



## Review

# An update on fermented plant-based dairy alternatives: Advances towards consumer acceptability and modern diets

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## ABSTRACT

Plant-based dairy alternatives (PBDA) represent a significant portion of the consumption of plant-based products, mainly to replace or complement the diet in relation to dairy milk, due to their health properties, the absence of lactose, environmental impacts, and consumer preference. These products have attracted a wide range of consumers, as they can be derived from different plant sources such as soy, rice, almonds, oats, cashews, and many others, offering different options for different needs and preferences. With the significant increase in the consumption of products that replace animal sources, other important concerns arise, mainly regarding the nutritional deficiency that each vegetable product offers, in addition to undesirable properties regarding adaptation to new foods. Fermentation technology is an attractive alternative as it can mitigate these sensory attributes that are present in plant-based products, as well as ensure greater food safety and health benefits of fermented products. Therefore, this review article will focus on the consumption of plant-based beverages as possible alternatives to dairy products. The consumer perception of PBDA will also be addressed, along with the differences between the fermentation of dairy milk and plant-based beverages, the challenges in the development of PBDA regarding rheological and sensory properties, and the health effects of the consumption of these fermented products.

## 1. Introduction

Fermented foods, defined as foods produced or processed by microbial activity, are estimated to account for a third of the human diet (Ibrahim et al., 2023), in which their diversity depends on ancient people and their dietary components (Leeuwendal et al., 2022). These foods are consumed throughout the world, with evidence registered from wine production during the Neolithic period (7000 BCE), and later from the Mediterranean regions of Europe, in the production of baked goods, beer, and other foodstuffs (Gänzle, 2022). There is also evidence from the Neolithic period in Asia, where fermentative processes occurred in rice, honey, fruits, and cheese (Leeuwendal et al., 2022).

Many fermented foods are consumed nowadays for their unique flavor and health benefits, but fermentation appeared as a method of preserving and processing foods to ensure no deterioration, being also the main responsible for the transition from hunting to agriculture (Gänzle, 2022; Tamang et al., 2020). The fermentation process can preserve food through three mechanisms: 1) the production of

compounds with antimicrobial activity; 2) the reduction in pH; and 3) the competition from microorganisms in the substrate. Compounds produced by microorganisms can reduce the pH of food, such as lactic and acetic acid, or may have antimicrobial activity (bacteriocins and exopolysaccharides) (Aguirre-Garcia et al., 2024; Huang et al., 2022). Moreover, the fermentation process may even have the potential to reduce the use of synthetic additives to increase the shelf life of foods (Dopazo et al., 2023).

The health effects may vary depending on the microorganism involved in the fermentation process. *Propionibacterium* bacteria can produce compounds that improve human health, such as folic acid, vitamin B<sub>12</sub>, proline, and others (Cichonska et al., 2022). Lactic acid bacteria (LAB) can increase vitamin content and bioavailability, as well as reduce antinutrients through the activity of microbial enzymes (Zapasnik et al., 2022) and have antioxidant and immunomodulatory effects (Deveci et al., 2023). Furthermore, *Saccharomyces cerevisiae* has beneficial effects on immune responses, in addition to having antibacterial and antiviral properties (Ansari et al., 2023).

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The production and increased bioavailability of these nutrients can be attributed to: 1) reduction in pH; 2) enzymolysis, proteolysis and hydrolysis processes; 3) metabolization and reduction in molecular weight; 4) increased solubility; and 5) separation of bioactive compounds bound in fibers and other cellular components of plant matrices (Annunziata et al., 2020; Zhao et al., 2021).

Dairy products and fermented foods have evidence of their consumption during the Mesolithic-Neolithic transition period in Europe, as the nutrient profile of milk makes it a complete food for all ages (Bickle, 2018; Ebringer et al., 2008). Furthermore, milk is rich in macronutrients, in addition to compounds responsible for supporting the immune system (vitamins, minerals and peptides) (Ahvanooei et al., 2022). These compounds (proteins and peptides, lipids, and oligosaccharides) help protect against intestinal pathogens, have antioxidant effects, and prevent various diseases such as obesity, osteoporosis, cardiovascular problems, and intestinal cancer (Ebringer et al., 2008). These fermented foods originating from dairy milk may not fit completely into the human diet due to health limitations or personal beliefs such as lactose intolerance, environmental impact, or the intention of not consuming animal-derived foods.

To improve overall knowledge about PBDA and fermentation effects, this manuscript complements many review articles that are already published. Mäkinen et al. (2016) and Gupta and Abu-Ghannam (2012) despite certain information being outdated with recent advances, they have relevant data regarding mathematical models and nutritional value of fermented PBDA, but lack on discussion about consumer acceptance and relation between metabolites produced by LAB, such as a deeper understanding into texture properties. On the other hand, recently published review articles focus on technological properties or specific compounds such as polysaccharides, but with little discussion on sensory and nutritional aspects and effects of fermentation (Erem & Kilic-Akyilmaz, 2024; Liu et al., 2024; Tanguy et al., 2019a, 2019b).

The review addresses and updates current research, focusing on the rheological and sensorial properties of fermented plant-based drinks, whether to obtain properties similar to fermented milk, but also to reduce off-flavors present in plant-based drinks. The health effects of ingesting metabolites from the fermentation process and the relationship between fermentation and the bioavailability and biodigestibility of nutrients are also presented. This review is structured as follows: 1) details the production of fermented dairy products, 2) discussion about consumer perception and challenges in PBDA products, 3) fermentation

process of PBDA and comparison with dairy-based beverages; 4) rheological properties of fermented PBDA; and 5) aspects related to the health of PBDA and fermentation of these products.

