O.)
2 LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
3 REQUIMTE/LAQV, ESS, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal
4 UMIB—Unit for Multidisciplinary Research in Biomedicine, ICBAS—School of Medicine and Biomedical Sciences, University of Porto, 4050-313 Porto, Portugal
5 ITR—Laboratory for Integrative and Translational Research in Population Health, 4050-600 Porto, Portugal
6 CEAUL—Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
* Correspondence: clp@ess.ipp.pt; Tel.: +351-222061000

Abstract: Beer is considered one of the most consumed beverages worldwide and a potential vehicle for probiotics. However, there are several technical challenges to overcome during the production and storage of beers, as probiotics must remain viable until the moment of consumption. Therefore, this work aims to discuss how the incorporation of probiotics improves or adds value to beer and which variables influence the viability of the process. This is a narrative review of the literature with research in the PubMed, Web of Science, and b-on databases for articles related to the incorporation of probiotics in beer and the variables that influence the process. The results demonstrated that the incorporation of probiotics into beer faces technical challenges such as probiotic selection, pH, the presence of alcohol, and beer's production and storage temperatures. However, strategies such as immobilizing probiotics in alginate, alginate–silica, and durian husk powder, fermentation with the yeast *Saccharomyces cerevisiae* var. *boulardii*, and co-fermentation with probiotics permit us to overcome these barriers. Thus, incorporating probiotics into beer brings added value, potentially increasing antioxidant activity and phenolic compound content and providing unique flavors and aromas. Nevertheless, strict control of the technical conditions involved is necessary to ensure probiotic viability and the health benefits they confer.

Keywords: probiotics; beer; encapsulation; fermentation; co-fermentation; health benefits

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1. Introduction

Beer is among the most widely consumed and popular beverages globally. In 2022, around 1.89 billion beer hectoliters were manufactured, with China (≈360.41 million hectoliters) and the United States (≈194.1 million hectoliters) being the global leaders in beer production [1,2]. In the European Union, beer consumption was around 313 million hectoliters in 2022. Germany leads beer production in Europe, with a total of 87,832 hectoliters, while Spain ranks second, with 41,138 hectoliters. Portugal has a beer production of 7787 hectoliters. Regarding beer consumption, Germany leads with 79,094 hectoliters, followed by the United Kingdom (45,870 hectoliters) and Spain (42,312 hectoliters). Portugal occupies the 13th place in the ranking, with a beer consumption of 6005 hectoliters in 2022. Finally, the number of active breweries in the European Union increased from

9500 breweries in 2021 to 9680 in 2022. In addition, nowadays, non-alcoholic beer represents over 5% of the European beer market [3,4].

Excessive alcohol consumption is harmful; however, beer with functional ingredients, when moderately consumed (12 g/day of ethanol for women and 24 g/day of ethanol for men), can provide benefits and minimize the deleterious effects linked to alcohol consumption [4,5].

The brewing process has the following nine unit operations (some are not mandatory for beer production): milling, mashing, wort filtration, wort boiling, wort treatment, fermentation, maturation, filtration, and pasteurization [6]. However, there are some technical challenges to the incorporation of probiotics in an alcoholic drink, such as beer, that is frequently pasteurized and filtered [7].

Beer pasteurization aims to extend beer's shelf-life, but it may also adversely impact its organoleptic characteristics (i.e., color, aroma, and flavor) [8]. However, in the production of beer with probiotics, filtration and pasteurization processes should be avoided, as they can remove or kill, respectively, the probiotics, unless their addition is only carried out after those mentioned steps [9,10]. Thus, the absence of the filtration and pasteurization steps in the manufacture of craft beers gives them a greater advantage when it comes to production with probiotics, to the detriment of industrial beers [9,11].

The composition of beer can be different from one beer type to another. Nevertheless, the average beer contains nutrients, such as minerals, vitamins, amino acids, carbohydrates, and proteins [12]. The present need for health benefits, which would draw consumers to search for new products and special beers, is a prospective market gap. Using health-promoting probiotic microorganisms in the brewing process is one technique to combine beer production with health criteria [13].

The definition of "functional food or drink" refers to a non-alcoholic drink product whose constituents include minerals, vitamins, amino acids, and plants (vegetables or fruits) with nutritional value and health benefits. Beverages with more than 1.2% v/v ethanol must not be characterized as having any health or nutritional benefits. As a result, a beer that contains a probiotic microorganism can only be classified as either a low-alcohol beer with a maximum of 1.2% v/v ethanol or an alcohol-free beer with 0.5% v/v ethanol. Therefore, non-alcoholic beer can be viewed as a functional beverage, and there is an increasing interest in creating innovative functional beers that offer enhanced health benefits [14–16]. Numerous articles refer to functional beers, such as probiotic, xanthohumol, kefir, and estrogenic beers, among others [13,17,18]. Biologically active components can be added to beer in numerous ways, and probiotic beers represent a new variety of significant interest [14]. Probiotics can be described as live microorganisms that are ingested in sufficient quantities to potentially provide the host with health benefits [19]. Therefore, their incorporation into functional foods is of particular interest, given their ability to maintain healthy intestinal microbiota and immune systems [20], potentially preventing and reducing the incidence of many diseases [21].

