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Review

# Vegan Fermented Drinks as an Alternative to Milk: Trend or Challenge?

**Giovanna Camile Vaz Gonçalves[1], Cláudia Moreira Santa Catharina Weis[1], Élide Rebechi Wolff[1], Vanessa Alves[1], Flavia Letícia Sanches[1], Luciano Tormen[1], Helen Treichel[1,2*], Larissa Canhadas Bertan[1]**

[1]Food Science and Technology, Federal University of Fronteira Sul, BR 158, km 405, 85319-899, Laranjeiras do Sul, PR, Brazil
[2]Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Environmental Science and Technology, RS 135, km 72, 99700-000, Erechim, RS, Brazil
E-mail: helen.treichel@uffs.edu.br

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**Abstract:** The attention given to food has increased in recent decades due to consumers' interest in the possible therapeutic and nutritional properties of foods. Eating habits are associated with the composition of the individual's gastrointestinal microbiota, so changes in the intake of macronutrients and fiber can induce changes in bacterial diversity. A healthy microbiota pattern tends to be observed when an individual includes fermented foods. However, most fermented foods are dairy products. They cannot be consumed by specific population groups, such as people who are lactose intolerant, allergic to milk protein, or for lifestyle and diet reasons, such as vegans and vegetarians. From this fact, there is a need to offer consumers an alternative non-dairy fermented product, exploring new substances to supply dietary probiotics. In this context, water kefir, a symbiotic culture of lactic acid bacteria, acetic acid, and yeast, stands out as a viable and affordable option, providing benefits similar to dairy versions without allergenic components that are undesirable for specific groups. Given the above, the objective of this work is to conduct a literature review on vegan fermented drinks, providing an overview of the fermentation process and matrices used, as well as presenting the factors that impact their adequate development, in addition to encouraging a discussion about the vegan fermented drinks market, including the issue of promoting research and development of new products from non-traditional sources, such as water-soluble vegetable extracts made from quinoa, cashew nuts, pistachios, among others.

***Keywords*:** kefir, fruits, plant base, prebiotics, probiotics

## 1. Introduction

Interest in food has expanded significantly in recent decades, encompassing nutrition in providing nutrients and supporting metabolic activities and its therapeutic properties. Such aspects stand out for aspects related mainly to functional foods, comprising functions beyond those inherent to their chemical composition, but rather those potentially beneficial in reducing the risk of chronic degenerative diseases [1]. Thus, consumers' willingness to use functional foods has increased mainly due to greater health awareness. Among these functional foods, we can mention probiotics, which are live microorganisms that promote a healthier intestinal microbiota when administered in adequate quantities.


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This interest in modulating the intestinal microbiota has introduced probiotic foods into the market as an essential dietary niche on the rise [2]. Still within this niche are non-digestible food components, such as prebiotics, which promote intestinal bacteria's growth and activity, producing a group of functional products called symbiotics [3]. Thus, dietary interventions with probiotic strains, prebiotics, and their associations in appropriate proportions have proven advantageous, as they improve the balance of the host's gastrointestinal microbiota, favoring the relative and absolute quantity of bacteria and their growth kinetics [4, 5].

Eating habits are associated with the composition of the individual's gastrointestinal microbiota, so changes in the intake of macronutrients and fiber can induce changes in bacterial diversity [6]. This plasticity characteristic makes a "healthy" microbiota pattern identifiable, making it possible to use microbial stability markers to indicate intestinal health due to the inverse association with chronic diseases and metabolic changes [5].

Consuming fermented foods has proven effects on the intestinal microflora, resulting in a healthy intestinal microbial balance and improving the absorption of vitamins and other antioxidant compounds into the bloodstream, consequently improving the digestive and immune systems [7]. Despite the benefits mentioned, most fermented foods are dairy products. They cannot be consumed by specific population groups, such as people who are lactose intolerant, allergic to milk protein, or for lifestyle and diet reasons, such as vegans and vegetarians. Therefore, consumers must be offered an alternative non-dairy fermented product, exploring new substances to supply dietary probiotics [8].

When analyzing the change in dairy beverage consumption patterns in the United States, a reduction in milk consumption is observed, which has occurred since 2013, with a gradual decrease in sales revenue from 19 million dollars to 16 million in 2018. At the same time, it was possible to observe a rise in alternative drinks, such as those made from plants, which, in the same period from 2013 to 2018, grew 61%, and retail sales reached US$ 2.3 billion [9]. This fact can be proven, as plant-based drinks have been one of consumers' most popular functional food categories, with an expanding market estimated to reach US$ 35,805 million by 2026 [10, 11]. As a result, the food industry has been improving in research and technologies that encompass the development of new products, ingredients, and production methods that serve this niche of consumers [12]. Therefore, aiming to increase the number of people with a predominantly vegan diet and the technological characteristics of products of plant origin, several studies have been carried out to use formulations with water-soluble plant extracts ("milk") such as quinoa, cashew nuts, lupins, hazelnuts, chickpeas, coconut, among others for the preparation of different types of products [13-21].

Considering the above cited, some differences between fermented and non-fermented beverage products are worth mentioning. Both differ primarily in the way they are produced, their nutritional profiles, flavors, and potential health benefits. Some essential points are the production process, where fermented beverages are produced through fermentation, a metabolic process in which microorganisms (yeast, bacteria, or molds) convert sugars into alcohol, acids, or gases. Common fermentation agents include *Saccharomyces cerevisiae* (yeast) and *Lactobacillus* (bacteria), and non-fermented beverages are produced without microbial fermentation. The sugars or ingredients in these drinks remain unchanged in their chemical composition, i.e., fresh juices, soft drinks, tea, coffee, sports drinks, and flavored water.

Another critical point is the alcohol content, where some fermented beverages contain alcohol due to the breakdown of sugars (e.g., beer, wine). Others, like kombucha, have shallow alcohol content (below 0.5%) and are often considered non-alcoholic. The Non-Fermented Beverages do not contain alcohol unless specifically formulated to include it (e.g., adding liquor to a cocktail). Regarding the flavor, fermented Beverages have complex, tangy, or sour flavors resulting from fermentation byproducts, like acids and esters. For example, kefir has a sour, yogurt-like taste, and kombucha can taste vinegary or fruity, while non-fermented beverages tend to have a straightforward flavor profile. Fresh juices are sweet or tart based on the fruit, while carbonated soft drinks tend to be sugary and fizzy. The health benefits are different, too. At the same time, fermented beverages could be probiotics, improved nutrient bioavailability, and potential for antioxidants; the non-fermented beverages present nutritional simplicity and are less acidic. Both beverages differ regarding shelf life and preservation, carbonation, sugar content, cultural and historical significance, and microbial content. In this sense, fermented beverages offer complexity in flavor, potential health benefits like probiotics, and lower sugar content. In contrast, non-fermented beverages provide hydration and nutrient content without fermented options' tangy flavors or microbial activity. Both categories have unique roles in diets and lifestyles, and the choice depends on personal preference, health goals, and cultural factors [22].

Because of the above, the objective of this work was to carry out a literature review on vegan fermented drinks.

The review provides an overview of the drinks, the elaboration and fermentation process, and the matrices used. Furthermore, it presents the factors that impact their adequate development and encourages a discussion about this market.

## 2. Beverage fermentation

Fermentation is considered one of the oldest ways of preserving drinks and food, beginning in the Middle East between 2,000 and 3,000 BC [23]. Among fermented products, beverages represent a growing sector in the food industry, as health-conscious consumers recognize such products as healthy, refreshing, convenient, and a probiotic vehicle that promotes well-being. Among fermented drinks, the best known are those made with milk. Still, their consumption is falling due to physiological factors such as lactose intolerance and allergies to milk proteins ($\alpha$-lactalbumin, $\beta$-lactoglobulin, and casein) [24]. It is essential to highlight that this type of allergy is more common in children than adults. Therefore, more research must be conducted to ensure that these beverages are safe and suitable for children's consumption since children represent the group most affected by milk protein allergy. In addition, it is essential to investigate the impact of potential antinutrients in some of these beverages and the nutritional suitability for this specific population, given that children have different and more delicate dietary needs.

Therefore, the most effective treatment is excluding milk and dairy products from the diet [25]. This fact has driven an increase in interest in vegan alternatives for the production of products such as cheeses and yogurts; however, there is some difficulty in creating products with acceptable texture and flavors [26], as many consumers still associate the sensory characteristics of such products, which are also made with milk.

The fermentation of milk by lactic acid bacteria is well elucidated; however, the molecular processes involving the fermentation of water-soluble plant extracts have received little attention [27]. Despite presenting little information on this aspect compared to milk, water-soluble plant extracts have been a viable and promising alternative to replace fluid cow's milk and prepare products, such as fermented drinks [28].

Fermented water-soluble plant extracts tend to have a reduced saturated fat content, which is attractive for consumers looking for this alternative. Furthermore, they contain fiber in their composition, which contributes to nutritional aspects not found in milk. On the other hand, they generally have low protein content, except for soy [29, 30], when compared to milk. Furthermore, one of the disadvantages of using water-soluble plant extracts when compared to milk is the presence of antinutritional factors such as saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, among others [31], which can reduce nutrient absorption. Additionally, as already mentioned, it presents undesirable flavors and textures, for example, with water-soluble vegetable extracts from beans [32].

Due to these aspects, the processing of water-soluble plant extracts often involves steps and the addition of ingredients, which frequently aim to meet the consumer's needs for a product that resembles milk in terms of color, texture, and flavor. For example, we can mention heat treatment (90 °C/1 h) as an alternative in the case of cyanogenic compounds (HCN) to make water-soluble cassava plant extracts safe for consumption [33].

Many raw materials are being researched to produce water-soluble plant extracts, as seen in Figure 1.

The fermentation process of these extracts varies according to the environment, pH, sugar content, and temperature. Furthermore, its quality is directly affected by the material used as an energy substrate, which promotes the development of bacteria in a biotransformation process, generating organic acids or alcohols as a reaction product [34]. Thus, despite being similar in appearance, their composition presents differences (Table 1), which can be minimized by modifying and inserting steps into the process.

Fermentation of water-soluble plant extracts improves the flavor of products. It assists in the formation, digestion, and absorption of essential nutrients and phytochemicals, increasing their shelf life. In this way, associating these points, practicality, and change in the market in the search for foods that provide health benefits creates a promotion for the production of drinks based on these vegetables [35], as well as research on raw materials that enable their production if necessary. Furthermore, the microbiological action of the cultures added for the fermentation process also guarantees the inhibition of contamination of deteriorants and pathogens through acids that reduce the pH, maintaining the biosafety of the prepared product [36].

