Vegetable Beverages from Carob, Tiger Nut and Rice

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Rice, tiger nut and carob are Mediterranean products suitable for developing new foods, such as fermented beverages, due to their nutritional properties. These crops have a high carbohydrate content, are gluten and lactose-free and have a low allergenicity index. The development of fermented beverages from these crops can contribute to the Sustainable Development Goals by promoting human health and sustainable production and consumption.

fermented beverage carob tiger nut rice antioxidants microbiota lactic acid bacteria yeast

1. Introduction

Carob, tiger nut and rice beverages are produced from different cultures and come from different regions around the world. They are rich in nutrients and have contributed to feeding millions of people.

The production of plant-based beverages involves several stages, starting from the collection of raw materials and culminating in the production of final beverages. Generally, the production process begins with the cleaning and selection of the raw material, followed by grinding and filtration to obtain plant-based milk. However, the specific method used at each stage of the process can vary significantly depending on the raw material employed.

Figure 1 provides an overview of the main production methods for beverages made from tiger nuts, carob, and rice. In the case of tiger nuts, a prolonged soaking process is required to soften the tubers, while to produce carob-based beverages, a roasting stage of the beans before grinding is advised. In the case of rice-based beverages, the raw material undergoes a soaking and cooking process before grinding.

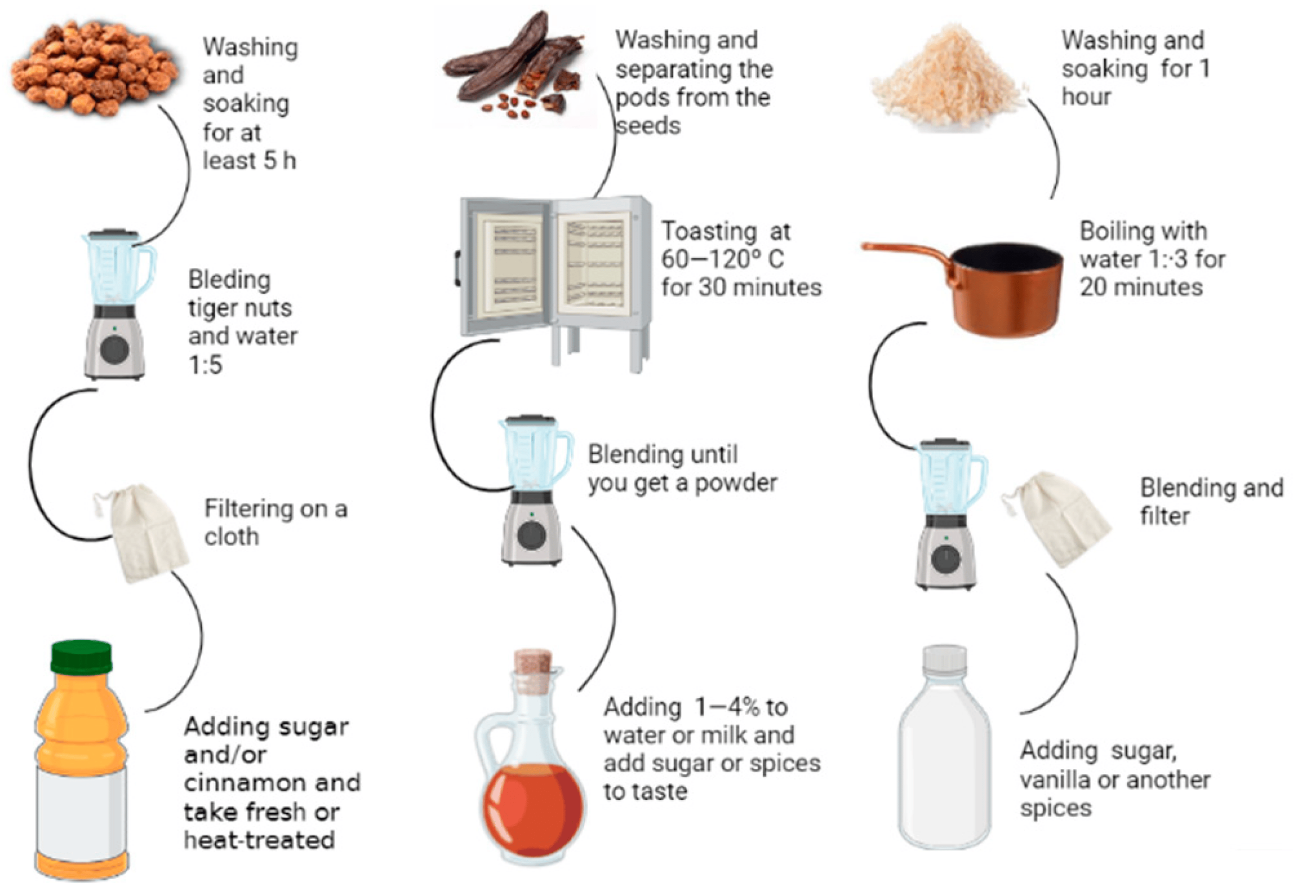

Figure 1. Different production stages for the plant-based beverages obtained from tiger nut, carob and rice, respectively (Image created using BioRender.com, version 2023).

In summary, the production of plant-based beverages consists of a multistep process specific to each raw material. The adequate selection of methods for each production stage is essential to achieve a high-quality and pleasant-tasting beverage.

2. Carob Beverages

The carob tree (*Ceratonia siliqua* L.) is a perennial plant belonging to the Fabaceae family, widely spread throughout the Mediterranean basin. It is an extremely drought-tolerant plant, requiring minimal care. Spain has a long-standing history of producing and consuming carob. Previously relegated to being a second-class plant, mainly used for animal feed or human food in case of food scarcity, carob cultivation is now experiencing exponential growth in modern markets due to its primary uses as a source for the extraction of carob bean gum, widely used as stabilizer and thickener (E410) in the food industry or as antidiarrheal in the pharmaceutical industry [1]. Carob is also used as a cocoa substitute, as it has a similar color and taste, making it a valuable raw material in the Spanish economy.