## 2. Manufacturing of fermented dairy products

As fermented foods are simply defined as any food processed through microbial activity, almost all foods can be fermented, depending on their fermentation conditions, including vegetables, meat, dairy products, cereals, and many others (Diez-Ozaeta & Astiazaran, 2022). The fermented food groups and examples are shown in Fig. 1. Some examples of microorganisms responsible for fermentation in foods are *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Saccharomyces cerevisiae*, *Acetobacter*, *Propionibacterium*, and some species of *Enterococcus* and *Leuconostoc* (Leeuwendaal et al., 2022).

For dairy products, Codex Alimentarius (2011) defines fermented milk as milk produced “by the action of suitable microorganisms and resulting in reduction of pH with or without coagulation (iso-electric precipitation). These starter microorganisms shall be viable, active and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation the requirement for viable microorganisms does not apply”. Also, according to Codex Alimentarius, the limit defined by the sum of starter cultures for fermented milk, yogurt and kefir must be at least  $10^7$  CFU/g.

The selection of probiotics depends on several criteria, such as surviving the conditions of the intestinal tract, health benefits, being safe for consumption and viable during production, distribution, and storage (Ibrahim et al., 2023). Furthermore, probiotics must also be able to perform the fermentation process in the desired foods. For dairy products, they can be divided into probiotic lactic acid bacteria (LAB) or non-LAB probiotics, both capable of fermenting dairy products (Gao et al., 2021).

Although there are other fermented drinks that use dairy products, the two most common are yogurt and kefir. For yogurts, the fermentation process is observed through the acidification of milk and the consequent formation of curds, attributed to the consumption of lactose and the production of lactic acid (Sharma et al., 2023). During fermentation, acetic acid, ethanol, exopolysaccharides, and compounds responsible for the flavor and aroma of yogurt are also produced, such as aldehydes, esters, furans, and aromatic hydrocarbons. Fermentation can

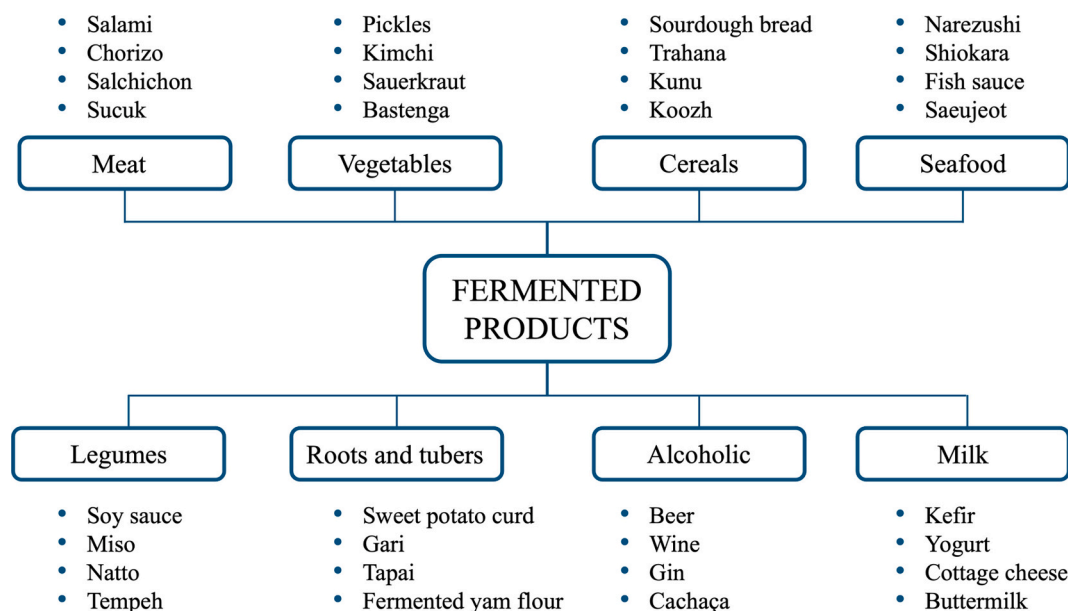


Fig. 1. Types of fermented foods.

occur through two paths: homofermentation and heterofermentation, which differ by the type of microorganism used, the type of substrate and environmental conditions, and each fermentation pathway produces different compounds (Chen et al., 2017).

Generally, yogurts are produced through fermentation with 2–3 % of the starter cultures, incubation at 40–45 °C until pH 4.6. However, the temperature and fermentation time can vary depending on the rheological and sensory properties of the final product (Meybodi et al., 2020). Increasing the temperature to 42–45 °C, for example, can result in an increase in the content of amino acids and monounsaturated and polyunsaturated fatty acids, in addition to having better digestibility (Yang et al., 2021).

Typically, the most common LAB used in the production of yogurts are *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, due to a synergistic action of these bacteria in fermentation, both strains being able to survive independently in yogurt (Ayivi & Ibrahim, 2022). The interaction between these two bacteria occurs due to the production and consumption of many compounds responsible for the metabolism of each LAB, such as peptides, free amino acids, organic acids, and fatty acids, responsible for the formation of the flavor and texture of the final product (Dan et al., 2023). The importance of using adequate microorganisms relies on the ability to avoid changes in physicochemical and nutritional properties that can affect consumer acceptability through its acidity, texture, and nutrient content (Gao et al., 2021).