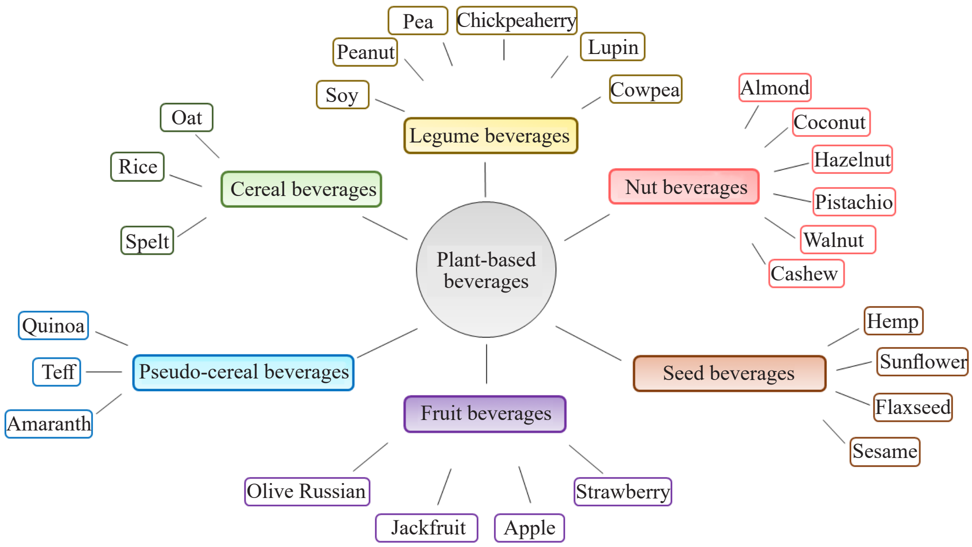


**Figure 1.** Plant-based beverages

Although dairy products present a better substrate for the fermentation process and consequently for probiotics, there is the aspect of hypersensitivity, be it allergies or intolerance to any of the milk constituents [37]. Thus, one alternative to enable non-dairy fermentation is using water kefir [38].

**Table 1.** Composition of fermented water-soluble plant extracts and fermented milk drink

| | Vegan extracts | | | | | | | Fermented milk drink [43, 44] |
|---|---|---|---|---|---|---|---|---|
| | Soy [39, 40] | Rice [41] | Almond [42] | Cashew nut [14] | Coconut [17] | Quinoa [13] | Lupin [15] | |
| Energy (Kcal/dL) | 95.0 | 130.0 | 35.0 | 70.0 | 45.0 | 28.0 | 40.0 | 55.0 |
| Proteins (%) | 8.0 | 1.0 | 1.0 | 1.83 | 2.90 | 0.30 | 0.70 | 3.41 |
| Lipids (%) | 4.5 | 2.5 | 2.5 | 3.97 | 4.5 | 0.06 | 0.36 | 0.56 |
| Carbohydrates (%) | 4 | 26.0 | 1.0 | 5.43 | 3.3 | 12.64 | 8.64 | 9.21 |
| Fibers (%) | 1.0 | 0.0 | 0.75 | ND | 2.2 | ND | ND | ND |
| Ash (%) | 0.44 | 0.48 | 2.87 | 0.26 | 0.16 | 0.1 | 0.2 | 0.48 |
| Calcium (mg $kg^{-1}$) | 330 | 315 | 1.581 | ND | 220 | ND | ND | 1.188 |
| Phosphorus (mg $kg^{-1}$) | 90.0 | 63 | 128.0 | ND | ND | ND | ND | 926.0 |
| Magnesium (mg $kg^{-1}$) | 52.5 | 35 | 70.50 | ND | 35 | ND | ND | 122.0 |
| Iron (mg $kg^{-1}$) | 0.52 | 0.065 | 1.030 | ND | 0.13 | ND | ND | 0.191 |
| Potassium (mg $kg^{-1}$) | 360 | 50 | 227.0 | ND | 40.0 | ND | ND | 1.787 |
| Zinc (mg $kg^{-1}$) | 0.75 | 0.75 | 0.595 | ND | 0.66 | ND | ND | 3.320 |

* Of which: References used: [13, 14, 15, 17, 39-44] ** MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. ND: Not determined

## 3. Water kefir

Water kefir, also known as sweetened kefir, is a fermented drink obtained from the fermentation process of kefir grains in an aqueous sucrose solution [45]. Its cultivation medium is versatile since its grains grow on different substrates in addition to the sucrose solution, such as molasses, honey, fruit, and vegetable juice, which is one of its main characteristics [46]. Kefir grains are a cluster of bacteria, especially lactic acids and yeast. These microorganisms act in symbiosis and are used to prepare fermented drinks whose characteristic presence is polysaccharides, B vitamins, carbon dioxide, lactic, acetic, and gluconic acids, and, in some cases, ethyl alcohol [13, 47-48].

Kefir grains are formed by a matrix of dextran exopolysaccharides (EPS), in which microorganisms (lactic and acetic bacteria and yeasts) are incorporated [49]. This EPS matrix contains glucose polymers consisting mainly of linear $\alpha$-D-1,6 bonds and, in smaller quantities, $\alpha$-1,3-linked side chains. This is the main difference with milk kefir grains, formed by branched heteropolysaccharides called kefiran or kefiran [50]. The grains have irregular shapes and sizes between 0.5 and 3.5 cm and are translucent, mucilaginous, fragile, and insoluble in water [46, 49], as shown in Figure 2.

**Figure 2.** Water kefir grains

One way to obtain water-soluble plant extracts to create vegan products is by extracting the extract and then fermentation [3]. Fermentation of water kefir grains (Figure 3) into water-soluble vegetable extracts is generally carried out at temperatures between 20 to 30 °C under anaerobic conditions for 24 to 96 hours until a pH of approximately 4.6, resulting in a slightly sweet fermented drink, acidic and effervescent with a fruity aroma [51]. During fermentation, lactic acid, carbon dioxide ($CO_2$), ethanol, acetic acid, gluconic acid, and several other products are produced, including volatile aromatic compounds formed by the symbiotic association of microorganisms in the grains [45, 52]. These substances arising from fermentation come from the consumption of the substrate, such as sucrose added to the plant extract, by the microorganisms present in the kefir grains, which confer desirable sensory characteristics to the final fermented drink, such as smooth texture and somewhat thick consistency (due to the glycerol, polysaccharides, etc.), slightly acidic and effervescent flavor (due to the presence of ethanol) and fruity aroma (due to esters) [46].

The most common types of fermentation of kefir grains are lactic, alcoholic, and acetic [51]. The fermentation of kefir grains is carried out through a symbiotic association of lactic acid bacteria (LAB), acetic acid (BAA), and yeast [45]. The most common microorganisms cited in the literature are LAB (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* spp.), BAA (*Acetobacter*), and yeasts (*Kluyveromyces*, *Saccharomyces*, *Candida*, and *Torula*) [46, 52]. However, the composition and association of these microorganisms in kefir tend to vary according to the origin of the grains, the cultivation method, and the substrates used in fermentation [45]. In industrial production, kefir cultures have limited microorganisms, and strains are selected and isolated for fermentation. Given this, it is clear that there is a diversity of species in kefir grains, and an approach to studying microbiology is necessary [53].

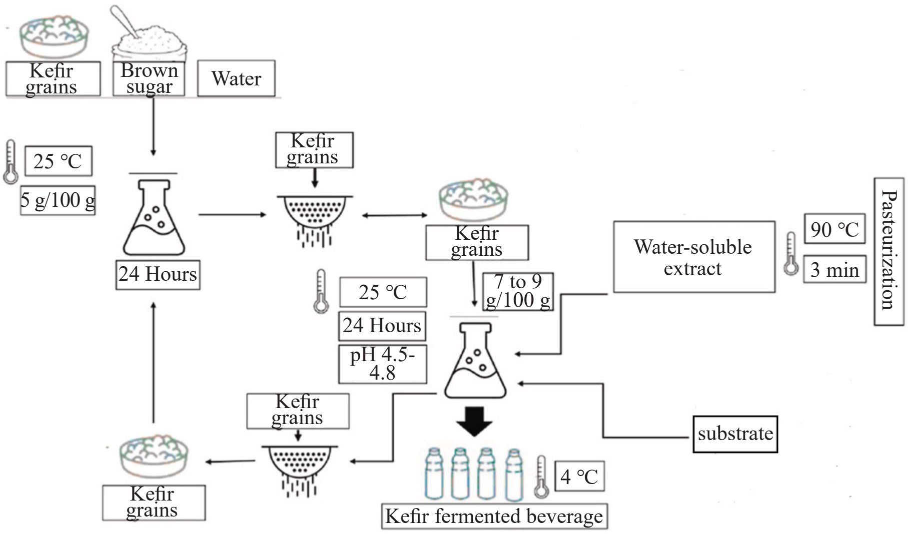


**Figure 3.** Vegan fermented drink production flowchart

Table 2 presents some microorganisms more frequently found in water kefir grains. These microorganisms can be a source of probiotics, prebiotics, and antioxidants for people allergic and intolerant to dairy products and vegans. When talking about Kefir, it is also interesting to evaluate the enzymatic activity, which is a determining factor in optimizing the fermentation process and producing the fermented drink. Enzymatic activity is directly involved in the degradation of compounds for the metabolic route of these microorganisms, the release of byproducts of more excellent nutritional value, and sensory changes [54].