The nutritional value of carobs stands out since they are rich in fiber and minerals.

The high presence of simple carbohydrates makes it an optimal substrate for fermentation by microorganisms. In fact, in the scientific literature, it is possible to find a vast amount of studies on industrial fermentations of carob to produce a wide variety of compounds of interest, ranging from the production and purification of substances such as lactic acid or succinic acid to the production of biohydrogen [2][3][4].

The most common way of using carob in the production of beverages is by adding it as an ingredient in small amounts, especially in dairy drinks, to sweeten and flavor them as a substitute for cocoa [5]. Carob does not contain caffeine or theobromine, so it can be consumed by a larger population, such as the elderly, children or hypertensive people. The use of carob as the main substrate for the production of both fermented and non-fermented beverages is traditional in some cultures.

Most carob-based preparations are artisanal mixtures that follow traditional recipes. Aloja is the only traditional fermented beverage made from carob, and it is typical in Argentina. After being soaked in water for over 48 h, fermentation is firstly driven by lactic acid bacteria (LAB) responsible for lowering the pH, followed by fermentation by yeast. Carob is a rich source of fiber, inositols (D-pinitol), and phenolic compounds (gallic acid, caffeic acid, catechin, myricetin, epigallocatechin and epicatechin). Its phenolic content may vary greatly depending on several factors (ripening time, genetic, environmental factors, etc.), but it can reach amounts of 500 mg gallic acid equivalent (GAE)/100 g [6]. The phenolic content of carob-based beverages is usually high, probably due to their dispersion in the liquid medium [7]. Although its bioavailability decreases in the intestinal phase during digestion simulation, the bioavailability of the phenolic compounds appears to increase slightly when the size of the carob flour particles is reduced [8]. During lactic acid fermentations, polyphenolic compounds are reduced, likely due to LAB metabolism and conjugation to lacteal casein [9]. However, in the case of alcoholic fermentations, these compounds slightly increase, likely due to the presence of ethanol as a yeast metabolite [10].