In the food industry, there are two common problems associated with probiotics, one related to their susceptibility to processing conditions and the other related to sensitivity to gastrointestinal stress [21]. It is known that, in contrast with other beverages (such as juice, coffee, or water), combined probiotic cultures in beer matrices demonstrated greater viability after digestion, making them effective carriers for probiotics consumption [22]. However, there are still several technical challenges to be overcome during the production and storage of industrial or craft beers with probiotics, in order to produce beverages that are both beneficial and appealing to consumers [12]. For the production of probiotic-enriched beers, it is important to develop strategies in order to adapt, tolerate, or resist compounds that may create a challenging environment within beer [23].

Therefore, the present literature review aims to critically discuss how the incorporation of probiotics improves or adds value to beer and which variables influence the viability of the process.

2. Materials and Methods

A narrative review was carried out between May 2023 and September 2024, using the PubMed, Web of Science, and b-on databases and the keywords "beer", "probiotic", and "co-fermentation" combined with the boolean operators "OR" and "AND". The inclusion criteria were reviews and research papers focusing on studies with reference to the effect of incorporating probiotics into beer, written in English and Portuguese, and without established temporal limits.

3. Probiotic Microorganisms

Traditionally, beer fermentation is performed by the two yeasts widely used as starter cultures, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, which produce the two main categories of industrial beer, 'ale' and 'lager' beers, respectively [24]. However, a growing number of studies have been conducted in order to use probiotic microorganisms in beer fermentation, given the consumer demand for beers with additional nutritional value as well as a differentiated flavor and aroma [25–28].

The best-known probiotic microorganisms are lactic acid bacteria, especially Lactobacilli, Bifidobacteria, Enterococci, or Streptococci [26]. Lactic acid bacteria are often associated with beer spoilage due to the production of undesirable compounds that can negatively affect the taste, aroma, and stability of the beverage [23,29]. However, when properly selected and added, lactic acid bacteria can be used beneficially in the production of sour beers, as they increase the acidity of the beer [23,29]. In turn, the yeast *Saccharomyces cerevisiae* var. *boulardii* (or *S. boulardii*) is also commonly used given its probiotic properties [26]. Table 1 presents some of the most common microorganisms related to probiotic activity.

Table 1. Most common microorganisms related to probiotic activity.

	Present Scientific Classification/Basionym or Homotypic Synonym	References
Lactobacilli	*Lactobacillus acidophilus/Bacillus acidophilus* *Lacticaseibacillus casei/Lactobacillus casei* *Lacticaseibacillus rhamnosus/Lactobacillus rhamnosus* *Limosilactobacillus reuteri/Lactobacillus reuteri* *Lactobacillus helveticus/Thermobacterium helveticum* *Companilactobacillus farciminis/Lactobacillus farciminis* *Lactobacillus curvatus* *Levilactobacillus brevis/Lactobacillus brevis* *Lactobacillus gasseri* *Ligilactobacillus salivarius/Lactobacillus salivarius* *Limosilactobacillus fermentum/Lactobacillus cellobiosus* *Lacticaseibacillus paracasei/Lactobacillus paracasei* *Lentilactobacillus buchneri/Lactobacillus buchneri*	[10,11,20,27,28,30–33]
Bifidobacteria	*Bifidobacterium bifidum* *Bifidobacterium breve* *Bifidobacterium longum subsp. Infantis/Bifidobacterium infantis* *Bifidobacterium longum* *Bifidobacterium animalis subsp. lactis/Bifidobacterium lactis* *Bifidobacterium thermophilum Bifidobacterium adolescentis* *Bifidobacterium animalis*	[32,34]
Other Bacteria	*Enterococcus faecium/Streptococcus faecium* *Escherichia coli Nissle 1917* *Lactococcus lactis/Bacterium lactis* *Propionibacterium freudenreichii/Propionicibacterium freudenreichii* *Shouchella clausii/Bacillus clausii* *Bacillus oligonitrophilis*	[32,34]
Yeasts	*Saccharomyces boulardii* *Saccharomyces cerevisiae/Mycoderma cerevisiae* *Lachancea fermentati/Zygosaccharomyces fermentati* *Cyberlindnera subsufficiens* *Lachancea thermotolerans/Zygosaccharomyces thermotolerans* *Monosporozyma unispora/Kazachstania unispora*	[26,28,32]

4. Incorporating Probiotics into Beers: Challenges and Strategies

Consumer demand for better quality and healthier beverages and foods has forced innovations in the brewing industry; therefore, the idea of functional beer has come into existence. Strategies for the development of functional beers include the incorporation of botanicals (plants or fruits), micronutrients (such as selenium), active ingredients for a healthier beer (phytoestrogens, L-carnitine, β-glucan, or spirulina), and microorganisms (probiotics), but also the removal of certain compounds to result in gluten-free, low-calorie, low-alcohol (0.5–1.2% *v*/*v*), or alcohol-free beers (<0.5% *v*/*v*) [14,35].