**Table 2.** Microorganisms commonly found in water kefir grains

| Group of microorganisms | Genus | Microorganisms |
|---|---|---|
| Bacteria | *Acetobacter* | *A. fabarium, A. orientalis, A. lovaniensis, a. peroxydans, A. persici, A. pasteurianus, A. lambici, A. indonesiensis* |
| | *Gluconobacter* | *G. cerinus, G.oxydans, G. frateurii. G. morbifer, G. albidus, G. liquefaciens* |
| | *Lactobacillus* | *L. brevis, L. buchneri, L. casei sub sp. casei, L. casei sub sp. rhamnosus, L. diolivorans, L. fermentum, L. harbinensis, L. hilgardii, L. hordei, L. kefiranofaciens, L. kefiri, L. lactis, L. mali, L. nagelli, L. paracasei, L. parafarraginis, L. perolens, L. plantarum, L. satsumensis, L. urvarum. L. sakei, L. oeni, L. curvatus* |
| | *Leuconostoc* | *L. pseudomesenteroides, l. carnosum, L. mesenteroides* |
| | *Other species* | *Lysinibacillus sphaericus, Oenococcus kitaharae, Bifidobacterium psychraerophilum, Bacillus cereus, Xanthomonas citri, Eubacteriales bacterium* |
| Yeasts | *Saccharomyces* | *S. cerevisiae, Saccharomyces sp.* |
| | *Pichia* | *P. membranifaciens, P. kudriavzevii* |
| | *Lanchancea* | *L. fermentati, L. meyercii.* |
| | *Kluyveromyces* | *K. lactis, K. marxianus.* |
| | *Kazachstania* | *K. aerobia, K. unispora.* |
| | *Hanseniaspora* | *H. valbyensis, H. uvarum.* |
| | *Cladosporium* | *C. herbarum, C. delicatulum* |
| | Other species | *Zygotorulaspora florentina, Issatchenkia orientalis, Zygosaccharomyces fermentati, Dekkera bruxellensis, Candida etchellsii, Brettanomyces anomalus, Torulaspora delbrueckii, Lasiodiplodia brasiliensis, Debaryomyces hansenii* |

## 3.1 *Enzymatic activity in kefir fermentation*

During the fermentation of kefir grains, enzymatic processes occur, which hydrolyze more significant substances into more minor compounds (poly and disaccharides to monosaccharides) that will be metabolized more efficiently to produce organic and acidic compounds [55]. Thus, evaluating the enzymatic activities involved in fermentation becomes vital since the various enzymes in this process are mainly responsible for catalysis, hydrolysis, and metabolism for forming new compounds [56]. This enzymatic activity is influenced by the type of substrate, which can be observed in Table 3 when quantifying several enzymes in fermented vegetable extracts based on coconut, quinoa, cashew nuts, and lupins.

Amylases are one of the main enzymes used in food production and are responsible for the catalysis and hydrolysis of the conversion of starch into sugars. *α*-Amylase and *β*-amylase, through the hydrolysis of glycosidic bonds, are the enzymes responsible for the most significant production of reducing sugars from starch [57]. Specifically, *α*-amylase, which acts on (*α*-1→4) glycosidic bonds, is the enzyme responsible for the production of glucose molecules from the hydrolysis of oligosaccharides. During the kefir fermentation process, glucose can be metabolized to produce acids and organic compounds and act in the grains' cellular growth [55]. Inulin (non-structural polysaccharide) is the most common energy source in nature after starch, and inulinases can be found in different groups of microorganisms, plants, and animals [58]. The bacteria are *Xanthomonas* sp., *Streptomyces* sp., *Bacillus* sp.; the fungi *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., *Rhizoctonia* sp.; and the yeasts *Cryptococcus* sp., *Phichia* sp., *Kluyveromyces* sp., the primary potential sources studied for inulinases [59]. Inulinases are metabolized by two types of enzymes: exo-inulinases (*β*-D-fructans) that separate fructose molecules through cleavage of the non-reducing terminal *β*-(2→1) of the inulin chain and endo-inulinases (2→1 *β*-D-fructans) that release fructooligosaccharide (FOS) from the hydrolysis of inulin's internal bonds [59]. In the fermentative process, the catalytic action of inulinases in inulin makes glucose and fructose available for the metabolization and production of organic acids and ethanol [58]. According to Konkit and Kim [55], kefir microorganisms contribute to the enzymatic activity of amylases and inulinases since these bacteria more easily metabolize available sugars through enzymatic hydrolysis for the production of ethanol when compared to other sugar sources such as starch, cellulose, arabinose, and xylan.

**Table 3.** Determination of enzymatic activity in vegan fermented extract

| Fermented plant extracts | | Enzymatic activity (U/mL) | | | | | | Authors |
|---|---|---|---|---|---|---|---|---|
| | | Amylase | Inulase | Peroxidase | Cellulase | Lipase | Protease | |
| Fermented coconut extract added with | 2.0% (m/v) coconut sugar and 3.0% (m/v) inulin | 9.13 | 2.03 | ND | ND | ND | ND | Alves et al. [17] |
| | 10.0% (m/v) coconut sugar; 2.0% (m/v) inulin and 0.26% (m/v) xanthan gum | 9.73 | 1.28 | ND | ND | ND | ND | |
| Fermented quinoa extract added with | 2.0% (w/v) sucrose; 2.5% (w/v) inulin | 14.05 | ND | 19.87 | 19.87 | 0.58 | 56.11 | Sanches et al. [13] |
| | 0.0%(m/v) sucrose; 3.5% (w/v) inulin; 0.16% xanthan gum | 423.85 | ND | 101.54 | 101.54 | 0.00 | 51.39 | |
| Fermented cashew nut extract added with | 10.0% (m/v) sucrose and 2.5% (m/v) inulin | 247.91 | ND | 67.43 | 67.43 | 3.36 | 53.89 | Weis et al. [14] |
| Fermented Lupines extract added with | 6.0%(m/v) sucrose; 3.0% (w/v) inulin; 0.08% xanthan gum | 264.63 | ND | 11.83 | 56.93 | 5.06 | 0.56 | Weis et al. [15] |

Where: ND-not determined

Peroxidases have enzymatic action through the oxidation of peroxides (hydrogen peroxide cofactor), and their primary sources are microorganisms, animals, and plants [60]. Oxidation-reductive enzymes are also responsible for the oxidation, reduction, and degradation of various substrates, such as aromatic and phenolic compounds [61]. Furthermore, according to Aguiar, Ferreira, and Monteiro [62], the enzymatic action of peroxidase is related to the increase in the availability of total phenolic compounds since peroxidase in the reactive presence of $O_2$ degrades and breaks the polymeric bonds of phenolics through co-oxidation, thus making free phenolics available. Peroxidases can also be attributed to the greater bioavailability of micronutrients since, due to their oxidation-reduction characteristics, they have active binding sites in minerals such as magnesium, vanadium, selenium, and iron [60]. Aguiar, Ferreira, and Monteiro [62] analyzed bioprocessed soy milk yogurt formulations supplemented with milk kefir cultures based on the enzymatic action of peroxidase. The authors observed that in 48 hours of fermentation, the enzymatic activity was 2.33 U/mL, lower than the enzymatic activity for peroxidase obtained for this study in 24 hours.

Cellulases are enzymes produced by microorganisms, such as bacteria and fungi, during their development on cellulosic substrates, in which they catalyze the conversion of cellulose into glucose. There are three main types of cellulases: $\beta$-glucosidase, endo-1,4-$\beta$-D-glucanase (endoglucanase) and exo-1,4-$\beta$-D-glucanase (exoglucanase), which act synergistically, carrying out hydrolysis efficiency of cellulose, with endoglucanase responsible for acting on the internal sites of oligosaccharides, while exoglucanase hydrolyzes the non-reducing ends of crystalline cellulose, resulting in cellobiose or glucose, and $\beta$-glucosidase acts on the non-reducing ends of cellobiose and cellodextrin. The complex structure of cellulases presents a hinge region rich in proline (Pro), threonine (Thr), and serine (Ser) residues, a cellulose binding domain (CBD) with O-glycosylated proteins, and a catalytic domain with proteins N. In bacterial cellulases, a peptide linker typically connects several functional domains. In aerobic organisms, the CBM binds to the catalytic domain; in anaerobic organisms, the dockerin domain binds to the catalytic domain [63]. The main bacteria known for their ability to degrade cellulose include *Streptomyces* sp., *Pseudomonas coleopterorum* and *Herbinix hemicellulosilytica*, and *Thermobifida fusca* and *Bacillus* sp., which also demonstrate adaptability and resistance to high temperatures, being classified as thermostable. In the fermentation process, the diversity of conditions and substrates can influence cellulase production by different microorganisms. For example, *Bacillus subtilis* AS3 demonstrates a maximum cellulase activity of 0.75 IU/mL at 39 °C and pH 7.2 after 48 hours of fermentation; using carboxymethylcellulose (CMC) as carbon substrate under thermophilic conditions, cellulose production can reach 2,392.2 IU/mg of cell with a cell yield of 0.28 mg/mL, using 0.2% of raffinose at 50 °C and pH 6.0 for 10 hours [64].

Lipases are characterized by an $\alpha/\beta$ hydrolase fold, composed of a twisted central $\beta$-sheet and surrounding $\alpha$-helices; their catalytic site contains the sequence GXSXG, typical of serine hydrolases, and other structures such as the lid, the binding pocket, the oxyanion hole, and the disulfide bridges [65]. The variability in the structure of the binding pockets determines the specificity of the substrate, influencing the selectivity of lipases in hydrolysis reactions and synthesis of lipids, such as triacylglycerols (TAG), resulting in the release of free fatty acids, diglycerides (DAG), monoglycerides (MAG) and glycerol, this preference for long-chain fatty acids. However, they act on short and intermediate chains, giving them greater biocatalytic adaptability. In addition to hydrolysis, they can catalyze several reactions, including esterification, transesterification, interesterification, acidolysis, and aminolysis; they are classified as enantioselective, substrate-specific, regioselective (1,3 and 2-regiospecific) and non-regioselective, these enzymes exhibit distinct release patterns of fatty acids from TAGs, directly influencing the synthesis of specific compounds [66]. The production of microbial lipases presents versatility, stability, and low cost, and it is considered a promising source of lipases due to its ability to multiply and manipulate genetics and the regular production of enzymes. Production occurs mainly by fermentation, whether in solid state (SSF) or submerged (SMF), with the choice influenced by the availability of water and characteristics of the producing microorganism; submerged offers ease of handling on a large scale, and solid-state uses substrates renewable sources and is more economically and environmentally sustainable [67].

Finally, proteases are enzymes that catalyze the breaking of peptide bonds in proteins, characterized by their stereospecificity, biodegradability, and ability to produce natural products and operate under mild conditions. All living organisms are essential for cell differentiation, growth, digestion of food proteins, cell division, protein renewal, blood clotting, apoptosis, signal transduction, and replication of retroviruses. Industrially, they are applied in detergents, leather, food, meat processing, cheese manufacturing, paper and cellulose, silver recovery from photographic films, and bioremediation. Proteases are classified based on substrate specificity, similarity to known proteolytic enzymes, pH range of activity, catalytic mechanisms, and hydrolysis sites [68]. They are divided into protease endopeptidases, which cleave internal bonds in polypeptide chains, with aspartic ones, those that use aspartate residues, have an active site with two aspartate residues, necessary in the degradation of proteins in acidic environments, cysteines, found both in prokaryotic and eukaryotic organisms, are active at neutral to slightly acidic pH and can be inhibited by oxidizing agents, metalloproteases, essential in the structural and functional stability of several proteins, depend on metal ions such as zinc for their catalytic activity, and serines, known for its ability to cleave peptide bonds at specific locations within polypeptide chains and exopeptidases, which catalyze the division of peptide bonds into distinct proteins adjacent to the carboxyl and amino termini incorporated in the substrate, such as aminopeptidases that act on the free N-terminus of polypeptide chains, release small peptides or amino acids, and carboxypeptidases that catalyze cleavage at the terminal C, removing specific amino acids and being fundamental in modifying proteins and removing terminal markers. Adding proteases and multiple microbial strains during fermentation promotes microbial growth, stimulates the secretion of organic acids and amylase, and reduces sugar metabolism, increasing the ethanol content by 2.4%. These effects are predominantly observed in processes with high gravity and no cooking, resulting in a 48-hour reduction in fermentation time [69].