Regarding biological activity, studies associate carob with health benefits at different levels. The first and most well-known benefit of carob is its capacity to improve gastrointestinal disorders, especially acute diarrhea [11]. In these initial studies on carob and acute diarrhea in children, Akşit and colleagues [12] demonstrated that the concomitant administration of an oral rehydration solution and carob juice reduced the frequency of diarrhea by 45% in children. Carob also seems to be able to act by protecting the stomach from ethanol-induced toxicity, modulating the microbiota and antioxidant status, and exhibiting antihelminthic and antibacterial activity [13][14][15][16][17]. Carob could also be implemented to treat metabolic alterations related to both the lipid and the glycemic profile. Many in vivo and clinical studies have shown that carob pulp and fiber can reduce plasma insulin levels, lowering LDL- and increasing HDL-cholesterol levels in both healthy and hypercholesterolemic subjects by consuming 10–15 g daily. These benefits could be due not only to the possible cholesterol clearance in the intestinal lumen by the fiber but also to the phenolic compounds, which seem to act in the inhibition of inflammation by increasing the expression of *SIRT1* and activating the peroxisome proliferator-1- alpha receptor [18][19][20][21][22][23].

The phenolic compounds of the carob could also be responsible for its possible anticancer activity, although further research is needed in this regard [24][25]. Lastly, carob is also gaining the attention of researchers as a food against infertility, particularly male infertility, as various studies have shown that carob extract can improve fertility parameters. In mice, the administration of carob extract is able to restore testicular function and testicular regeneration after the administration of doxorubicin, and in mice, it prevents sperm DNA fragmentation after the administration of cyclophosphamide [26][27]. In three clinical trials, the administration of carob capsules (1500 mg) or carob syrup in infertile men improved the count, morphology, and motility of spermatozoa [28][29][30].

Based on the current articles, no carob-based fermented vegetable beverages without alcohol, yeast or animal products have been developed, so it would be interesting to consider the development of a fermented beverage free of animal products and low in alcohol beverages. Despite the fact that the consumption of alcoholic beverages is present in many gastronomic cultures, ethanol is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), and therefore, there is no safe minimum level that is not 0 [31]. The consumption of non-alcoholic beverages is also suitable for children, elderly and pregnant women. Moreover, no studies have been conducted regarding the health benefits of carob fermented beverages. Carob-based beverages are well-received and organoleptically accepted, especially if the carob has undergone a previous toasting phase [32]. Carob is a widely used medium for industrial fermentation and as a thickener, yet its presence as food in the food industry remains low; thus, carob can be considered to innovate new food.

3. Tiger Nut Beverages

Tiger nut (*Cyperus esculentus* L.), “chufa” in Spanish, is a tuber that has been grown in the Iberian Peninsula for over 5000 years, and it has adapted to the local ecosystem. As the world’s leading producer, Spain is spearheaded by the Valencian Community. Remarkable for its high oil content, tiger nut is mainly composed of monounsaturated fatty acids, which are far superior to other tubers and cereals, although its carbohydrate content, mainly starch, is still quite high (40%). The protein fraction, higher than other tubers, is mainly composed of albumins.

Tiger nut is an underutilized crop that could be used to develop various food products, being of special interest in less developed countries due to its ease of growth and the possibility of storing throughout the year. Tiger nuts can be consumed raw or cooked, but its primary use is in the traditional Valencian beverage called “horchata” according to Royal Decree 1338/1988 [33]. “Horchata de chufa” is a nutritive product of milky appearance obtained mechanically from healthy, mature, selected, and clean *Cyperus sculentus* L. tubers. It is rehydrated, milled and extracted with potable water, with or without the addition of sugar, sugars or their mixtures, to give it a typical color, aroma and flavor. The minimum content of starch, fat and sugars must be specified for each type of “horchata de chufa” [33]. If the product contains added sugars, it is called “horchata” or natural “horchata”. If it does not contain them, it is called “chufa” beverage, although the legislation also contemplates the term sugar-free “horchata”.

Tiger nut has scarce research in evaluating fermentation as an alternative method for the development of new foods or as a substrate for the production of industrially interesting compounds. One of the earliest studies exploring tiger nut fermentation was to produce a 50% tiger nut yogurt [34]. The results showed that the 50% milk and 50% tiger nut yogurt had rheological and sensory characteristics similar to conventional yogurt, and it was the most accepted by the tasting panel, followed by cow’s yogurt and, lastly, the one containing only tiger nut beverages.