Beer is the most preferred alcoholic beverage with sensory, nutritional, and medicinal properties, and it is recognized that the incorporation of probiotics into this beverage will confer positive health outcomes that a conventional beer cannot provide [36]. However, this incorporation triggers several technical challenges since probiotics are sensitive microorganisms. Therefore, the selection of strains and the quantity of inoculum for beer production are fundamental criteria for obtaining a product with viable probiotics and desirable and distinct sensory properties [27,30].

Water is the most prevalent ingredient present in beer; however, due to a multi-step brewing and fermentation procedure, this beverage is also rich in nutrients, which can be favorable to the viability of probiotics. However, there are also a number of factors that create a challenging environment within beer, including high CO_2 levels, low pH, low oxygen availability, and antimicrobial ingredients such as hops and ethanol [23,34].

Most beers' ethanol level is expressed as the amount (in percentage) of alcohol by volume (ABV). The ethanolic concentration in beer ranges from 3–9% and is known to inhibit bacterial growth by denaturing proteins and disrupting membrane stability [34]. However, *Levilactobacillus brevis* and *Schizosaccharomyces pombe* are among the microorganisms that can withstand an ethanol level of up to 18%, along with yeasts, acetic acid bacteria (AAB), and lactic acid bacteria (LAB) [23].

Hop cones are female flowers that contain iso-α acids extracted while the wort is boiling. The iso-α acids contribute to the bitterness and acidity of beer, and due to their role as ionophores, which dissipate the pH gradient across the membranes of bacteria and diminish proton motive force, they are capable of inhibiting Gram-positive bacteria [34].

Therefore, for the incorporation of probiotics in beer, the microorganism selected should be resistant to bitter iso-α acids. Given the growth inhibition of lactic acid bacteria with iso-α acids, the use of these compounds is limited to a scale of 25 International Bitterness Units (IBU) as the maximum bitterness [17]. However, more tolerant microorganisms, such as *Saccharomyces boulardii*, are required to create additional probiotic beer styles with increased concentrations of α acids (such as stouts, lagers, and India Pale Ale) [17,23]. It is also crucial to consider the time when hops are added to beer because introducing hops during the initial stages of boiling causes their compounds to undergo isomerization, which may affect probiotic growth [37].

In addition to all the conditions mentioned, the metabolites formed by probiotics during fermentation and storage, as well as the production and storage temperature, are also challenges that need to be addressed [11,27,34]. The beer matrix can be a stressful environment for probiotics, resulting in the accumulation of unwanted substances such as diacetyl and L-lactate, which impart undesirable flavors to beer [11,27,34]. In addition, temperature also plays an essential role, as in refrigerated conditions (such as 4 °C), probiotics have a better survival rate [34].

Despite technical challenges, strategies have emerged to overcome these barriers, namely probiotic immobilization, co-fermentation, and fermentation with *S. boulardii*, enabling the creation of functional beers that are potentially beneficial for consumers' health.

4.1. Encapsulation/Immobilization of Probiotics

Encapsulation or immobilization technologies refer to the process of trapping anything in a matrix, which can increase the growth and survival of probiotics in products, including

beer. One advantage of this approach is maintaining greater cell viability despite the stomach's acidity. Various immobilization techniques have been explored, with particular emphasis on biopolymers and natural supports [20,38]. To discuss these techniques, three studies were included in this section, as shown in Table 2 [20,30,34].

Table 2. Probiotic encapsulation/immobilization techniques.

Probiotics	Encapsulation/Immobilization Matrix	Reference
Lactobacillus rhamnosus GG	Alginate and alginate–silica	[30]
Lacticaseibacillus rhamnosus GG, *Escherichia coli* Nissle 1917, and *Bifidobacterium longum*	Alginate	[34]
Lactobacillus brevis	Powder of durian (*Durio zibethinus*) rind	[20]

The most used material for probiotic encapsulation is alginate due to its natural origin, biocompatibility, low cost, and mild gelation when combined with divalent cation crosslinkers [32,39].