On the other hand, traditional kefir production adds 'kefir grains' to pasteurized milk. Small grains are formed by aggregation of lactobacilli and yeast (e.g., *L. kefirifaciens* and *Kazachstania turicensis*) with other microorganisms and milk components (proteins and polysaccharides) to form large granules. The mixture between polysaccharides and the biofilm forms a complex network defined by kefir grains (Nejati et al., 2020).

Since kefir grains have little industrial interest due to low growth, high cost, and poor reproducibility, starter cultures are also used to produce kefir, consisting of a mixture of LAB, yeast, and acetic acid bacteria. Fermentation occurs at 25 °C and commonly for 20–24 h until pH 4.6 (Guzel-Seydim et al., 2021; Nejati et al., 2020; Yilmaz et al., 2022). The LAB population in dairy kefir depends on several factors, such as fermentation time, type of milk (cow, goat, sheep, camel, or buffalo), and production method (artisanal or industrial), but the most common species found in kefir are *Lentilactobacillus kefir*, *Lactocaseibacillus casei*, *Lactocaseibacillus rhamnosus*, *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, and *Lc. lactis* (Yilmaz et al., 2022). To produce artisanal kefir, there is a drawback that some parameters of the fermentation process cannot be fully controlled, such as pH, microorganism growth rate, substrate consumption, and metabolite production (AcOH, EtOH, lactic acid, and other flavor/aroma compounds) (Lynch et al., 2021).

### 3. Fermented plant-based dairy alternatives (PBDA)

#### 3.1. Consumer perception towards PBDA

The consumer perception of new products can depend on health benefits, familiar flavor, nutritional composition, sensory characteristics and environmental aspects. The demand for PBDA has been growing due to the desire for a healthier life, lactose intolerance issues, less environmental impact, and being against animal cruelty, thus avoiding the consumption of animal-derived products (Aydar et al., 2020). The production of PBDA as new alternatives to dairy milk has been growing, with a projected significant increase in sales in the coming years (Plamada et al., 2023).

In 2022, Europe produced approximately 160 million tons of raw milk, an increase of 10 million tons compared to 2014, in which Germany, France, and the Netherlands represented almost 50 % of all dairy milk produced in Europe. Despite the increase in milk production, only 8.5 % of whole milk was destined for drinking, while cheese and butter production represent more than 70 % of all whole milk destinations in

Europe (Eurostat, 2022). Regarding the United States, in a survey conducted with almost 1000 households, the exclusive use of PBDA exceeds 20 %, in addition to more than 15 % of the population who are flexible regarding the consumption of both plant-based and dairy milk. Despite the reduction in milk consumption in recent decades, total consumption of dairy products has increased, particularly cheese, butter, and yogurt (Wolf et al., 2020).

The perception of food and its nutrients by consumers is correlated with diet and lifestyle. Vegans and vegetarians, for example, have greater concern and knowledge about the nutrient content of foods. On the other hand, people with flexible diets care more about quality of life and health than animal welfare and sustainability (Noguerol et al., 2021). Despite these factors, the desire for plant-based products often comes from the need to obtain a beverage with properties similar to those of dairy milk. For instance, drinks based on oat, rice and coconut had better sensory acceptance as they are characterized by 'white appearance' and 'milky flavor' (Cardello et al., 2022). The texture of PBDA can also cause an undesirable mouthfeel for the consumer and seeking solutions that influence viscosity and reduce the sensation of 'chalkiness' and 'grittiness' (astringent mouthfeel sensation) can be an interesting alternative, in addition to reducing the undesirable 'beany' flavor of some plant-based beverages (Harper et al., 2022; Moss et al., 2023).

Generally, the consumer also prefers PBDA as an alternative that is free of lactose, cholesterol, and animal protein. However, research is also required to achieve an energetic and nutritional balance, to compensate for the lack of benefits compared with milk products (Bocker & Silva, 2022). Except for soy milk, other common alternatives, such as rice, coconut, oat, and wheat milk offer a low protein content, despite having other properties such as low cholesterol and high fiber content (Cardello et al., 2022). According to the Dietary Guidelines for Americans (U.S. Department of Agriculture (USDA), 2020), only soy beverages have the key nutrients to mitigate the lack of dairy milk, but are only compared when fortified with calcium, vitamins A and D. Other vegetable drinks, although they contain some of the nutrients present in dairy milk, are not sufficient to meet the recommendation of the dairy group.

Although macronutrients (mainly proteins) and energy content are the main concerns when looking for plant-based alternatives to milk, there are also other obstacles such as mineral and vitamin content in vegetable beverages (Silva & Smetana, 2022). The fortification in micronutrients (vitamin B<sub>12</sub>, vitamin D, calcium and  $\omega$ -3 fatty acids) are essential in plant-based drinks as they represent a deficiency in almost all vegetable substitutes for dairy milk (McClements, 2020). Finally, plant-based beverages also have antinutritional compounds, such as phytic acid, oxalate and phytate, which can reduce the bioavailability of nutrients (Aydar et al., 2020).