## 3.2 *Vegetable extracts fermented with water kefir*

Given the above, water-soluble plant extracts fermented with water kefir emerge as an alternative to fermented milk-based drinks. Therefore, it can be consumed by the vegan public, but not as a drink exclusive to this public [70-72]. Furthermore, the drink fermented with water kefir is easily combined with different natural sources of flavors and has great probiotic potential [54]. This fact has driven research using various sources such as amaranth, almonds, peanuts, rice, oats, hazelnuts, hemp, cashew nuts, coconut, spelt, cowpea, sesame, sunflower, linseed, pistachio, quinoa, soybeans, teff, lupins, among others, as a way to obtain fermented plant-based drinks.

### 3.2.1 *Water-soluble almond plant extract*

Originally from the Mediterranean, sweet almonds (Prunus dulcis) are the region's main nut crop. Notable for its high nutrient content, including calcium (3,067.53 mg/kg), potassium (9,796.08 mg/kg), phosphorus (8,190.75 mg/kg), magnesium (4,002.85 mg/kg), as well as essential fatty acids, vitamin E, fiber and phytochemicals. Its proximate composition varies from 17.14 to 25.12 g/100 g of proteins, 13.34 to 18.59 g/100 g of carbohydrates, and 51.12 to 56.26 g/100 g of lipids [73, 74].

The cooked paste method can be used to produce the almond extract. Initially, 200 grams of almonds are immersed in 1 liter of sterile, chlorine-free water at 25 °C for 8 hours. Afterward, the mixture (nuts + water) is filtered to heat treatment at 85 °C for 10 minutes. Then, the almonds are added to 1 liter of drinking water, mixed, and filtered to obtain the extract without dispersed particles. After filtration, 1% refined sugar is added, and the extract is pasteurized at 85 °C for 15 minutes, resulting in a final pH of 6.46. To prepare the fermented drink, 2% water kefir grains are added to the sweetened extract at 25 °C for 17 hours, obtaining a pH of 4.52.

The drink obtained by Ustaoğlu-Gençgönül et al. [75] using the methodology mentioned above contained 8.3 logs CFU/mL of *Lactobacillus* spp., 7.73 logs CFU/mL of *Lactococcus* spp. and 5.36 logs CFU/mL of yeast. The sensory evaluation of the drink considered the criteria of color, appearance, consistency, flavor, and odor on a scale of 1 to 5. The almond extract obtained scores of 4, 4, 5, 3, and 3, respectively.

Gocer and Koptagel [76] also prepare water-soluble plant extracts from almonds. Initially, the almonds were soaked in water for 12 hours in a ratio of 1:5 (w/v) and then mixed in a blender for 10 minutes. The mixture was filtered through a standard American cotton cloth bag, and the extract obtained was used to prepare the fermented drink. The extract was heated to 90 °C for 5 min, cooled to 25 °C, inoculated with commercial kefir starter culture (0.015 g/L), and incubated at 25 °C until the pH reached 4.6. The author above did not report the fermentation time.

The proximate composition of the almond extract is represented on average by 1.33% proteins, 2.81% lipids, mainly mono- and polyunsaturated fatty acids, such as oleic, linoleic, palmitic, stearic, and palmitoleic, and 4.15% of carbohydrates, primarily isomaltotriose, sucrose, glucose, and fructose [42]. Almond proteins belong to the oleosin family. They have low molecular weight and solubility in water, creating difficulty in dissolution and sensory changes. However, when fermented, due to the aggregation of proteins with the liquid, they form larger structures in a weak gel format, being better solubilized [77].

### 3.2.2 *Cashew nut water-soluble plant extract*

The cashew nut, scientifically named *Anarcadium occidentale* L., originates in Brazil and is the third most-sold nut globally [78]. Its nutritional composition presents 23% protein and 29.8% carbohydrates, with nine essential amino acids and 44% lipids, predominantly mono- and polyunsaturated fatty acids. In addition to vitamin E, which helps with high antioxidant capacity, it also has vitamin K, carotenoids and minerals such as iron (70.6 mg/kg), copper (21.8 mg/kg), zinc (67.8 mg/kg) and (19.9 mg/kg), manganese [79-80].

To produce a water-soluble vegetable extract from cashew nuts, following the method described by Comak and Koptagel [81], the nuts are left to soak for 12 hours without specifying the proportion of water. After removing the shells, the peeled nuts are mixed with water in a blender at a 1:5 (w/v) ratio for 10 minutes, resulting in milk with 10 g of total solid content per 100 g. The resulting liquid is then filtered using cotton cloth to obtain a milky extract. This is pasteurized by heating it to 90 °C for 5 minutes and then cooling until it reaches 25 °C. To start the fermentation stage, a starter culture of commercial kefir is inoculated at a concentration of 0.015 g/L. Fermentation occurs at 25 °C until the pH reaches 4.6, although the time required for this process is not specified. After fermentation, the kefir samples are packaged in 200 mL plastic cups with lids and stored at 4 °C for 30 days. At the end of the storage period, the drink reaches a pH of 4.76. The drink obtained contained 8.78 logs CFU/mL of *Lactobacillus* spp., 8.86 log CFU/mL of *Lactococcus* spp., and 3.60 logs CFU/mL of yeast.

Comak and Koptagel's [82] study sensorially evaluated vegetable drinks fermented with kefir, comparing milk and water-soluble vegetable extracts (cashew nuts, almonds, hazelnuts, and peanuts). The criteria of color, appearance, consistency, flavor, and odor were considered, with a score from 1 to 5. For cashew nut extract, the results were color (3), appearance (3), consistency (1), flavor (3) and odor (3), with positive results.

Weis et al. [14] used another method to produce cashew nut extract, starting with cleaning the nuts in a sodium hypochlorite solution (200 mg/L) for 15 minutes, followed by maceration in water in a 1:2 ratio (nuts and water, w/v) at room temperature for 24 hours. After discarding the maceration water, the chestnuts were briefly heated in water for 5 minutes. Subsequently, the nuts were processed in a ratio of 1:10 (walnuts and water, w/v) in a Vegan Milk Machine, crushing, shaking at 30,000 rpm, and heating at 80 °C for 26 minutes. The extract was filtered to remove solid residues, pasteurized at 95 °C ± 2 °C for 3 minutes, filled into sterilized glass bottles, and stored refrigerated. For fermentation, kefir grains were conditioned in an aqueous sucrose solution (5 °Brix) at 25 ± 1 °C for seven days, with a continuous supply of nutrients every 24 hours. After this period, 5% m/m of the kefir culture was inoculated into the extract and incubated at 25 ± 1 °C for 22 hours until reaching a pH between 4.3 and 4.5. The study does not describe the microorganisms found during fermentation.

The extract's proximate composition is 0.26% ash, 1.83% proteins, 3.97% lipids, and 5.43% total carbohydrates. Due to the longer fermentation time, the pH does not change with fermentation, as this substrate has a low carbohydrate content. Consequently, it remains stabilized during storage to be microbiologically safe for 60 days [78, 83].

### 3.2.3 *Water-soluble coconut vegetable extract*

Coconut (*Coccus nucifera* L.) is a dried drupe originating from Asia, considered the primary source of fat in South and Southeast Asian diets, with 33% of its content composed of oils, of which around 90% consist of fatty acids medium-chain saturates, 5.7% proteins, 64.2% carbohydrates. In addition, it has vitamin E, antioxidants and minerals, such as potassium (122.6 mg/100 g), calcium (39.4 mg/100 g), phosphorus (36.2 mg/100 g), magnesium (129.4 mg/100 g), copper (0.18 mg/100 g) and zinc (0.9 mg/100 g) [84].

To prepare the water-soluble coconut extract, Alves et al. [17] used 100 g of dry coconut pulp in 1,000 mL of filtered water processed in an industrial blender for 10 minutes at medium speed. The mixture was filtered through a sieve covered with cotton fabric to remove particulate matter. The resulting extract was pasteurized at 90 ± 1 °C for 3 minutes in a thermostatic bath with internal circulation, followed by cooling to 20 ± 1 °C. For fermentation, 5% (w/w) of the kefir culture was incubated in the extract at 25 ± 1 °C, with soluble solids (SS) between 7 and 9 g/100 g, without shaking in a shaker incubator. The Erlenmeyer flasks were covered with sterile gauze during the process. After fermentation, the fermented drink was filtered to separate the kefir grains. These were removed and incubated in a new aqueous substrate with 5% sucrose for maintenance. The fermented drink was then stored in sterilized glass bottles under refrigeration (4 ± 1 °C) for 72 hours, obtaining a pH of 4.51 to 4.80. The analysis of the bacterial species present in kefir grains revealed the presence of 37.50% of Lactobacillus uvarum bacteria, 36.60% of Lactobacillus mali, 8.46% of Acetobacter lambici, and among the yeasts, the majority were 31.34% was composed of Saccharomyces cerevisiae.

Another way to use coconut to prepare drinks fermented with kefir is from coconut water, as described in the methodology of Limbad et al. [72]. Using coconut water (300 mL) and kefir grains (1.5 g/L), fermentation occurred aerobically at 30 °C for 96 hours in a LabServ incubator (Thermo Fisher Scientific). Samples were collected every 24 hours for pH analysis, viable cell count (CFU/mL) for lactic acid bacteria (LAB) and yeast, and determination of residual sugars, carboxylic acids, and amino acids. The results showed that total titratable acidity increased significantly over 96 h of incubation at 30 °C, which correlated with a significant drop in pH from 4.5 to 2.8, in addition to the addition of sucrose and glucose, significantly increasing the count of viable LAB cells throughout the fermentation time.

The drink supplemented with sucrose at 12 g/L showed the highest growth in BAL (5.72 ± 0.04 log CFU/mL) after 96 hours, followed by the one containing glucose and sucrose in a 1:1 ratio (5.64 ± 0.02 log CFU/mL). For yeast, growth was lower, with an increase of approximately 1 log after 96 hours, influenced mainly by the fermentation time and the sucrose concentration in the fermentation media. The growth of acetic acid bacteria (AAB) was observed after 48 hours of incubation, with the most significant increases occurring in media containing sucrose at higher concentrations [72].