In their study, Haffner and Pasc (2018) studied the viability of lyophilized *Lactobacillus rhamnosus* GG (LGG) (free and encapsulated) in alginate or in alginate–silica microcarriers, upon storage, in beer containing 5% (*v*/*v*) alcohol and another beverage, at 4 °C [30]. The encapsulation approach was developed based on the observation that alginate forms a relatively flexible network suitable for cell proliferation. However, relying solely on polymer protection is insufficient to avoid bacterial leakage and ensure that cells are shielded from the acidic external environment. As a result, the use of a silica shell allows the system to be supported while assuring compatibility with bacteria and human consumption [30]. Regarding beer and the results from encapsulation, beer with 5% (*v*/*v*) alcohol was mixed with alginate and silica-coated beads, and the release of LGG from the carriers was monitored following residence periods of 3 h and one week in each tested beverage. Furthermore, the authors evaluated the number of bacteria retained within the carriers. Results demonstrated that this encapsulation approach limits the release of probiotics over time, and alginate–silica beads demonstrated greater efficiency compared to alginate beads on their own. After the silica-coated beads were left in beer for a week, the filtrate only presented 2.7×10^2 CFU/mL. After the same one-week period, the alginate beads values in the filtrate were augmented 10-fold in beer. In fact, after just 3 h in beer, the viability of the bacteria within the alginate beads dropped by about two logs (from $\approx 10^5$ to 10^3 CFU/mL). After 3 h and one week, silica-coated beads showed viability of 2.5×10^6 CFU/mL and 1.4×10^5 CFU/mL, respectively, in 5% (*v*/*v*) beer. Compared to alginate, these silica-coated beads were shown to be a more effective choice, as the pores in the alginate matrix may allow increased ethanol diffusion into the beads, resulting in toxicity to probiotics [30].

Recently, Tan et al. (2023) verified that the viability of unencapsulated and alginate-encapsulated probiotics (*Lacticaseibacillus rhamnosus* GG, *Escherichia coli* Nissle 1917, and *Bifidobacterium longum*), alone, in commercialized beers (lager and stout) and other beverages, and at different exposure conditions, was investigated [34]. Results demonstrated that, under the evaluated conditions, alginate encapsulation increased the viability of all probiotics in beer. This can be explained by alginate's pH-buffering effect, which reduces the antagonistic effects of acidity combined with bactericidal compounds found in beers (such hops' iso-α acids). In addition, alginate encapsulation protected the probiotics from the gastric passage. Overall, all unencapsulated probiotics decreased the production of L-lactate (a probiotic metabolic by-product) in refrigerated conditions and displayed better survival at 4 °C than at 25 °C within the 14 days of the experiment. Therefore, for the development of functional beers with encapsulated probiotics, and in order to minimize viability losses and prevent flavor alterations that may result from probiotic metabolism, it is advised to keep them in an environment with a refrigeration temperature [34].

Other natural matrices have been used for probiotic immobilization [20]. For example, a highly popular tropical fruit in Southeast Asia is the durian (*Durio zibethinus*). Only

around one-third of the fruit is eaten, with the fiber-rich rind making up over half of the fruit's weight [20,40]. Consequently, probiotics may be immobilized using powder made from durian rind, a by-product that is now wasted [20]. Calumba et al. (2021) aimed to produce an ale beer using durian rind powder to function as a delivery system for free *Lactobacillus brevis* (FLB) or immobilized cells (ILB). The final carbonation of beer was 2.8 volumes CO_2 at 24 days of storage, which is typical for ales, and it was demonstrated that *L. brevis* in beer (both free and immobilized) was resistant to CO_2 levels up to 2.8 volumes. In addition, immobilization in durian rind powder did not have a significant impact on the total soluble solids, specific gravity, and alcohol content in beer after 24 days of storage at an ambient temperature (21 °C). The pH of FLB and ILB remained at about 4.40 throughout the period of 24 days [20]. These results are in accordance with those of Sakamoto and Konings (2023) (pH from 3.8–4.7), and are considered an unsuitable environment for microbial development [41]. After 24 days of storage, the titratable acidity of both beers, expressed in lactic acid, was not significantly different (4.59 mg/mL lactic acid for beer with ILB in comparison with 4.26 mg/mL for beer with FLB), which means that immobilization in durian rind powder had minimal impact on lactic acid production. Finally, before beer inoculation, the initial cell counts of FLB and ILB were 8.92 ± 0.04 log CFU/mL and 9.94 ± 0.04 log CFU/mL, respectively, regarding the viability of FLB and ILB in ale beer during storage at 21 °C. Over the period of 24 days, values fell to 4.89 log CFU/mL and 5.00 log CFU/mL for FLB and ILB, respectively. However, during storage days 0, 6, and 12, the immobilized cells exhibited higher counts ($p < 0.05$) compared to free cells. This suggests that immobilization effectively protected *L. brevis* for up to 12 days of storage at 21 °C when compared to the control. During the storage period in beer, more than 1 million CFU of *L. brevis* remained viable, indicating that the beverage can potentially provide probiotic benefits as it satisfies the minimum required concentration (10^6 CFU/mL or gram) for a probiotic product to deliver its positive effects [20,42].