Although consumers understand that PBDA are considered healthier and more sustainable, the market price remains a barrier as dairy milk is approximately 50 % cheaper than PBDA, with the cost associated with processing plant-based ingredients and nutrient supplementation (Lee et al., 2024; Siegrist et al., 2024). Fortification of plant-based beverages can ensure greater consumer attraction through their nutrition, but higher costs also affect the affordability of the product and can discourage purchase (Siegrist et al., 2024).

#### 3.2. Fermentation and composition of PBDA

In relation to fermented plant-based products, PBDA represented more than 25 % of all sales in the global market in 2022 (Boukid et al., 2023). Fig. 2 presents the fermentation process of PBDA and comparison with the traditional process in dairy milk.

Unlike milk, plant-based dairy alternatives require pretreatment before pasteurization and cooling. Depending on the vegetable product, such as cereals, grains, or oilseeds, the peeling, soaking in water, and blanching/steam cooking processes must be carried out. Wet milling

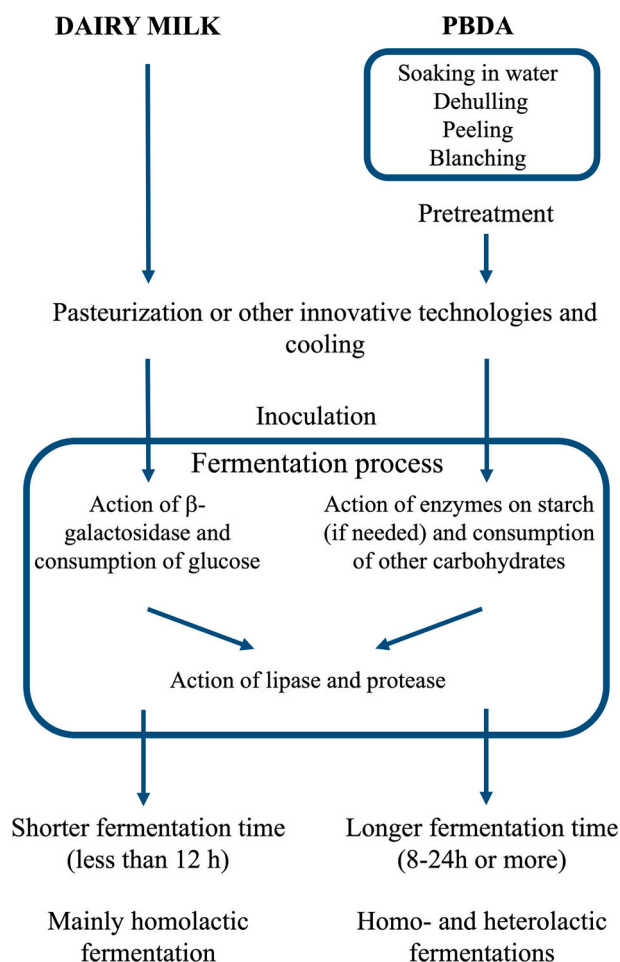


Fig. 2. Production of fermented dairy milk and PBDA.

also occurs until the desired concentration of vegetable beverage is obtained (Aydar et al., 2020). Although the dairy milk process involves pasteurization, this can undesirably modify several properties of PBDA. Therefore, the use of other technologies such as ultrasound, high pressure processing, pulse electric field, UV radiation and other methods can be alternatives to avoid the negative effects of pasteurization (Bocker & Silva, 2022).

The type of fermentation process applied strictly depends on the nutrients present in the vegetable beverage. Although nuts and seeds represent the class with the highest fat content, some legumes (soy and peanuts) are also rich sources of fat. Finally, the main sources of proteins and carbohydrates are legumes (soy, lupine, and chickpea) and grains (rice, barley, and maize), respectively (Pua et al., 2022). Table 1 shows the different plant-based beverages consumed under different fermentation conditions, and results regarding viable LAB, effects in nutrient content, and sensory characteristics.

For lactic acid bacteria, homolactic and heterolactic fermentations can occur and are divided into obligately or facultatively species, in addition to environmental factors that affect their priority in the fermentation pathway (Prückler et al., 2015). Although homolactic fermentation has lactic acid as its main product, heterolactic fermentation results in the production of acetic acid, ethanol, carbon dioxide, besides lactic acid. Due to the compounds produced, in dairy milk the homolactic fermentation prevails, whereas in plant-based products heterolactic fermentation occurs more frequently depending on the microorganisms used (Pua et al., 2022). Sugar fermentation is also more effective in dairy products than in plant-based beverages, as LAB bacteria can use lactose in dairy milk more efficiently than other sugars in vegetable sources, even when supplemented (Harper et al., 2022). The

fermentation process can increase the antioxidant activity of plant-based beverages through the production and metabolization of antioxidant compounds, the release of bioactive compounds bound to cell walls, and the conversion of phytochemicals into forms with enhanced antioxidant activity (Saritas et al., 2024).

Traditional fermentation in dairy products occurs through proteolysis, lipolysis, and carbohydrate metabolism, and although most LAB are isolated from dairy milk, there is evidence of their successful use as probiotic carriers in vegetable beverages. Carbohydrate metabolism in plant-based products occurs through the consumption of sugar and the production of lactic acid (homofermentation), in addition to the production of exopolysaccharides, which are also capable of producing in plant-based beverages through LAB (Harper et al., 2022). Fermentation can also stimulate enzymes (amylase and maltase) to break down starch in some plant matrices, transforming them into simple sugars, facilitating glucose consumption and favoring fermentation (Alrosan et al., 2023).