The sensory analysis by Dwiloka et al. [85] revealed that the fermentation time of coconut water kefir significantly influences its sensory attributes. The 24-hour and 48-hour treatments showed lower acidity acceptability, with average scores of 4.2 (T2) and 4.3 (T4), compared to 5.1 for the 36-hour treatment (T3). The sensation of soda did not vary significantly between treatments with variation in fermentation time, with average scores of 2.8 (T1), 2.9 (T2), 3.0 (T3) and 3.1 (T4). The sour aroma increased with fermentation time, ranging from 2.5 (T1) to 4.6 (T3). Viscosity remained stable, with average scores of 3.0 to 3.2, while turbidity increased over time, ranging from 2.0 (T1) to 3.9 (T4). These results indicate that longer fermentation times may negatively affect the sensory quality of coconut water kefir.

The proximate composition of coconut milk extract after fermentation has 0.95% proteins, 4.46% carbohydrates, and 2.84% lipids. Regarding sugars, glucose is not detected, which can be attributed to complete metabolization by microorganisms during fermentation or to the low amount available after 24 hours. On the other hand, fructose appears in higher concentrations (5.30 g/L), suggesting that it can be converted into acetic acid or ethanol during the fermentation process [17].

#### 3.2.4 *Water-soluble chickpea plant extract*

Chickpea (*Cicer arietinum* L.) is a legume from the Fabaceae family originating from the Middle East region, consumed globally, especially in Afro-Asian countries, being the world's third most produced legume crop. Chickpeas are in high demand due to their high nutritional value and because they are an accessible source of protein, containing 7.87% of their composition, 2.22% of lipids, and 5.57% of carbohydrates, in addition to containing phytochemicals and minerals such as sodium (38.3 mg/kg), copper (0.09 mg/kg), chromium (0.36 mg/kg), iron (0.29 mg/kg), phosphorus (0.28 mg/kg) and magnesium (0.90 mg/kg) [86].

To prepare chickpea extract, Ustaoğlu-Gençgönül et al. [75] initially immersed 200 g of grains in 1 liter of sterile chlorine-free water and kept at 25 °C for 8 hours. After this period, the mixture was subjected to heat treatment at 85 °C for 10 minutes and filtered to remove water. Then, 1 liter of sterile water was added to the grains, and the system was homogenized with a mixer and filtered again to obtain the chickpea extract. After this process, 1% refined sugar was added to the extract pasteurized at 85 °C for 15 minutes. To start fermentation, the sweetened and pasteurized chickpea extract was adjusted to 25 °C and inoculated with 2% (m/v) kefir grains, remaining in these conditions for 17 hours. After fermentation, the fermented plant extracts were stored at 4 °C in the refrigerator. The microbial count of chickpea kefir showed 7.73 logs cfu/mL of *Lactococcus* spp., 7.3 logs CFU/mL of *Lactobacillus* spp., and 4.38 log CFU/mL of yeast.

An alternative to making the extract is to use mucilage extracted from chickpeas. To do this, the grains, after being cleaned, are air-dried and crushed first in a traditional stone mill, followed by an electric mill. They are then sieved to pass through a 500 µm screen. The ground grains, stored under refrigeration in sealed plastic bags, are then added to distilled water in a ratio of 10:400 (w/v) and stirred for three hours at 60 °C. After cooling, the extracts are centrifuged at 4,000 g for 20 minutes. The supernatant is lyophilized to obtain crude mucilage. For fermentation, 3% kefir is added and incubated at 37 °C for 24 hours until a pH of 4.2 is reached. No microbiological count was performed [87].

The sensory evaluation was carried out using a hedonic scale ranging from 1 to 5, which covered criteria such as color (2), appearance (3), consistency (3), flavor (2), odor (2), and general criteria (2). The drink received low scores across the board, mainly due to the residual chickpea odor, which negatively impacted its sensory properties. Given the results achieved and the complexity of the production process, this alternative of obtaining a water-soluble vegetable extract from chickpeas for application in fermentation processes for the development of new products appears to be unfeasible and requires improvements in methodology [88].

Chickpea extract has a proximate composition of 1.27% proteins, 1.53% lipids and 3.7% carbohydrates. Furthermore, it is a significant source of fiber, especially galactooligosaccharides, which act as prebiotics, promoting microbiological growth and complementing probiotic drinks [89].

### 3.2.5 *Water-soluble hemp plant extract*

Hemp originating from Central Asia, cultivated mainly for its resistant fibers and seeds, is obtained from the dried fruit of *Cannabis sativa* L.; however, its tetrahydrocannabinol content is low (0.3%), so it does not present toxic effects or hallucinogens. It is composed of 25 to 35% lipids, 85% being unsaturated fatty acids (oleic, linoleic, linolenic and palmitic), 20 to 25% proteins and 20 to 30% carbohydrates, in addition to minerals such as phosphorus (1,650 mg/100 g), potassium (1,200 mg/100 g), magnesium (700 mg/100 g), calcium (70 mg/100 g) and bioactive compounds [90-91].

To prepare the hemp extract, according to the methodology of Li et al. [60], fresh seed should be mixed with water in a ratio of 1:9 (w/w), supplemented with 5% fructose corn syrup (w/w), and homogenized under high pressure for 5 minutes at 20 MPa using ultra-high voltage equipment. The resulting suspension was sterilized at 102 °C for 25 minutes, cooled to 25 °C, and then kefir grains were added at a concentration of 3% (w/w). Fermentation occurred for 24 hours at 28 °C under aerobic conditions, obtaining a pH of 3.92. Subsequently, the mixture was homogenized until uniformity was reached, sterilized again at 102 °C for 10 minutes, and stored at 4 °C. The study did not perform analysis of microorganisms.

For the preparation of the fermented drink, using the methodology of Lopusiewicz et al. [33], hemp was ground in a processor and mixed with distilled water at 90 °C in a ratio of 7:3 (w/v); the mixture was then boiled for 20 minutes, cooled and homogenized using a domestic mixer, then was pasteurized at 60 °C for 30 minutes. The pasteurized material was packaged and cooled until reaching 25 °C with a pH of 6.3 to be inoculated with 10% (w/w) commercial kefir grains (containing 2.14 × 107 ± 0.36 CFU/g of lactic acid bacteria (LAB) and 1.08 × 107 ± 0.41 CFU/g of yeast). Fermentation took place in a sterile low-density polyethylene cup (50 mL capacity), hermetically sealed, and incubated for 24 hours at 28 ± 1 °C until reaching a pH of 4.82. After incubation, the samples were cooled and stored at 5 ± 1 °C in the refrigerator for 28 days. The drink contained 1,010 CFU/mL of lactic acid bacteria and 106 CFU/mL of yeast.

The hemp seed extract, after fermentation, had a proximate composition of 3.2 g/100 g of proteins, 2.5 g/100 g of lipids, and 5 g/100 g of carbohydrates (fructose). Furthermore, a significant increase ($p < 0.05$) was observed in the levels of aldehydes, especially hexanal, mainly responsible for the characteristic herbal “green grass” flavor. This sensorial attribute is essential for the acceptance and quality of the final product.

### 3.2.6 *Water-soluble lupine vegetable extract*

Lupine (*Lupinus* L.) is a legume originating from the Mediterranean region, subdivided into four primary species: *L. albus* L. (white or sweet lupine), *L. luteus* (yellow lupine), *L. angustifolius* L. (blue lupine) and *L. mutabilis* (Andean lupine). This legume stands out for its low lipid content, approximately 9.34%, and high protein content, approximately 37.87%, with most proteins being globulins and albumins in a 9:1 ratio. Additionally, it contains 38.92% carbohydrates and is a significant source of minerals such as potassium (1.010 mg/100 g), phosphorus (440 mg/100 g), magnesium (198 mg/100 g), calcium (176 mg/100 g), iron (4.36 mg/100 g) [91-92].

Despite its nutritional properties, lupine contains alkaloids, nitrogenous organic compounds that taste bitter and can be toxic in high concentrations. These alkaloids, a natural defense against herbivores and pests, must be removed to make the lupine more palatable and safe for human consumption. A detailed treatment and extraction process eliminates alkaloids from lupine beans. It begins with immersing 40 g of grains in water in a 1:5 (m/v) ratio at 4 °C for 12 hours. After the initial immersion, the water is discarded, and the grains are heated at 60 °C, in the same proportion of 1:5, for 6 hours in a 0.5% tartaric acid solution, with changes of this solution every 30 minutes. Subsequently, the grains are treated in a 0.5% sodium carbonate solution for 15 minutes at the same proportion and temperature, with two changes to this solution. Then, the grains are boiled for 30 minutes in the same solution, with two additional changes. After these treatments, the grains are immersed again in water at 4 °C overnight for a final leaching of the remaining alkaloids. Finally, the beans are boiled twice for 30 minutes, dried in an oven at 30 °C for 24 hours, and stored in glass jars at room temperature until use [15].

In the preparation of the water-soluble lupine vegetable extract, according to the methodology of Weis et al. [15], approximately 100 g of previously treated lupine grains are used to extract alkaloids, homogenized with 500 mL of water in a specific container for the preparation of plant extracts (Vegan Milk Machine), with crushing and stirring (3,000 rpm) and heating (80 °C) for 26 minutes. It is then subjected to a double filtration process and then pasteurized at 95 °C ± 2 °C for 3 minutes, being packaged in sanitized and sterilized glass bottles and stored in a refrigerator. For fermentation, 5% (m/m) of the kefir culture was inoculated into the extract and incubated at 25 ± 1 °C for 22 hours until reaching a pH of 4.06. The study did not quantify microorganisms.

Lopes et al. [88] also prepared a lupine extract without carrying out the alkaloid extraction process, which was done through the process of soaking 150 g of dry seeds in warm water (30-35 °C) twice and in cold water (15-20 °C) once for 16 hours, with water discarded. The seeds were then cooked for 30 minutes after boiling in a pressure cooker with 1.5 L of water, then divided into three equal parts and processed: the first fraction was drained, added to 500 mL of fresh water, and crushed in a processor of food at 20,500 rpm for 4 minutes; the second fraction was drained, the cooking water replaced with fresh water up to 500 mL and crushed like the first; the third fraction was divided into two equal parts, one with and the other without cooking water. Trituration involved grinding the seeds with 200 mL of water (cooking or new) at 20,500 rpm for 4 minutes, followed by colloidal grinding at 70 rpm for 15 minutes, with the remaining volume of water. The drinks were sieved, bottled in sterilized bottles (100 °C, 10 minutes), pasteurized with thermal shock in a pressure cooker for 1 minute in boiling water, and stored at 4 °C for up to 7 days for subsequent analysis. The study did not perform fermentation. The sensory analysis carried out evaluated on a scale of 1 to 5 aspects such as flavor (2), aroma (4), color (3), consistency (4), appearance (2), and general aspects (3), with high marks for aroma and consistency.