4.2. Co-Fermentation

Co-fermentation with probiotics is a technique used in beer production, in which strains of yeast or bacteria are added to the beer wort to work in its fermentation. Co-fermentation with probiotics aims to create beers that contain cultures that are beneficial to the consumer's health, as well as imparting unique flavors and aromas to the beer. To discuss these topics, eight studies were included in this section, as shown in Table 3 [9–11,28,31,33,43,44].

Table 3. Most common probiotics used in co-fermentation.

Non-Probiotic Strain	Probiotic Strain	References
Saccharomyces cerevisiae	*Saccharomyces cerevisiae* var. *boulardii*	[9]
Saccharomyces cerevisiae S-04	*Lactobacillus paracasei* L26	[11]
Saccharomyces cerevisiae S-04	*Saccharomyces cerevisiae* var. *boulardii* and *Lactobacillus paracasei* DTA-81	[33,43]
Saccharomyces cerevisiae WB-06 and *Saccharomyces cerevisiae* CNCM I-3856	*Lactobacillus paracasei* Lpc-37	[44]
Saccharomyces cerevisiae US-05	*Lactobacillus paracasei* F19	[10]
Saccharomyces cerevisiae WLP001	*Lachancea fermentati* KBI 12.1, and *Cyberlindnera subsufficiens* C6.1, *Lactobacillus plantarum* FST 1.7	[28]
Saccharomyces cerevisiae US-05 and *Streptococcus thermophilus* TH-4	*Lactobacillus paracasei* F19 and *Lactobacillus paracasei* 431	[31]

Capece, Romaniello, Pietrafesa, et al. (2018) studied the impact of combining the probiotic strain of *Saccharomyces cerevisiae* var. *boulardii* with the strains of *S. cerevisiae* that are commonly used in mixed cultures. The probiotic yeast outnumbered the *S. cerevisiae* strain

at the conclusion of nearly all mixed fermentations, and the experimental beers had a significant amount of live *S. boulardii* cells (ranging between 8×10^6 and 7.0×10^7 CFU/mL), with a concomitant rise in the levels of polyphenols and antioxidants. Therefore, *S. boulardii* strains could be a useful tool for making probiotic beer and enhancing the product's health benefits. However, probiotic stability and viability during storage were not assessed [9].

Chan et al. (2019) investigated the stability, growth, and viability of *Lactobacillus paracasei* L26 in unhopped wort when co-cultured with *Saccharomyces cerevisiae* S-04 [11]. After that, the isomerized hop extract was incorporated and the mixture was stored between 5 °C and 25 °C [11]. As opposed to probiotic yeasts that can withstand hop iso-acids, incorporating probiotic lactic acid bacteria in beer may be a much more challenging task [45]. According to the results, in co-cultured *S. cerevisiae* S-04 within unhopped wort, *L. paracasei* L26 showed good stability and growth, generated significant amounts of lactic acid ($p < 0.05$), and kept up large levels of viable cells (above 8 log CFU/mL), showing that it is able to contribute to health benefits [11].

In their study, Silva et al. (2020) assessed beer production to act as matrices for probiotic delivery and developed appropriate fermentation systems to assure their viability [43]. Wheat beer and sour beer were generated by fermentation in an axenic (*S. cerevisiae* var *boulardii* 17) or semi-separated co-culture environment (*L. paracasei* DTA-81 and *S. cerevisiae* S-04). Acids may also be intentionally added to sour beers by incorporating fruit juice or produced during the maturation time to develop an acidic profile following fermentation. Co-fermentation systems with yeasts and lactic acid bacteria appear to be a substitute for the addition of acids. In this study, the semi-separated co-culture system led to symbiotic commensal interaction, allowing for the growth and survivability of *L. paracasei* DTA-81. On the other hand, the co-culture (without separation) was not an appropriate system for manufacturing probiotic sour beer because of the competition between yeast and lacticaseibacilli. Therefore, in probiotic sour beer production, *L. paracasei* DTA-81 needed to be inoculated in a semi-separated co-culture system before *S. cerevisiae* S-04, and grown axenically, to enhance probiotic viability and allow acidification. After, *S. cerevisiae* was incorporated to create CO_2, alcohol, and several other secondary compounds.