Finally, in the lipolysis process, fatty acids are released and, for protein, since there is no casein, this occurs at a lower concentration since vegetable proteins are less accessible to protease due to the larger particle size and multiple interconnected proteins (Harper et al., 2022). The fermentation process also generates an increase in the nutritional value of proteins through changes in the secondary structure, in addition to improving related properties such as water solubility, water holding capacity, emulsion stability, foaming capacity and stability (Alrosan et al., 2023).

Most plant-based products are fermented through *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Enterococcus* bacteria (Boukid et al., 2023). The choice of microorganisms is based on how the technological, functional, and sensorial properties of the product will be affected. LAB, for example, produces organic acids in metabolism, capable of increasing the shelf life of fermented products, in addition to flavor compounds and exopolysaccharides that improve overall sensory quality (Erem & Kilic-Akyilmaz, 2024). Mixed culture can exhibit a 100-fold increase compared to monoculture fermentation and can also present a high acidification rate (Tangyu et al., 2019a, 2019b). The mixture of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* can ferment several beverages prepared from vegetable matrices (almond, rice, oats, soybean), despite not having stable LAB viability during 28 days of storage, when compared to dairy milk (Deziderio et al., 2023).

The combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* is also effective in plant-based fermentation due to the mutualistic interaction between the substrates consumed and the compounds produced between both bacteria (Harper et al., 2022). *Lactobacillus helveticus* is capable of metabolizing galactose, in addition to rapid acidification and strong proteolytic activity, fermenting fruits, vegetables, beans, cereals, and nuts (Zhao et al., 2024).

The fermentation time may also depend on the material to be used as a substrate and the microorganism that will be responsible for the process. For *S. thermophilus* and *L. bulgaricus* subsp. *delbrueckii*, optimal conditions can differ from 37 to 43 °C and between 6 and 10 h to obtain rapid acidification, while for other microorganisms such as *Lactobacillus helveticus*, they can ferment up to 24 h or days (Horlacher et al., 2023; Zhao et al., 2024). However, a fermentation process longer than 24 h can produce nutritionally and sensorially undesirable compounds (Boukid et al., 2023).

### 3.3. Rheological and sensory properties of fermented PBDA

Although PBDA consumers are concerned about environmental impacts, animal welfare, and better health conditions, organoleptic properties have a significant impact on consumer attraction towards new food products, especially when they aim to replace food from animal sources (Pua et al., 2022). Plant-based products have three major challenges to consider: a light taste, insufficient nutritional content, and poor stability. As plant-based beverages are defined as colloids (small



**Table 1**  
Plant-based beverages and fermentation properties.

| Plant-based beverages | Microorganism  | Supplementation  | Fermentation Conditions | Findings  | References                   |
|-----------------------|--|--|-------------------------|---|------------------------------|
| Almond                | <i>S. thermophilus</i> , <i>L. acidophilus</i> , and <i>Bifidobacterium BB12</i> | Sucrose  | 37 °C for 12 h          | Viable LAB after 1-month storage with and without sucrose supplementation   | Deziderio et al. (2023)      |
| Oats                  | <i>S. thermophilus</i> and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>        | –  | 40 °C for 24 h          | No increased viscosity<br>Increase in total phenolic content  | Lopusiewicz (2024)           |
| Soy                   | <i>S. thermophilus</i> and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>        | –  | 40 °C for 24 h          | Increased viscosity<br>Increased total phenolic content<br>Supplementation of skim milk improves the fermentation process | Lopusiewicz (2024)           |
| Broken rice           | <i>L. bulgaricus</i> , <i>L. casei</i> or <i>L. acidophilus</i>                  | Skim milk powder   | 37 °C for 8 h           | <i>L. bulgaricus</i> presented the best pH reduction and highest viability count  | Hozzein et al. (2023)        |
| Brown rice            | <i>Lactobacillus pentosus</i> 9D3  | Yeast extract, isolated soy protein and pyridoxine hydrochloride | 30 °C for 16 h          | Stable for 30 days during refrigeration<br>Good acceptability when flavored<br>Higher phenolic content                    | Kittibunchakul et al. (2021) |
| Quinoa                | <i>S. thermophilus</i>   | –  | 37 °C for 6–8 h         | Viable LAB after 21-days storage<br>Increased total phenolic content<br>Good overall acceptability                        | Soumya et al. (2024)         |
| Cashew                | <i>S. thermophilus</i>   | –  | 37 °C for 6–8 h         | Viable LAB after 21-days storage<br>Increased total phenolic content<br>Bad acceptability                                 | Soumya et al. (2024)         |
| Cowpea-Peanut         | <i>Lactocaseibacillus rhamnosus</i> Yoba   | –  | 37 °C for 24 h          | Increase in antioxidant activity<br>Reduction of antinutritive compounds<br>Good acceptability                            | Chawafambira et al. (2022)   |
| Blue lupin seeds      | Kefir grains (LAB and yeast)   | –  | 25 °C for 24 h          | Viable after 21-days storage<br>Excellent radicals scavenging activities<br>Less viscosity and firmness after storage     | Lopusiewicz et al. (2024)    |
| Chickpea              | <i>L. rhamnosus</i>  | Sucrose  | 37 °C for 24 h          | Fermentation reduced off-flavors<br>Papain treatment improved the plant-beverage flavor                                   | Zhang et al. (2020)          |
| Coconut               | <i>Lactobacillus plantarum</i>   | –  | 33 °C for 15 h          | Stable shelf-life and viable LAB after 21-days storage<br>Fermentation enhanced antimicrobial and antioxidant properties  | Qadi et al. (2023)           |

particles dispersed in the medium), phase separation and other physical stability problems can occur during shelf life, and the use of stabilizers is recommended in soy beverages, for example (Patra et al., 2021).