Furthermore, when Bukola and colleagues [35] subjected the tiger nut to different fermentation methods (solid or liquid with spontaneous fermentation or inoculated with starter), the results showed that the microbial count during fermentation was in accordance with other fermented foods. Although the current objective of this research was not the development of a fermented tiger nut beverage, the results showed that the previously ground and fermented tiger nut in liquid medium contained the highest number of microbial counts and at a pH lower than 4, which could be taken into account when developing fermented drinks based on tiger nut. In 2021, Madsen and colleagues [36] conducted a similar study to develop a dessert similar to a “cold milk soup” made with tiger nut drink. To make the product more similar to commercial milk soup, vanilla, lemon juice, xanthan gum, sucrose, lecithin, and potato proteinated from various vegetable sources were added, as well as a commercial yogurt inoculum (containing *Lactobacillus delbrueckii* sp. *bulgaricus* and *Streptococcus thermophilus*). The commercial inoculum caused the least drop in pH compared to other strains previously isolated in the laboratory from different plant sources, indicating a worse fermentation performance. The authors hypothesized that commercial strains selected and developed from cow’s milk sources might have greater difficulty in metabolizing sugars in a vegetable matrix. Similarly, the lower pH drop resulted in a sweeter taste than the sample fermented with isolated bacteria from vegetable, since the latter increased the acetic acid content, which was unpleasant. The use of thickeners such as xanthan gum helped the product to approach the rheological characteristics of commercial products, but without forming the typical gel structure of dairy derivatives, as found in earlier studies [36].

One of the earliest studies on tiger nut fermentation was conducted by Wakil and colleagues [37], where tiger nut drink was subjected to spontaneous fermentation and fermentative strains were isolated. LAB strains *(Lactobacillus plantarum*, *L. brevis*, *L. cremoris*, *L. bulgaricus* and *Lactococcus thermophilus*) were isolated, as well as *S. cerevisiae* and *Candida kefyr* yeasts. Subsequently, the isolated strains were re-inoculated into another tiger nut beverage to evaluate fermentation kinetics, microbial growth, and consumer acceptability. Results showed an adequate growth of the starter cultures for proper fermentation, a low bacterial count of coliforms and a high acceptability of the panelists when the beverage was fermented with a mixed culture of LAB [37]. Similar results have been found in Patent ES2335783T3, where different LAB strains were tested for “horchata” fermentation, evaluating fermentation time, bacterial count, viscosity and flavor. The best results were obtained with the combination of the strains *S. thermophilus BS5* and *L. acidophilus BL228*, producing a creamy beverage with an excellent taste, according to panelists [38].

The use of a microbial consortium as a starter culture can be beneficial to improve fermentation processes in plant matrices, taking advantage of the diversity of microbial metabolism. One study examined the behavior of a tiger nut beverage inoculated with kefir grain (containing a mixture of LAB and yeast) with respect to several parameters [39]. Fermentation reduced anti-nutritional compounds and improved the synthesis of B vitamins, including vitamin B12, although a reduction in vitamin C caused by both pasteurization and fermentation was observed. Furthermore, fermentation increased the antioxidant status of the beverage from 77.8 to 101.8 mg gallic acid (GAE)/L, which highlights the potential of the use of kefir grains in tiger nut-based beverages.

In Nigeria, a traditional beverage made from tiger nuts, known as Kunun-Aya, is consumed. This beverage is generally prepared with tiger nuts, coconut, dates, cinnamon and clove. The production process is similar to that of the Valencian "horchata" and other tiger nut-based drinks: after a soaking period that can go from 5 h to a full day, the tuber is ground with water and additional ingredients and subsequently passed through a mesh. Kunun-Aya is predominantly consumed unfermented, although it can also be found fermented [40]. The main microorganisms isolated are LAB and *S. cerevisiae.* Due to the lack of control of the fermentation process, which is completely artisanal, it is not possible to define a specific consortium of microorganisms for the production of this type of drink due to the variability associated with spontaneous fermentations, and the presence of pathogenic microorganisms can even be detected [41].