In another study from Silva et al. (2021), with the purpose of obtaining appropriate fermentation systems for industrial-scale production, the authors intended to create a probiotic-containing functional wheat beer (PWB) using an axenic culture system with potential probiotic *S. cerevisiae* var *boulardii* 17 and to develop a probiotic-containing functional sour beer (PSB) through a semi-separated co-cultivation system with potential probiotic *S. cerevisiae* S-04 and *L. paracasei* DTA-81 [33]. The study also aimed to discuss the ability of these beers to promote antidepressant behavior in vivo in rats [33]. As mentioned before, co-fermentation systems with lacticaseibacilli and *S. cerevisiae* S-04 have been employed as a substitute for adding acids in sour beer production [11]. However, the competition between microorganisms may adversely impact the viability of lactic acid bacteria, which might be enhanced by the use of semi-separated co-cultivation systems, emphasizing the significance of cultivating lacticaseibacilli in the wort prior to the addition of yeast to initiate fermentation [33,43]. Regarding PWB, during wort fermentation by *S. boulardii* 17, glycerol production remained below the threshold of perception, while acetic acid production was approximately six times higher than the sensory limit associated with the taste of beer [33,46,47]. For PSB, *L. paracasei* DTA-81 growth significantly influenced the acidity of the wort, with values ranging from 5.71 to 3.30 [33]. Behavioral tests were also conducted, in vivo, using *Swiss Webster* rats, which allowed the authors to conclude that PWB or PSB may exhibit antidepressant effects in mice, likely by increasing short-chain fatty acids, which can modulate the serotonin–brain-derived neurotrophic factor system [33].

Loh et al. (2021) demonstrate the use of probiotics as starter cultures in beer fermentation with the production of phenolic compounds and amino acid metabolites. Both cocultures of *S. cerevisiae* WB-06 with *L. paracasei* Lpc-37 and *S. cerevisiae* CNCM I-3856 with *L. paracasei* Lpc-37 revealed higher total phenolic content. However, only the cocultures of *S. cerevisiae* WB-06 with *L. paracasei* Lpc-37 showed the highest antioxidant activity [44].

Praia et al. (2022) evaluated four sour beer formulations with bagasse and/or *Spondias mombin* L. fruit juice while using the probiotic strain *L. paracasei* subsp. *paracasei* F19 (F19) and the yeast *S. cerevisiae* US-05 (US-05) [10]. The formulations containing *S. mombin* exhibited the lowest F19 counts, indicating that the survival of probiotics from these components was at risk. After 30 days of storage, the population values of the control formulation (with no ingredient added) were the highest, reaching 6.85 log equivalent CFU/mL [10]. This was the only formulation that maintained adequate levels, suggesting possible probiotic action (from 8 to 11 log CFU/mL) [48]. These results disprove the original theory that additional *S. mombin* bagasse and/or juice would increase the probiotic bacteria's viability [10].

In a recent study from Nyhan et al. (2023), in order to produce an non-alcoholic beer (NAB), wort was fermented with different yeasts (*S. cerevisiae* WLP001, *Lachancea fermentati* KBI 12.1, and *Cyberlindnera subsufficiens* C6.1), isolated, and combined with *Lactobacillus plantarum* FST 1.7. The use of *Lactobacillus* with non-*Saccharomyces* yeasts in limited co-fermentation is an innovative strategy to produce NAB with differing flavor and aroma qualities. Co-fermentation of *L. fermentati* KBI 12.1 and *C. subsufficiens* C6.1 with *L. plantarum* FST 1.7 was recognized as a positive approach for NAB production (< 0.5% ethanol). This can be explained by the fact that the study's yeasts had a lower fermentative capacity, which resulted in lower ethanol production and longer fermentation time (24 and 96 h, respectively) compared with the control yeast, *S. cerevisiae* WLP001 (17 h). The short co-fermentation of *L. fermentati* KBI 12.1 with *L. plantarum* FST 1.7 resulted in an NAB characterized by lower residual sugar concentrations, elevated lactic acid levels, reduced amounts of diacetyl, an undesirable volatile compound that is described as "fruity" and "acidic". In this way, the short co-fermentation of *L. fermentati* KBI 12.1 with *L. plantarum* FST 1.7 made it possible to overcome some of the undesirable aspects pointed out in beers that result from limited fermentation, namely the buttery taste conferred by diacetyl and the sweet taste related to elevated levels of residual sugars, and the beer was classified as the most favorable in the sensory assessment. In addition, co-fermentation of *C. subsufficiens* C6.1 with *L. plantarum* FST 1.7 yielded the lowest pH (3.21) and the highest total titratable acidity (3.77 mL) due to the high concentrations of lactic acid, which are characteristics that can be utilized to create a sour NAB beer [28].