To guarantee a strong flavor and the presence of sufficient nutrients (compared to milk derived beverages), other components are also added to fortify these aspects (Liu et al., 2023). On the other hand, to maintain 'clean label' products, some technologies are developed to avoid the use of additives in plant-based drinks, such as thermal, mechanical, chemical and biological treatments, ensuring texture stability of food and removing off-flavors, adapting products to achieve consumer acceptability (Pua et al., 2022). Some treatments such as ultrasonication, high hydrostatic pressure, and pulse electric field can improve texture and stability properties, while thermal processes (high and ultra-high pasteurization) can affect sensorial aspects, in addition to inactivating enzymes responsible for producing antinutritional and off-flavor compounds (Liu et al., 2023; Pua et al., 2022).

The appearance and aroma of the vegetable beverage are the first attributes to be observed by the consumer, as it must have a creamy appearance similar to dairy milk and with the absence of compounds responsible for lipid oxidation and protein hydrolysis (Grossmann et al., 2021). Texture and flavor are also very important properties, which must have a certain viscosity and little off-flavor. The macronutrients of plant products are also directly related to their sensory properties. Specifically, proteins are correlated with physicochemical parameters and flavor precursors and fats affect mouthfeel and flavor-carrying capacity (Pua et al., 2022).

To obtain desirable flavor and functionality properties in fermented soymilk, the authors suggested selecting *Lactobacillus plantarum* NCU001563 and *Streptococcus thermophilus* NCU074001, revealing the best fermentation temperature of 39 °C, which had a 10-fold increase in viable bacteria, while 24 h of fermentation presented the highest viable

bacteria in the fermented beverage. The authors also correlated the higher abundance of flavor compounds (2-butanone, acetoin, and acetic acids) with a higher sensory score, presented after 24 h of fermentation (Madjirebaye et al., 2022). The substrate used for fermentation can also affect the fermentation properties, such as the use of okara flour in soy milk, which presented a continuous decrease in pH for 48 h, correlated with the growth of *Lactobacillus acidophilus* LA3 and *Lactobacillus plantarum* BG 122 during fermentation (Filho et al., 2016).

For rice beverages, it also depends on the rice type, as the authors describe that Basmati rice has higher viscosity compared to Krasnodar, red and black rice. When considering fermentation and sensory properties, *B. bifidum* and *B. longum* B379M showed the best results (Shiriaev et al., 2024). For fermented oat drinks, the most notable acidification occurred in the first 6 h but continued until 24 h of fermentation. From all studied mixes between different starter cultures (*S. thermophilus*, *L. bulgaricus*, *L. lactis*, *B. lactis*, *L. acidophilus*, and *L. paracasei*), although the manufacturer did not provide the proportion information, *S. thermophilus* was the dominant species in all mixes and fermentation times. Regarding sensory properties, all preferred volatile compounds (such as diacetyl and acetoin) increased during fermentation, as well as lactic acid content, attributed to sour odor and taste (Kütt et al., 2023).

The fermentation process can change all the properties of plant-based beverages through the consumption and production of certain compounds by microorganisms (Lopusiewicz, 2024). In terms of carbohydrate metabolism, the production of flavor compounds occurs, such as lactic acid, diacetyl, acetaldehyde, and exopolysaccharides. Exopolysaccharides are also responsible for increasing the viscosity of the product during fermentation. The released fatty acids (methyl ketones, alcohol, lactones, and esters) are also directly related to the flavor of fermented PBDA (Harper et al., 2022).

The rheological properties of plant-based yogurts can be affected by

the low protein content in most PBDA, impacting the coagulation properties and the consequent need for food thickeners (Harper et al., 2022). For instance, in oat milk, an increase in viscosity may not occur during fermentation due to the low content of  $\beta$ -glucan and gelated starch (Łopusiewicz, 2024). Reducing pH during fermentation also affects the solubility and functionality of plant proteins, which can make beverages more acceptable to consumers (Pua et al., 2022). In soy-based beverages, gel formation may occur due to the electric point of soy protein (soy glycinin) being similar to casein in dairy milk (Łopusiewicz, 2024).

In soy beverages, there is also the ‘beany’ flavor, developed by methanol, hexanal, pentanal, and ethanol, which is undesirable compared to dairy milk (Almghawesh et al., 2022; Harper et al., 2022). This off-flavor can be removed or reduced through fermentation by some species of *Bacillus*, *Lactobacillus*, and edible fungi, reducing bitterness (Almghawesh et al., 2022; Boukid et al., 2023). On the other hand, in cereals, fermentation by some microorganisms can produce a ‘salty’ and ‘fishy’ flavor, undesirable for fermented beverages (Liu et al., 2023).