The extract's approximate composition contains 0.54% lipids, 4.05% proteins, and 3.27% carbohydrates, most of which are polysaccharides like galactose and xylose. So, it is rich in glucose despite not having large amounts of starch. Its protein digestibility is high, with around 96% bioaccessibility [93].

### 3.2.7 *Water-soluble pistachio plant extract*

Pistachio (*Pistacia vera* L.) is a nut originating in Western Asia that belongs to the *Anacardiaceae* family, and the main species are *P. atlantica*, *P. cabulica*, *P. chinensis*, *P. falcata*, and *P. integerrima*. It is composed of lipid (45 g/100 g), protein (20.5 g/100 g) and carbohydrate (27.7 g/100 g). This nut stands out for its high content of amino acids and essential minerals such as calcium (117 mg/100 g), iron (3.47 mg/100 g), potassium (947 mg/100 g), magnesium (110 mg/100 g) and phosphorus (500 mg/100 g) [91].

According to the methodology of Sánchez-Bravo et al. [94], pistachio extracts can be prepared from 400 g of shelled pistachios soaked in distilled water at room temperature (25 ± 2 °C) for 4 hours. The nuts were crushed and mixed with hot water (80 °C) in a 1:5 (m/v) ratio using a blender. The mixture was then filtered to remove all solid particles to obtain the drink. Then, the pistachio drinks were pasteurized at 70 °C for 30 min and kept in a refrigerator (4 °C). The kefir grains (5% m/v) were then placed and incubated in an oven at 25 ºC for an average of 20 hours, reaching a pH between 4.5 and 4.1. *Lactococcus lactis*, *L. cremoris*, and *L. biovar diacetylactis* grew more significantly. The study did not quantify microorganisms.

Another way to prepare the extract is by following the methodology of Pakzadeh et al. [95], where the pistachio shell is initially manually removed, leaving only the kernel. This is then mixed with distilled water in a ratio of 1:4.5 (m/v) and kept at rest for 24 hours at 4 °C. After this period, the mixture is processed in a blender for 8 minutes. The resulting mixture is filtered through a mesh sieve for smooth paste. This paste is the basis for preparing water-soluble pistachio extract. Later, xanthan gum (0.25% m/v) and pistachio paste (20-40% v/v) are added, together with a composition of Tween 80/Span 60 (0.5-1% m/v, ratio 1:1). The xanthan gum is first gradually incorporated into the water and placed on a magnetic stirrer for 2 hours to ensure complete hydration. Emulsifiers are added separately to the pistachio paste and heated to 40 °C. After the complete dissolution of the emulsifiers, the oil phase is slowly incorporated into the xanthan solution, and the mixture is homogenized using a homogenizer at 10,000 rpm. Finally, the water-soluble pistachio extract is subjected to an ultrasonic device for 10 minutes at 200-400 W power to reduce particle size.

The proximate composition of the fermented drink presented 2% protein, 4.6% lipids, 0.21% carbohydrates, and 2.9% fiber. Furthermore, it can be considered that, due to the high-fat content, pistachio extract naturally has a protective effect on the matrix on the development of microbial cells, as observed in analyses of the viable counts of all cultures, which they remained at 8 log CFU/mL after 30 days of storage [96]. No sensory analysis studies were conducted on the drink fermented with water kefir in water-soluble pistachio extract.

### 3.2.8 *Water-soluble quinoa plant extract*

Quinoa (Chenopodium quinoa Wild) originates from the Andean countries of South America, belonging to the Amaranthaceae family. It is a pseudocereal that has gained prominence on the world market due to its nutritional value, especially the high protein content (14.1 g/100 g) and quantity of carbohydrates (64.2 g/100 g), in addition to having higher levels of calcium (47 mg/100 g), potassium (563 mg/100 g), magnesium (197 mg/100 g) and iron (4.57 mg/100 g) among grains [91, 97].

Karovičová et al. [98] state that the seeds must be desaponified to prepare the quinoa extract. The chaponized quinoa seeds were dried at 60 °C for 8 hours and ground into flour. In the next step, quinoa flour was mixed with water to a concentration of 5% (w/v), then the mixture was gelatinized at 95 °C for 10 min and cooled to 20 °C. Subsequently, the samples were fermented at 37 °C for 6 hours, resulting in an extensive development of lactic acid bacteria (LAB). After fermentation, the drinks are stored in sealed glass containers for 21 days at 5 °C.

For the preparation of the water-soluble quinoa plant extract following the methodology of Sanches et al. [13], first, the quinoa grains are soaked in water in a ratio of 1:2 (w/v) for 1 hour in refrigeration at 5 ± 2 °C. After this period, the water is discarded, and new water is added in a 1:5 (w/v) ratio, subjecting the contents to the boiling process on a domestic stove for 17 minutes. Then, a fine plastic sieve separates the solid content from the liquid. The cooked grains are added again with water in a proportion of 1:10 (w/v) and crushed using a blender until all the contents are well crushed. Again, a plastic sieve separates the solid content from the liquid. The water-soluble quinoa plant extract is then pasteurized at 95 ± 2 °C for 3 minutes, filled in sterilized glass bottles, and stored in a refrigerator at 5 ± 2 °C. To carry out fermentation, the kefir grains were previously activated for seven days in an aqueous brown sugar solution with soluble solids of approximately 5 °Brix at 25 ± 1 °C, with solution changes every 24 hours. After conditioning, 5% (m/m) of the kefir culture is inoculated into the plant extract. The mixture is then incubated at 25 ± 1 °C for 12 hours.

The main organic compounds produced in the process are lactic and acetic acid; in addition, fermentation increases the protein content of the drink [98]. The proximate composition of the fermented drink contains 13.63% proteins, 4.53% lipids, and 69.40% carbohydrates [13]. No sensory analysis studies were conducted on the drink fermented with water kefir in water-soluble quinoa extract.

### 3.2.9 *Rice water soluble vegetable extract*

Originating in Asia, rice (*Oryza sativa* L.) is a highly energetic grain, made up of approximately 75.86% carbohydrates, 80% starch, 7.28% proteins, containing eight essential amino acids, 1.82% lipids, in addition to B vitamins, fiber and minerals, such as phosphorus (433 mg/100 g), potassium (427 mg/100 g), magnesium (177 mg/100 g), calcium (21 mg/100 g ) and iron (1.96 mg/100 g) [91].

To prepare the water-soluble rice vegetable extract, using the methodology described by Magalhães-Guedes et al. [99], 200 g of rice grains should be washed in water to reduce contamination. Then, the grains were added to 400 mL of sterile water in a stainless steel container, maintaining the proportion of 1 part of the grain to 2 parts of water (1:2, w/w). The mixture was heated to 85 °C and cooked for 30 minutes. After cooking, the rice grains were filtered to separate the liquid solution and homogenized in a high-power blender until a homogeneous puree free of visible particles was obtained. Subsequently, the rice extract was pasteurized at 85 °C for 15 minutes and packaged. To start the fermentation process, sweetened Kefir grains are added at a proportion of 5% for 24 hours at 28 °C until a pH of 3.5 is reached. The PCR-DGGE technique investigated the microbiological composition of sweetened Kefir grains and Kefir drinks based on rice extract. The results revealed the presence of several microbial species, including *Lactobacillus paracasei*, *Lactobacillus kefir*, *Lactococcus lactis*, *Leuconostoc citreum*, *Acetobacter lovaniensis*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Lachancea meyersii* and *Kazachstania aerobica*.

Ustaoğlu-Gençgönül et al. [75] also prepared and fermented rice extract with water kefir. In the same way, as in the almond extract, they used the paste methodology in the same concentrations and conditions. The rice extract had a pH range of 6.10 (without fermenting) and 5.72 (fermented). The concentration of rice kefir was 5.64 log CFU/mL, but an increase was observed during refrigerated storage, probably due to the slow metabolism of rice starch. For fermentation, water kefir grains were added to rice extract in a proportion of 2% at 25 °C for 17 hours. The drink had a low level of *Lactobacillus* spp. but a high level of *Lactococcus* spp. (8.5 logs CFU/mL in rice kefir). Regarding sensory aspects, rice kefir obtained average scores in all criteria (color, appearance, consistency, flavor, odor, and general criteria); however, it demonstrated an acceptable level of palatability.

The sensory evaluation of the drink was carried out by analyzing attributes such as flavor, color, aroma, and consistency using a 9-point hedonic scale. Scores ranged from 1 (I don't like it very much) to 9 (I like it very much). The results indicated that the flavor received an average score of 5.2, indicating a neutral response. Color and aroma scored moderately, with 6.4 and 6.7, respectively. Consistency obtained a higher score of 7.1, indicating good texture [100].

### 3.2.10 *Water-soluble soy plant extract*

Soy (*Glycine max*), originating in Asia, is considered the most commercially common among all grains and legumes. It has high protein content (40%), in addition to 30.16% carbohydrates, 19.94% lipids, minerals such as potassium (1,797 mg/100 g), phosphorus (704 mg/100 g), magnesium (280 mg/100 g) and iron (15.7 mg/100 g). It is also rich in bioactive compounds, such as isoflavones, saponins, oligosaccharides, plant sterols, protease inhibitors, lectins, phytic acid, and polyamines [91, 101, 102].

To prepare the soy extract, according to the methodology of Tu et al. [103], serum obtained as a byproduct of tofu production was used. The process began with 250 mL Erlenmeyer flasks containing 200 mL of sterilized soy whey, whose Brix value was adjusted to 9.5 °Brix. We then added 5% (w/v) water kefir grains to each fermentation bottle and incubated at 25 °C over five days. During this period, samples were collected every 24 hours for subsequent analysis. The microbial growth dynamics during the fermentation of soy whey with water kefir revealed that yeasts, LAB, and AAB reached more than 7 logs CFU/mL after two days of fermentation. The pH decreased from 5.31 to 3.47, while the TSS reduced from 9.20 to 4.43 °Brix in 2 days. Fructose and glucose concentrations increased to 6.78 ± 0.17 g/L and 15.6 ± 1.25 g/L after 1 and 2 days, decreasing to less than 1 g/L after five days. Organic acids such as acetic acid and lactic acid also increased during fermentation. The analysis of volatile compounds showed the reduction of undesirable compounds, such as 2-pentyl furan and hexanal, after fermentation. The total contents of phenolics, flavonoids, and isoflavones increased significantly during fermentation, improving the antioxidant capacity of soybean whey [103].