Finally, Herkenhoff et al. (2023) evaluated the usage of *Streptococcus thermophilus* TH-4 (TH-4) as a starter alongside the probiotics *L. paracasei* F19 and 431, in combination with *S. cerevisiae* US-05, both with and without fruit juices (passion fruit and peach), for the production of *Catharina sour* beer. In addition, this study also aimed to assess the mechanisms behind the survivability of the bacteria lactic acid [31]. Sour beer production possesses an additional step of lactic acid fermentation before the alcoholic one, which is performed by yeasts, causing reduced pH levels (3.0 to 3.9) in comparison with "regular beers" (pH = 4.2) [49]. Consequently, while traditional beer fermentation is typically constrained to single-strain yeast fermentation, the production of sour beer can be accomplished through the fermentation of multiple microorganisms, incorporating both yeasts and bacteria [50]. *Catharina sour*, a Brazilian sour-type beer, is a beer style that incorporates fruit or fruit juice into the fermentation process. According to the results, the strains *L. paracasei* F19 and *L. paracasei* 431 possess multiple defense mechanisms, including membrane adhesion proteins and H^+ pumps, which render them suitable candidates for utilization in breweries. In addition, proteome analysis indicated that the *L. paracasei* 431 strain may be the most adaptable to beer conditions, since it has proteins related to carbohydrate metabolism, especially L-lactate dehydrogenase, with greater activity, which translates into the early synthesis of the proteins L-lactate dehydrogenase and D-lactate dehydrogenase, providing energy for rapid strain proliferation [31,51]. Proteomic approaches allow the identification of proteins associated with beer quality attributes, such as turbidity, foam formation, effervescence, taste, and color, as well as contributing to understanding the survival mechanisms of bacteria [52]. The co-culture with *L. paracasei* F19 or 431 did not demonstrate any influence on TH-4, with no differences in single or co-culture [31]. In

general, probiotic products have been observed to offer health benefits when consumed on a daily basis at a dosage of 10^8 to 10^{10} colony-forming units (CFU) [48]. All formulations demonstrated probiotic viability ranging from 5 to 8 log equivalents CFU/mL (equivalent to 8 and 11 log CFU per serving portion of 350 mL) during the production process, indicating the potential for beneficial health effects [31].

4.3. Fermentation with Saccharomyces cerevisiae var. boulardii

Due to its metabolic characteristics, which include resistance to bile and stomach acids, a large range of temperatures for fermentation, excellent viability in the human gastrointestinal tract, and intestinal defense against infections caused by bacteria, *S. cerevisiae* var. *boulardii* has proven to be a useful starter culture for the production of beer [13,17,25,53–55]. To discuss fermentation with *S. cerevisiae* var. *boulardii*, six studies are included in this section [13,17,25,53–55].

Manshin et al. (2022) revealed that the primary benefit of using *S. boulardii* is the potential to produce beer with probiotic properties. Furthermore, the introduction of *S. boulardii* allowed the production of low-alcohol or non-alcoholic beers, as it has low fermentation activity compared to traditional *S. cerevisiae* strains. However, given that the fermentative activity of *S. boulardii* (with an apparent degree of fermentation of 43%) is lower than the brewing strains commonly used (which have an apparent degree of fermentation of 50%), the fermentation process is longer. This situation can be overcome through process optimization, for instance, by changing parameters such as pitching rate, which is the initial density of *S. boulardii* added to the fermentation medium, and temperature [25].

The study by Díaz et al. (2023) corroborates these results, mentioning that *S. boulardii* gave rise to less turbid probiotic beers, which can facilitate filtration, and lower pH levels, reducing the risk of deterioration [17]. Furthermore, *S. boulardii* yeast produced beers with a slightly lower alcoholic content, which is a relevant aspect for the development of healthier beers [17]. In both studies, the quantity of live cells was significantly higher ($p < 0.05$) compared to the beer fermented with conventional yeasts, evidencing their probiotic properties [17,25].

The results obtained by Mulero-Cerezo et al. (2019) are consistent with the above-mentioned studies, demonstrating that craft beer produced with *S. boulardii* yeast in contrast to craft beer made using *S. cerevisiae* yeast has a lower alcohol content (1.65% ABV and 2.39% ABV, respectively), higher yeast viability ($8.3 \pm 1.4 \times 10^4$ and $1.1 \pm 0.2 \times 10^5$ CFU/mL, respectively), and greater acidification (significantly lower pH) ($p < 0.05$), which is positive as it reduces contamination risks in large-scale production. In addition, the craft beer produced with the probiotic *S. boulardii* yeast showed increased antioxidant activity (measured by DPPH Scavenging Activity) [53].

Ramírez-Cota et al. (2021) found that *S. boulardii* CNCM I 745 showed resistance to alcohol concentrations of up to 4% (v/v) at 37 °C and between 6 and 8% (v/v) at 28 °C, which may be promising for the development of functional craft beers [55]. It should be noted that the yeast membrane's fluidity can change in response to increased ethanol concentrations, consequently leading to the inhibition of cell growth and death [55].