Off-flavors can be divided into volatile (aldehydes, alcohols, ketones, furans) or non-volatile (phenolics, saponins, peptides and other nitrogenous compounds), in which lipids are the biggest precursors of off-flavors in plant-based products. Hexanal and other aldehydes, responsible for the “green odor”, can be reduced by microbial degradation during fermentation (Leonard et al., 2022). The mechanisms of action for off-flavors during fermentation may occur through biotransformation, enzymatic activity or consumption during the metabolic pathway of microorganisms. The reduction of off-flavors depends on the compound present in the plant matrix, such as glycosidic hydrolases capable of transforming saponins, or reductases/esterases for phenolic compounds. Aldehydes are among the main contributors to off-flavors in plant-based products, and during fermentation aldehydes are reduced in the metabolic pathway to produce additional ATP or act as a hydrogen acceptor for NADH regeneration (Molina et al., 2024). *Lactobacillus* fermentation can reduce aldehydes and ketones in plant-based products by up to 40 % to 60 %, respectively (Shi et al., 2021). With advances in lipidomics and genetics, a greater contribution to knowledge about off-flavors is expected (Leonard et al., 2022).

The plant matrix is also responsible for the microorganism’s ability to metabolize antinutrients, due to the bond between the antinutrient and the raw material (e.g., complex sugars such as raffinose and stachyose, or the formation of insoluble complexes between macronutrients and phenolic compounds). Therefore, the mechanism of action for off-flavor reduction during fermentation also depends on substrate specificity (Molina et al., 2024). Although plant matrices contain several compounds that contribute to off-flavors, in general, human perception of odor is attributed independently. The authors also report the importance of future work to evaluate the interaction of individual compounds (Ma et al., 2021).

#### 4. Fermented PBDA and its role in human health

Dairy milk is still considered an essential beverage to combat malnutrition, especially in children. In addition to macronutrients, milk also contains several bioactive compounds, enzymes, minerals and vitamins, and the presence of all these nutrients in milk can prevent osteoporosis, cancer, dental disease, contribute to the immune system, and more health benefits (Clark et al., 2022).

Except for methionine, soybean contains all essential amino acids and has anticancer and immunomodulatory properties, in addition to regulating blood pressure. Although protein content can be compared to dairy milk, soy milk is deficient in other nutrients such as water-soluble vitamins (B<sub>2</sub> and B<sub>12</sub>) and low isoflavone activity (Zhu et al., 2020). The health effect depends on the vegetable beverage consumed, nutritionally they do not contain saturated fats, are low in calories, and most of them have added nutrients such as calcium and vitamin B<sub>12</sub> and vitamin D to a

lesser extent (Craig & Fresán, 2021). Supplementation with other plant sources is also researched with the aim not only of increasing protein or micronutrient content, but also of increasing consumer acceptability (Oduro et al., 2021).

When the fermentation of PBDA is included, the list of health benefits becomes longer. Fermentation in vegetables can increase the phenolic content and antioxidant activity naturally present in these beverages (Liu et al., 2023). Consumption of active strains in fermented foods can help prevent diarrhea, gastroenteritis, lactose intolerance, and other gastrointestinal diseases. Fermentation can also increase vitamin content, which is responsible for preventing diseases such as rickets, chondromalacia, and osteoporosis (Liu et al., 2023).

Antinutrients are also compounds naturally present in plant-based products, which reduce the bioavailability of minerals and protein digestibility (Nath et al., 2022). The fermentation process can reduce the content of antinutritional compounds in plant matrices, such as tannins, phytic acid, and trypsin inhibitors, in addition to producing bacteriocins, increasing food safety (Horlacher et al., 2023).

The fermentation process also affects the macronutrients properties. In plant proteins, they can increase their bioavailability and produce antidiabetic, antihypertensive, and antithrombotic effects (Horlacher et al., 2023). Although the digestibility of vegetable proteins is lower than dairy proteins, it can be improved through the action of pectinases released by fermentation (Boukid et al., 2023).

Increasing protein digestibility through fermentation can increase by up to 10 % in various plant matrices such as rice, peas, and lentils (Alosan et al., 2023). The *in vitro* protein digestibility (IVPD) increase is dependent on the microorganism used, substrate and fermentation time. In many plant-based matrices (soybean, corn, pumpkin seeds, locust bean, and pea), fermentation with different cultures increase the IVPD between 10 and 240 %, with different fermentation times (5 h to 5 days). The fermentation process can make proteins more accessible, attributed due to heat treatment, lower pH, and hydrolysis of protein aggregates, enhancing their solubility in water, which is a challenge in plant-based product development (Shaghaghian et al., 2022). Furthermore, fermentation reduces phytic acid and saponins in plant-based formulations, which are also responsible for the lower digestibility (Xie et al., 2023).

The overall amino acid profile is also modified, being synthesized or released from peptides by microorganisms (Hidalgo-Fuentes et al., 2024). However, other authors also highlight the importance of evaluating each microorganism involved in the fermentation process. Strains of *L. paracasei* can partially consume L-glutamate/glutamine and L-alanine, while L-phenylalanine and L-leucine content has increased on fermented chickpea milk. Using *in-silico* screening of 31 strains of different genera, including *Lactobacillus* and *Bifidobacterium*, showed that the L-lysine content increase or decrease is not dependent on *Lactobacillus* strains, but all *Bifidobacterium* and *Corynebacterium* isolates decreased the L-lysine levels (Tangyu et al., 2021).