Another way to prepare soy extract is through the methodology described by Kundu et al. [104], where soybeans are initially immersed in a 0.5% $NaHCO_3$ solution for 12 hours, followed by draining the alkaline solution. Subsequently, the grains are boiled again in a new 0.5% $NaHCO_3$ solution for 30 minutes, and then, the mixture is drained. After this process, the soybeans are ground in water at room temperature, forming a soybean paste. This paste is then heated to 90 °C and filtered to remove okara, the residue composed mainly of insoluble fibers and proteins not solubilized during the extract manufacturing.

The proximate composition of the fermented extract presented 0.40 g of lipids, 0.65 g of protein, and 0.44 g of ash per 100 mL [103]. The texture and viscosity of soy-based drinks are essential parameters, considering that they are composed of a substantial amount of tiny lipid droplets dispersed in the aqueous phase, derived from soybean seeds, which can lead to a sensory change; however, it is possible to add substances such as xylitol to increase viscosity, even when subjected to stirring or mixing efforts, becoming more resistant and stable [39].

Sensory analysis was performed using the hedonic scale of 1 to 10 to evaluate the quality of the drink in terms of color (9), acidity (8), flavor (9), and overall acceptability (7) [103]. Previous studies highlight that soy-based beverages often have unfavorable results due to their acidic taste and bean profile, which can be mitigated by adding sugar. However, this effect may be exacerbated by the higher soy content in products.

### 3.2.11 *Other fermented extracts*

The Russian olive (*Elaeagnus* spp.) is a riverine tree that grows near rivers and is found mainly in Central Asia, Iran, Uzbekistan, Syria, and northwestern China. Furthermore, it is found exotically on riverbanks in central Spain, Canada, and the western United States [105].

To prepare the juice from pesticide-free Russian olives, the fruits were initially air-dried until they reached a moisture content of 11.8%, then the mesocarp was separated from the seeds and skin. Afterward, the dried mesocarp was ground in a blender until it became powder. This powder (15.4 g) was mixed with water (100 mL) and stirred for 30 minutes, then subjected to centrifugation and pasteurization at 75 °C for 5 minutes. For the fermentation process, reactors were used, in which a volume of 0.4 liters was added, consisting of 20 grams of water kefir grains (50 g/L) and 20-30% Russian olive juice pasteurized and 32 g of sucrose, being dissolved in a volume of deionized water, resulting in a final concentration of 8% w/v of sucrose in a 0.4-liter bioreactor. The prepared sucrose solutions were sterilized by filtration before being added to each bioreactor. During the experiments, the temperature was maintained at 20 to 32 °C while fermentation continued with pH monitoring for 24 to 48 hours.

At the end of the work, the authors concluded that the ideal conditions for the fermentation process were an incubation temperature of 31.2 °C, an incubation time of 24 hours, and a concentration of 30% Russian olive juice. Furthermore, the fermented drink obtained showed antioxidant activity with values of 0.199 µmol FSHE/mL for FRAP, 0.121 µmol Trolox Eq/mL for scavenging DPPH radicals and 101.939 µg GAE/mL for Total Polyphenol Content (TPC). Furthermore, high levels of microbial viability were observed, with values of 7.39 log 10 CFU/mL for BAA, 7.18 log 10 CFU/mL for BAL, and 7.08 log 10 CFU/mL for yeast [106].

The carnelian cherry, also known as Cornus mas L., is cultivated in regions such as Turkey, Romania, Bulgaria, Italy, southern Europe, and southwest Asia [107]. This fruit is used in the food industry to produce a variety of drinks and other traditional products. Furthermore, it contributes to a healthy diet due to its phenolic components, vitamin C, pectins, and essential minerals [108].

The extract can be prepared in two ways, as Ozcelik et al. [109] described. First, the Aronia pomace (300 g) is boiled with 3 liters of water for 10 minutes, followed by filtering the mixture through a sieve and cooling until it reaches 35 °C. Alternatively, commercially available ready-made aronia juice can be diluted with water at a ratio of 1:3 (v/v) to achieve a brix content of 10-11%, then pasteurized at 65-68 °C for 5 minutes and cooled until it reaches room temperature. For both fermentation, water kefir grains, previously activated for 72 hours at 25 °C, are added to fruit juices at a concentration of 5% (w/w). Fermentation is carried out at 25 °C for 48 hours until reaching a pH of 3.39.

Aronia pomace kefir had a high anthocyanin content (150 mg/L), while aronia juice kefir had a higher flavonoid content (180 mg/L). After 72 hours of fermentation, both kefirs showed a significant reduction in antioxidant compounds, with a decrease in antioxidant activity measured by DPPH (60% drop) and CUPRAC (55% drop). The drink made with aronia juice demonstrated a color change, taking on a more reddish hue. The analysis of volatile compounds revealed the presence of components such as acids, esters, alcohols, aldehydes, ketones, alkanes, hydrocarbons, terpenes, and phenols [109].

The sensory properties of the drink were evaluated on a scale of 1 to 10 concerning color (9), turbidity (3), attractiveness (optical) (8), attractiveness (taste) (7), aroma/odor (6), acidity (4), sweetness (8), sparkling (4), and favor (general acceptance) (7). In the general descriptive criteria, acidity was present, slightly alcoholic, and slight effervescence was present [109].

Jackfruit (*Artocarpus heterophyllus*) belongs to the *Moraceae* family and is native to Southeast Asia. It is cultivated in most tropical regions, such as Bangladesh, India, the Philippines, Pakistan, Malaysia, Thailand, and Latin America [110]. It has antioxidant, anti-inflammatory, and anti-cancer properties as it contains several nutrients and minerals, such as flavones and saponins. For the elaboration of jackfruit extract, as described by Pablo and Cimafranca [111], the process begins with obtaining the jackfruit concentrate, derived from dehydration processing, which was initially prepared by washing the peeled jackfruit fruits, the seeds Removed from the bulbs and cut into longitudinal slices, the slices were immersed in sugar syrup and left to soak for 30 minutes. They were then heated at 80 °C for 45 minutes. Afterward, they were left to soak again for 3 hours, with 2,000 ppm of potassium metabisulfite (KMS) added. The concentrate was diluted with drinking water to reach the desired concentration and pasteurized at 70 °C for 30 minutes to ensure food safety. After cooling the solution, water kefir grains were added to the mixture. Aerobic fermentation was conducted for 24 hours at room temperature, using glass bottles covered with muslin.

This study explored jackfruit concentrate as a substrate for fermentation with water kefir. The initial TSS of the material ranged from 10.5 to 15 °Brix, and the pH was 5.0, which was a favorable condition for fermentation. After fermentation, the TSS varied from 3.85 to 11.90 °Brix, while the final pH remained at 5.0. It was observed that increasing kefir grain levels reduced TSS due to the fermentation of sugars, mainly sucrose. The titratable acidity was 0.243 to 0.414% after fermentation due to the production of lactic and acetic acids. Microbial viability ranged from 7.55 $\times 10^6$ to $3.44 \times 10^7$ CFU/mL, ensuring a minimum of $7 \times 10^9$ live probiotics per 100 mL portion. Sensorially, the drink was described as “moderate yellow”, “perceptible jackfruit aroma”, and “moderately sweet”, with overall acceptability ranging from 6.66 to 7.88 on the nine-point hedonic scale. Optimization of jackfruit concentrate and kefir grain levels indicated that the ideal ratio is 75% v/v jackfruit concentrate and 9.75% w/w kefir grains [111].

## 4. Important points about fermented drink with water kefir

The challenges of producing plant-based or alternative dairy beverages revolve around three key factors: production costs, consumer acceptance, and replicating sensory characteristics similar to traditional dairy beverages [112].

### 4.1 *Production costs*

Some points are essential regarding production costs, such as using raw Materials. Sourcing plant-based ingredients (like almonds, oats, soy, or peas) often involves higher fees than traditional dairy. Some crops require significant resources (water, land), while others may need to be imported from specific regions, impacting prices. The kind of processing technologies is also a point to consider since creating a beverage miming dairy involves advanced processing techniques, such as enzymatic treatments, fortification with vitamins and minerals, and emulsification. These steps, combined with the costs of specialized machinery, can add to production expenses. The economies of scale are also a point since traditional dairy industries are highly scaled and efficient, benefiting from long-established infrastructure. Plant-based alternatives are newer, with many producers lacking similar economies of scale, raising costs.

### 4.2 *Consumer acceptance*

Regarding taste preferences, as discussed by the authors cited at the beginning of this section, dairy beverages have a well-established flavor profile, and consumers often strongly prefer Plant-based alternatives, which sometimes have distinct flavors that may not appeal to everyone. Overcoming these taste preferences can be difficult, especially with consumers accustomed to dairy. Besides, nutritional concerns should be considered since some consumers are concerned about whether plant-based alternatives offer the same nutritional benefits as dairy (like protein, calcium, and vitamin D). Ensuring that alternative beverages are fortified and perceived as healthy is essential for broader acceptance. Finally, cultural and psychological factors should be considered since dairy has deep cultural roots, and for many people, milk is seen as a staple of their diet. Overcoming this traditional attachment can be a barrier to consumer acceptance of alternatives.

### 4.3 *Difficulties in replicating sensory characteristics*

As cited by the authors in the literature, the sensory characteristics should be pointed out in flavor, considering that milk has a distinct, mildly sweet flavor that is hard to replicate. Plant-based ingredients often introduce unique tastes that differ from dairy. For example, soy milk may have a bean-like aftertaste, while almond milk might be nuttier, which some consumers dislike. Considering texture and mouthfeel, dairy’s creamy texture and rich mouthfeel come from its fat content and protein structure. Plant-based milks often struggle to replicate this, leading to products that can feel too thin or chalky. Creating a smooth, creamy texture mimicking dairy can require additional emulsifiers and thickeners, complicating production. The foaming and cooking performance, for applications like coffee, where milk is steamed and frothed, plant-based alternatives can struggle to perform similarly. Achieving stability and similar frothing capabilities is technically challenging.

These factors combined create a complex landscape for companies looking to develop alternatives that appeal to traditional dairy consumers and those seeking plant-based options. Balancing cost, sensory replication, and market appeal is crucial for success in this growing market.

## 5. Legislation of fermented drink with water kefir

The health benefits associated with consuming fermented kinds of milk have ancient roots but were highlighted in the 20th century by Élie Metchnikoff, who suggested that yogurt promoted longevity by replacing harmful bacteria with beneficial ones in Bulgarians. Researchers such as Henry Tissier, Alfred Nissle, Leo Rettger, and Minoru Shirota advanced the study of beneficial microorganisms. The definition of probiotics has evolved to describe live organisms that, in adequate amounts, benefit the host's health. Since the 1990s, scientific interest and international regulations, such as the Codex Alimentarius, have grown to ensure the safety and efficacy of these products [113].