Another research work found on lactic acid fermentation of tiger nut-based beverages was carried out by El-Shenawy et al. [42], who evaluated three different probiotic strains in different combinations (*L. plantarum*, *L. brevis*; *L. plantarum*, *L. acidophilus*;) on the probiotic behavior, rheological change and acceptability of a drink composed of 65% milk permeate, 30% tiger nut drink and 5% sugar. No major differences were found between the three samples, except for a lower acceptability of the mixture containing *L. acidophilus* and *L. brevis*, probably due to the higher production of acetaldehyde and diacetyl compared to the other two samples [42]. In another study, it was also shown how the addition of milk protein in fermented tiger nut beverages decreases syneresis and improves the aromatic profile, although the highest rheological characteristics were obtained by adding xanthan gum [43]. Additionally, other studies have evaluated the feasibility of the tiger nut for the elaboration of alcoholic beverages such as wine and beer, showing the viability of the process, although the alcoholic degree reached is lower than that of traditional wine and beer beverages. However, these beverages showed good organoleptic acceptability [44][45].

Tiger nut has been shown to possess various beneficial health properties beyond its optimal nutritional value. These include anticancer, immunomodulatory, cardiovascular, and improvement of intestinal microbiota effects. This is likely due to its macronutrient composition, consisting of soluble and insoluble fiber, monounsaturated and polyunsaturated fatty acids, as well as its high levels of phytocompounds [46][47][48]. The bioaccessibility of carotenoids, such as lutein, zeaxanthin, β-carotenes, and total polyphenols has been shown to be higher in tiger nut beverages than in flour, oil, and whole grains [49]. Additionally, gastrointestinal digestion has been shown to increase antioxidant activity, likely due to the release of polyphenols from the matrix due to hydrolysis or proteolysis. Further research has pointed out the potential health benefits of the phytochemicals contained in tiger nuts. Several studies have indicated that the polyphenolic compounds found in tiger nuts may be effective in reducing oxidative stress, which can lead to a variety of health issues, including cardiovascular diseases and cancer. In addition, compounds such as lutein and zeaxanthin, which are present in high concentrations in tiger nuts, have been suggested to have a protective effect on the eyes, reducing the risk of age-related macular degeneration. Finally, the high fiber content of tiger nuts has been linked to improvements in digestive health, as well as potential benefits in weight management and blood sugar control [50].

4. Rice Beverages

Rice (*Oryzasativa* L.) is one of the most nutritious and consumed foods in the world and has been cultivated and consumed for millennia. Rice is an important source of energy thanks to its high carbohydrate content, and it also contains essential nutrients such as proteins, vitamins, minerals and fiber [51]. This cereal is a versatile food that can be found in many ways, such as white rice, brown rice, red rice, wild rice, etc.

In addition to being a valuable source of nutrients, rice is also used as a base for many fermented products. In fact, due to the high consumption of rice in the world, probably it could have the largest number of fermented products, including dozens of fermented beverages [52][53]. Rice is not always an essential ingredient for these drinks, as its production can be made with a wide variety of cereals its production can be applied to a wide variety of cereals, depending on food availability. These fermented rice-based products are rich in probiotics, antioxidants and other beneficial compounds for health. They also have a unique flavor and texture that make them popular in Asian countries such as Japan, China, Korea, Thailand and several Latin American countries such as Panama, Ecuador, Costa Rica or Peru. Fermented rice-based beverages such as sake, mirin, amazake, shochu, huangjiu and “chicha” have been consumed for centuries [54]. These beverages are usually prepared with rice, water and microorganisms such as yeasts and bacteria but can also contain herbs, fruits or other cereals. The fermentation process begins with the washing and soaking of the rice, sometimes followed by cooking. Then, the microbial culture is added and left to ferment for several days or weeks. During this process, microorganisms break down the rice sugars and release aromatic compounds, organic acids and beneficial compounds that contribute to the unique flavor and texture of the drink.

Sake is a traditional Japanese alcoholic beverage, also known as Japanese wine. To prepare this beverage, rice is cleaned, cooked and ground to obtain a rice puree, which is mixed with a koji starter (*Aspergillus oryzae*), a solid-state culture used as an enzyme source and a raw material for different fermentation processes [55]. This mixture is added to a solution of water and yeast, which is left to ferment for several days. Then, the resulting liquid is filtered and bottled. Sake has an alcoholic content between 15–25% *v*/*v*. Varieties of Sake have been documented where there is a greater presence of microbial species depending on the fermentation method of each place, for example, due to the use of uncooked rice [56]. In these varieties of sake, the genera *Lactobacillus, Acinetobacter* and *Staphylococcus* have been detected during the first 24 h, followed by *Bacillus* and secondary populations of *Staphylococcus* and *Planococcaceae.* This type of sake has shown greater acceptability among consumers, but the lack of standardization limits its production to an artisanal level since there is a possibility that undesirable microorganisms will develop [57]. In order to also avoid possible post-preparation contamination of commercial sake, the beverage is pasteurized after the fermentation process. Similarly to sake, mirin is made from rice, water, koji and yeast, but it contains more sugar and less alcohol. Mirin is often used in cooking to add a sweet flavor to different dishes. Although sake and mirin could contain interesting bioactive compounds, the high presence of ethanol restricts its consumption to a moderate intake in certain population groups [58].