In the study performed by Senkarcinova et al. (2019), the possibility of using *S. boulardii* yeast in the production of non-alcoholic beer is described [13]. However, even at 2 °C, the yeast can convert fermentable sugars in the wort into ethanol, which makes it impossible to produce non-alcoholic beers containing fermentable sugars and active probiotic yeast. Therefore, they resorted to high-pressure processing, a non-thermal technology alternative, to steady the alcohol concentration of the beer. This processing not only rendered the yeast inactive, which halted fermentation, but also had little effect on the composition and flavor of the non-alcoholic beer [11].

It was also noted in the study by Paula et al. (2021) that probiotic beer can be made using *S. boulardii* yeast [54]. Although cellular stress caused by the beer and the gastrointesti-

nal tract could reduce the viability of probiotics, the culture remained above 6 log CFU/mL, thus having probiotic potential [49].

5. Effects of Incorporating Probiotics on the Sensory Characteristics of Beer

During fermentation by probiotics, some of the produced compounds can affect the beer's sensory characteristics. According to Tan et al. (2023), there is an accumulation of L-lactate when using encapsulated *L. rhamnosus*, stored at room temperature (25 °C), after 14 days, leading to undesirable flavors in beer. In addition, the usage of non-encapsulated *L. rhamnosus* does not result in significant L-lactate concentrations. To overcome this limitation, beer with encapsulated *L. rhamnosus* must be refrigerated (4 °C), thus minimizing the formation of L-lactate [34].

Dysvik et al. (2020) verified that the co-fermentation of *S. cerevisiae* with *L. plantarum* resulted in a greater fruity and dried fruit odor, while the co-fermentation of *S. cerevisiae* with *L. brevis* presented a higher intensity of acidic, sweet, and astringent flavors in beer [27]. The data also revealed that *Lactobacillus buchneri* is unsuitable for the production of sour beer because it produces high levels of the metabolite diacetyl, which imparts an unpleasant taste. Nyhan et al. (2023) demonstrated that the co-fermentation of *L. fermentati* KBI 12.1 with *L. plantarum* FST 1.7 resulted in a high-acidity beer (pH = 3.54 $\pm$ 0.00) with an acceptable sensory evaluation, given that it has a quantity of diacetyl below the threshold for perception. In turn, the non-alcoholic beer resulting from the co-fermentation of *C. subsufficiens* C6.1 with *L. plantarum* FST 1.7 exhibited a fruity flavor and aroma and high acidity (pH = 3.22 $\pm$ 0.01) [28].

In the study of Capece, Romaniello, Pietrafesa, et al. (2018), the incorporation of *S. boulardii* for co-fermentation with *S. cerevisiae* did not negatively affect the aroma of beer [9]. In turn, Manshin et al. (2022) revealed that the aroma of the beer obtained from fermentation with *S. boulardii* revealed notes of caramel, spices, and fruits and has honey as the main note, along with smoked and wine components [25]. The study by Mulero-Cerezo et al. (2019) corroborates these results, as it is mentioned that craft beer produced with *S. boulardii* yeast has acceptable sensory attributes similar to *S. cerevisiae* [53]. Paula et al. (2021) verified a tendency in acetic acid production by *S. boulardii*, so there must be control of the entire process in order to avoid unwanted flavors and aromas in the final product [54].

Canonico et al. (2021) verified that the probiotic *Lachancea thermotolerans* caused an increase in aromatic notes, namely acidic and fruity notes [26]. *Kazachstania unispora* exhibited effective and distinctive aromatic potential, while *S. cerevisiae* strain 2 PV provided a favorable aromatic profile to beer.

Therefore, in general, probiotic cultures do not harm beer's sensory profile. If the entire fermentation process is properly optimized and controlled, it is possible to obtain probiotic beers with unique aromas and flavors that are acceptable to consumers.

6. Conclusions

Probiotics have been added to beer in response to customers' desire for goods that offer distinctive sensory sensations and support health. Preserving the vitality of probiotics in food matrices prior to ingestion is crucial but still difficult, especially in beers due to their acidity and alcohol content. Studies reveal that the incorporation of probiotics in beer has the potential to add value to the beverage, given the microorganisms' ability to increase antioxidant activity and phenolic compound content, as well as impart unique flavors and aromas. It is also known that heat treatments can make probiotics unviable and, as such, alternatives to their incorporation into beer have been developed (e.g., storing probiotic alginate spheres separately from beer; the possibility of adding lyophilized probiotic powder to the beer's vessel lid, so that the probiotics and beer only come into contact at the time of consumption). Beer is a promising vehicle for probiotics; however, more studies are needed to evaluate its biological activities and probiotic potential.

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