The "vegan loophole" in regulations allowing products to be considered vegan without meeting the expected criteria is a global concern. In Europe, the Food Information to Consumers Regulation creates specific legislation on the "suitability of a food for vegetarians or vegans" by the European Commission. However, there is still no unified definition of "vegan". The European Vegetarian Union (EVU) has proposed a definition that excludes any product of animal origin at any production stage. The lack of an official definition has led to the growth of third-party certifiers and the offering of precision fermentation products [114].

The Codex Alimentarius, compiled by the Codex Alimentarius Commission (CAC) [115] of the FAO and WHO, presents specific standards such as CXS 243-2003 for fermented kinds of milk, establishing criteria for starter cultures (minimum 107 CFU/g) and other microorganisms (minimum 106 CFU/g), essential composition, analytical methods, and labeling. Additionally, regional regulations such as CXS 332R-2018 for doogh in Asia define standards such as maximum pH of 4.5, minimum titratable acidity of 0.3% (lactic acid), minimum non-fat solids of 3.0%, minimum milk protein of 1.08%, and sodium chloride with no specified maximum value. Starter cultures must have a minimum of 107 CFU/g, and specific labeled microorganisms, when present, must also have a minimum of 107 CFU/g.

In Europe, the regulation of fermented foods is covered by Regulation (EC) No. 178/2002 on general food safety. The microorganisms used are considered food ingredients and must comply with general food legislation prohibiting the sale of unsafe foods. Novel foods are regulated by Regulation (EU) 2015/2283, requiring risk assessment by EFSA before marketing. Recently, in Spain, the term "probiotic" has been used on labels without the need for specific health claims, reflecting a trend toward broader acceptance among Member States [116].

In the United States, the FDA regulates acidified foods like yogurt but not other fermented foods due to a lack of evidence of adverse risks. As the FDA defines, yogurt must contain at least 3.25% milk fat, 8.25% non-fat milk solids, and a titratable acidity of 0.5% (lactic acid). State regulations such as the 2010 "Draft Guidance for Industry: Acidified Foods" regulate the production and sale of fermented foods, which vary between states [116].

In Australia and New Zealand, to regulate water kefir, products must comply with standard 2.6.2: Non-alcoholic drinks and fermented soft drinks of the Food Standards Code, in which it is stipulated that the drink must not contain more than 1.5% alcohol by volume. This measure aims to guarantee its classification as non-alcoholic. Furthermore, the code states that drinks must contain a maximum of 75 g/L of sugars and not contain caffeine [117].

In Canada, live bacterial cultures, including probiotics, are food ingredients under the Food and Drug Regulations. Non-specific probiotic health claims are permitted if the product contains *Lactobacillus* spp. and *Bifidobacterium* spp. at minimum levels of 109 UFC per serving, maintained throughout the product's shelf life [118-119].

In South America, the regulation of fermented foods varies significantly between countries. In Argentina, the Food Code incorporates MERCOSUR and Codex Alimentarius standards, covering detailed specifications for fermented dairy products and kombucha. In Brazil, MAPA and ANVISA supervise processed foods, with regulations such as RDC nº 331/2019 establishing microbiological standards aligned with the Codex Alimentarius [120].

## 6. Advantages of using water kefir

Water kefir offers several significant advantages compared to traditional kefir made with milk. Firstly, it is an alternative for individuals with allergies to milk protein or lactose intolerance and the vegan public. As this market grows, there is an increasing demand for fermented products that meet these dietary preferences. Water kefir is a source of probiotics and nutrients similar to traditional fermented drinks. Furthermore, its fermentative matrix is versatile, allowing various extracts from different raw materials, each with other nutrients, such as amino acid variability, vitamins, and minerals [121]. Physiologically, water kefir shares many of the beneficial properties of fermented milk drinks, such as supporting intestinal health due to the presence of probiotics, so it provides a viable and healthy alternative for those with dietary restrictions, but also contributes to diversification and expansion of the fermented products market, promoting nutritious and functional options for a wide range of consumers [122].

## 7. Critical analysis

The development of vegan alternatives, especially in the food and consumer goods industries, presents several practical challenges for manufacturers and researchers. Here is a critical analysis of these challenges, highlighting key areas such as formulation, scalability, consumer acceptance, regulatory hurdles, and sustainability.

### 7.1 *Formulation challenges*

Replicating Animal-Based Products: One of the most significant difficulties is replicating animal-derived products' taste, texture, and nutritional profile. Achieving the same sensory qualities as meat, dairy, or eggs is complex because these products have unique chemical compositions. Plant-based proteins often lack the richness or "mouthfeel" of animal fats and proteins, requiring sophisticated techniques like fat encapsulation or novel emulsifiers.

Protein Structure and Functionality: Plant proteins differ in functionality from animal proteins. For instance, achieving the proper elasticity or cohesiveness in plant-based cheese or meat alternatives often requires additional binders or stabilizers, which can complicate the formulation. Similarly, specific plant proteins may not have the same solubility or foaming properties as animal proteins, making it challenging for dairy-free milk or egg-free baking applications.

Nutritional Adequacy: Another challenge is ensuring that vegan alternatives provide a balanced nutritional profile. Many plant-based proteins may lack certain essential amino acids found in animal proteins, such as lysine, which must be addressed by fortifying or blending different plant protein sources. Manufacturers also face issues of nutrient bioavailability, as plant-based sources may contain antinutrients like phytates, which hinder mineral absorption.

### 7.2 *Scaling production*

Supply Chain Limitations: The production of vegan alternatives is constrained by the availability of critical raw materials such as specific plant proteins, oils, and specialty ingredients like pea or soy proteins. Some ingredients, incredibly exotic or less commonly used in mass production, have limited supply chains that are not yet robust enough for large-scale manufacturing. This affects cost and consistency in product formulation.

High Production Costs: Plant-based alternatives are often more expensive than animal-based alternatives. This can be attributed to high ingredient costs, the need for advanced processing technologies, and the smaller production scale compared to traditional meat or dairy industries. Reducing the cost of production without compromising on quality or nutritional value remains a challenge.

Technology Access: Advanced processing techniques such as extrusion (for meat alternatives), fermentation (for dairy alternatives), or cell-culturing (for lab-grown meat) require significant investment in R&D and production technology. Access to these technologies, especially for smaller manufacturers or startups, can be limited due to high capital costs or intellectual property barriers.

### 7.3 *Consumer acceptance*

Taste and Texture Expectations: Consumers transitioning to vegan alternatives often expect the products to mimic their animal-based counterparts closely. Many plant-based products fail to meet these expectations, leading to lower repeat purchases. While early adopters may be more forgiving, mainstream consumers tend to demand a closer approximation of taste and texture.

Cultural Preferences: Different cultures have varying expectations around food. For example, meat-heavy cultures may be less open to plant-based alternatives unless the product closely mimics the real thing. In contrast, regions with a more extended history of plant-based diets, such as parts of Asia, may have different standards for what constitutes a good alternative. Manufacturers must account for these cultural differences when developing products for global markets.

Perception of "Naturalness": Many consumers equate plant-based products with health and sustainability, but this expectation can backfire if products are perceived as overly processed. Additives, preservatives, or synthetic ingredients to enhance flavor, texture, or shelf life can lead to concerns about the health implications of consuming vegan alternatives. This perception can hinder market adoption.

### 7.4 *Regulatory and labeling hurdles*

Regulatory Approval: Some novel ingredients used in vegan alternatives, such as specific plant proteins or lab-grown components, require approval from food safety authorities. The regulatory process can be slow, adding time and cost to the product development pipeline. Manufacturers may face different regulatory environments depending on the country, complicating efforts to launch products in multiple regions.

Labeling and Marketing: Clear and honest labeling is essential for consumer trust, but it can be challenging for manufacturers to balance transparency with appealing marketing. Terms like "meat-free," "plant-based," or "vegan" can sometimes confuse or mislead consumers. Moreover, many countries have ongoing debates over using terms like "milk" or "cheese" for plant-based products, with some regions requiring different terminology.

### 7.5 *Sustainability and environmental concerns*

Sourcing of Raw Materials: While vegan alternatives are often marketed as more sustainable than animal products, sourcing raw materials can still raise environmental concerns. The large-scale production of crops like soy or almonds can contribute to deforestation, water scarcity, and monoculture farming practices, which have adverse environmental impacts. Sourcing sustainable, ethically produced ingredients can increase costs and complicate supply chains.

Packaging and Waste: Another issue is that many vegan products rely on processed ingredients, which may come with significant packaging and production waste. For example, producing plant-based meats often involves using plastic packaging to extend shelf life, which can undermine the environmental benefits of choosing vegan products.

Life Cycle Analysis: While plant-based alternatives generally have a lower environmental footprint than animal products, conducting a comprehensive life cycle analysis (LCA) can reveal hidden ecological costs. This can include energy-intensive processing methods, transportation emissions, or unsustainable agricultural practices for critical ingredients.

The development of vegan alternatives presents challenges that span technical, economic, and social dimensions. Manufacturers and researchers must balance the need for high-quality, nutritionally adequate products with concerns about sustainability, cost, and consumer acceptance. Overcoming these challenges will require ongoing innovation in food science, supply chain management, and consumer education, alongside support from regulatory frameworks that facilitate the adoption of vegan alternatives. Some authors in the literature corroborate all points cited above about critical analysis [123-124].

## 8. Conclusion

Therefore, kefir grains are complex microbial ecosystems that perform multiple functions in fermentation. The diversity of microorganisms, including lactic acid bacteria, acetic acid bacteria, and various yeasts, contributes to the production of bioactive compounds and nutrients and directly influences the sensory and nutritional characteristics of fermented drinks. Furthermore, enzymatic activity during fermentation, such as amylase, inulinase, peroxidase, cellulase, lipase, and protease, plays a crucial role in the degradation of complex substrates, the production of functional compounds, and the improvement of nutrient bioavailability. These characteristics make kefir a promising option for consumers with dietary restrictions, such as vegans and lactose intolerants, and for the food industry interested in fermented products with well-established probiotic and prebiotic properties.

Regarding the trends, vegan fermented beverages are gaining popularity as health-conscious consumers seek plant-based, sustainable, and gut-friendly alternatives to traditional drinks. Several key trends are shaping the future of this category: Innovative Probiotic-Rich Beverages, Expansion of Varieties, Fermented Plant kinds of milk, Sustainable and Upcycled Ingredients, Alcohol-Free Fermented Beverages, Personalized Fermentation, International Fermented Beverages, Fermentation and Functional Health. These trends reflect the growing interest in health, sustainability, and culinary innovation, pushing vegan fermented beverages into the mainstream. The future will likely blend tradition and modern innovation, emphasizing functionality, flavor, and environmental impact.

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## Confilict of interest

The authors declare no competing financial interest.

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