Shochu is another typical Japanese beverage produced by a fermentation process similar to sake, but white koji (*Aspergillus luchuensis*) is used, and after fermentation, the drink is subjected to a distillation phase that greatly increases its alcohol content, up to 40% *v*/*v* [59][60]. In contrast, Amazake is a traditional Japanese non-alcoholic beverage made from a mixture of rice, water, and koji. This drink is naturally sweet, although some varieties include the addition of sugars. It is usually served cold and contains a great variety of digestible and indigestible sugars such as trehalose, maltose and raffinose. It also contains 20 amino acids, B complex vitamins, lipids and glycosphingolipids, as well as substances with antioxidant activity. Because it does not contain alcohol, it is suitable to be consumed at all ages, including children [61]. Amazake appears to have anti-fatigue activity, gut microbiota modulatory effect and the ability to improve intestinal motility and lipid metabolism, as well as to increase collagen synthesis. Unfortunately, the number of clinical trials on the benefits of Amazake for human health is limited, and current available studies have not yet been reproduced [62][63][64][65].

In addition to the typical Japanese rice beverages, there are other fermented beverages based on rice that are prepared using unique processes. Makgeolli, Bhaati Jaanr and rice beer are popular in Japan, China, Bhutan, Nepal, India and Korea. These drinks are prepared with rice malt and yeasts, as well as a variety of plant and vegetable ingredients, such as flowers and fruit peel ash, which act as fermentative bases for microbial growth. Studies have shown that LAB and yeasts are the main microbial components of these drinks. *Lactobacillus* and *Bifidobacterium* were the most usually found genera, with *Pediococcus acidilactici,* the main representative species, followed by yeasts of the species *S. cerevisiae* [53][66]. Some of the mentioned bacteria have been shown to maintain their viability intact during a gastrointestinal simulation, suggesting possible probiotic potential. However, no studies of the bioavailability or bioactivity of compounds of interest in these beverages are available. In addition to the described products, it is possible to find more fermented rice beverages in the Asian continent, but their production processes are similar to those described above or have not been well characterized, so they are not included in this research.

Fermented drinks based on rice are also found in South America, known as rice “chicha”. This drink is typical of countries such as Ecuador, Brazil, Costa Rica, Peru and Venezuela. “Chicha” can be prepared with different cereals, depending on their availability at the time of production, although the most frequent ones are corn and rice. The preparation of traditional “chicha” is carried out in two stages. Firstly, the corn or rice is cut and soaked for one night. Secondly, a cooking process is applied until the cereal softens, and it is mixed with water at room temperature to obtain a thick mixture. The mixture is strained to separate the liquid and any solid particles. The liquid is poured into a container where it is left to ferment for one or two days. Finally, the “chicha” is decanted to separate the yeast and the sediment. In a recent study, 27 samples of whole “chicha” produced in different regions of Peru were analyzed, showing that *Firmicutes* and *Proteobacteria* were the dominant phyla in most samples, followed by *Bacteroidetes* and *Actinobacteria*. At the family level, *Lactobacillaceae* and *Acetobateraceae* were the most representative families detected in most samples. At the genus level, *Lactobacillus* was the most representative genus in all samples, followed by *Weissella*, *Leuconostoc*, *Lactococcus* and *Streptococcus* [66]. These results provide information on the bacterial composition of this typical Andean fermented drink, revealing its diversity. “Chicha” is a type of beverage that undergoes fermentation; this results in a drink that contains varying levels of alcohol, typically ranging from 2–12% *v*/*v*, depending on the duration of the fermentation process. As a result, “chicha” is considered an alcoholic beverage [67].

Since most fermented rice drinks presented so far come from artisan recipes and present discrete amounts of ethanol, it is interesting to focus on the development of probiotic LAB beverages through well-established processes and without, if possible, the presence of ethanol. Upon reviewing the scientific literature, different studies were found that try to explore lactic acid fermentation for the production of rice-based drinks. In one of these first studies, carried out by Kim et al. [68], low-purity isomalto-oligosaccharide (IMO) syrup was produced from gelatinized organic rice through treatment with commercial amylolytic enzymes such as BAN, FUNGAMYL, PROMOZYME and transglucosidase. Afterward, the syrup was inoculated with five different strains of *Lactobacillus*, and primary fermentation was carried out in reconstituted skimmed milk broth. The highest number of viable cells was obtained for *L. plantarum*, with 7.2×10^8 CFU/mL, and the highest dry cell weight was also that of *L. plantarum* (13 mg/mL). Lactic acid was quantified in the highest amount of 8125.78 mg/kg. These results indicate that *L. plantarum* is a good strain for fermenting IMO syrup preparations. However, no further evaluation studies of this drink were carried out to support these findings.

The use of glycolytic enzymes before rice fermentation is common in other experiments. A more recent study by Yang et al. [69] added α-amylase and amyloglucosidase at 0.05% to beverages formulated with different cereals, subsequently inoculated with *Limosilactobacillus reuteri*. The rice drink presented pH values of 3.53 after 24 h of fermentation. Regarding the volatile compounds analysis of the drinks, 3-furaldehyde and benzaldehyde were detected in the coix seed substrate but not in whole rice. The 2-pentyl furan content was higher in coix seed than in whole rice [69]. 2-acetylthiazole and benzothiazole were detected in whole rice, while they were not detected in quinoa. These differences in the content of aromatic compounds depend on the substrates used. In addition, the results of this research showed that fermentation of the four cereal substrates produced significant differences in the flavor of the food products obtained. Fermented rice showed a more obvious sweet taste, which may be due to the carbohydrates fermented by the bacteria during the fermentation process. Moreover, fermented rice had a slightly lower flavor intensity than the other three substrates. These results suggest that fermented rice is a good option for those looking for a sweet and mild flavor in their food products.

In another study, the probiotic effects of nine isolates of LAB from the traditional drink Bhaati Jaanr were evaluated and subsequently inoculated into a rice drink prepared in the laboratory. Among them, the *L. plantarum* L7 isolate was the most promising, as it had desirable properties such as variable pH tolerance, hydrophobicity of the cell surface, autoaggregation, susceptibility to antibiotics, and antibacterial activities [70]. When used as a starter for fermentation, this strain increased functional components and mineral bioavailability, improved acidification and the generation of organic acids, reduced starches and increased its antioxidant potential. These characteristics make rice drinks a functional beverage, as both rice and fermentation products can contribute to the better nutritional status of the population.

The functional potential of fermented rice beverages was evidenced through another study in which a probiotic beverage was developed using the probiotic *L. pentosus* 9D3 strain isolated from Thai pickled herbs and whole rice as key raw material. This beverage contained high levels of gamma-aminobutyric acid (GABA), probiotic cells, inhibitory activities against oxidative stress and enzymes associated with obesity and diabetes [71]. The product also presented good sensory and nutritional qualities and remained stable at low temperatures for long periods. This drink could be an alternative for those with lactose intolerance or vegan/vegetarian diets.

Fermented rice beverages can also be enriched with other ingredients to increase their nutritional value and their possible functional effects, such as adding other protein sources to the beverage. Manus et al. [72] evaluated the effect of fermenting a probiotic beverage enriched with pea and rice proteins on its protein quality. It was demonstrated that fermentation increased the quality of the probiotic pea and rice proteins (PRF), as well as the level of live probiotics present in the feces. In another study, the same research group evaluated the physicochemical stability, sensory properties and microbiological quality of a fermented beverage (with the same bacterial strains) enriched with PRF during storage at 4 °C. The results showed that protein enrichment induced an increase in the pH, titratable acidity, and viscosity of the PRF products, while fermentation led to a decrease in pH and viscosity. In addition, the PRF products preserved a high level of viable probiotics throughout the storage, which improved their nutritional and nutraceutical value [73].

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