In carbohydrates, fermentation can improve digestion, provide anti-inflammatory effects, and reduce blood glucose spikes (Horlacher et al., 2023). In some vegetable beverages, such as soy, the presence of oligosaccharides (raffinose and stachyose) can cause intestinal discomfort and flatulence, reducing consumer acceptance (Almghawesh et al., 2022). When fermented, some LAB have the enzyme  $\alpha$ -galactosidase, capable of reducing the unwanted effects caused by these molecules (Harper et al., 2022).

Organic acids produced by fermentation not only extend the shelf life of foods, but also benefit the consumer’s health, such as hormone regulation, anti-inflammatory properties, promotion of calcium absorption, and consequent prevention of osteoporosis (Shi et al., 2022). The production of organic acids and fatty acids during fermentation can also enhance the absorption and solubility of some vitamins and minerals in plant-based beverages (Boukid et al., 2023).

Certain oilseeds are also used for beverage production and can cause allergies due to some proteins, such as peanuts (Ara h 1 ~ Ara h 18). LAB

fermentation can partially mitigate the allergenic properties of peanuts, suggesting a change in the primary structure of proteins by breaking or exposing peptide bonds through the action of enzymes and organic acids (Yang et al., 2024).

Many compounds from plant matrices are also known to reduce the bioavailability of minerals, such as oxalates, phytates, and phenolic compounds (Molina et al., 2024). Phytic acid is capable of chelate minerals ( $\text{Zn}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ) and form phytates, making them insoluble and reducing their bioavailability in the gastrointestinal tract (Fang et al., 2023; Molina et al., 2024). With the fermentation process and consequent reduction in pH, phytic acid is degraded through the activation of endogenous enzymes in grains. After 24 h of fermentation, the phytic acid content can be decreased between 40 % and 90 %, either for endogenous enzymes or for microbial phytase present in LAB (Fang et al., 2023).

Phenolic compounds are also known for their chelation properties, but they are dependent on each mineral and phytochemical studied (Zhang et al., 2020). *In vitro* assays indicate that certain plant matrices are effective in precipitating iron, in which fractional iron absorption studies demonstrate that patients who were supplemented with polyphenol (black tea powder, cocoa powder, and grape juice extract) showed lower iron absorption compared to placebo (Buerkli et al., 2022). The presence of polymeric polyphenols has also been reported to affect Ca absorption in rodents, but without association with bone metabolism (Zhang et al., 2020).

Therefore, the health effects of fermented PBDA are related to the nutritional composition of each vegetable beverage, but also to the compounds produced and consumed during the fermentation process, providing the population with better food safety and essential nutrients for a balanced and healthy diet covering consumers demands.

## 5. Conclusions

Plant-based beverages have gained significant popularity due to their nutritional properties, absence of lactose, animal welfare, and environmental sustainability. Although no plant-based beverage offers a nutritional profile that fully matches dairy milk, fortifying these beverages with nutrients such as vitamins, proteins, and minerals can help address this gap. Nonetheless, it is recommended to include both beverages to ensure a complete nutritional source for the human diet.

PBDA also represents a way to obtain nutrients from plant sources such as soy, rice, peanuts, almonds, and oats, but innovation challenges revolve around ensuring similar properties to dairy milk, such as texture, aroma, and subtle flavor. A strategic approach to address the limitation of plant-based beverages is through the fermentation process. Fermentation, in addition to reducing negative sensory attributes, also produces several compounds with proven effects on human health. The probiotic action of live cultures also affects intestinal health, nutrient absorption, and improves the immune system.

As each plant-based product has different nutritional and sensory properties and each microorganism can metabolize specific compounds, one of the challenges is to find a combination of specific strains capable of providing sensory properties that are considered acceptable to the consumer. In this review, the viability of different fermentation cultures of many plant-based beverages has already been extensively discussed, but studies are still needed to increase overall acceptability, such as ensuring product stability during shelf life.

Consumer acceptance may be different for people who are already vegetarian or vegan than for people who are in the process of changing or just want to consume plant-based products. Still, an overall drawback is the off-flavor present in many vegetable beverages, and although the fermentation process can reduce undesirable sensory aspects, there is a research gap in the search for individual interaction of off-flavors in plant-based foods and finding real solutions for their reduction or inhibition. Although fermented foods are available on all markets, with increased knowledge of the health benefits and safety of consuming

probiotic microorganisms, the consumption of these products continues to increase.

Therefore, the combination of plant beverages and fermentation can attract the attention of a wide range of consumers through the advantages of both technologies. To support the nutrition and wellness of the population, food research and development play a fundamental role in creating products that fulfilled consumers demands and ensure food safety and quality.

## Author contribution

Sandrina A. Heleno, Patricia Morales, Marcio Carochi: conceptualization; Jonata M. Ueda: writing – original draft; Sandrina A. Heleno, Marcio Carochi, Patricia Morales: Supervision; Sandrina A. Heleno, Márcio Carochi; Patricia Morales, Virginia Fernández-Ruiz: writing – review & editing. All authors have read and agreed to the published version of the manuscript.

## CRedit authorship contribution statement

**Jonata M. Ueda:** Writing – original draft. **Virginia Fernández-Ruiz:** Writing – review & editing. **Patricia Morales:** Writing – review & editing, Supervision, Conceptualization. **Anabela Ferreira:** Writing – review & editing. **Sandrina A. Heleno:** Writing – review & editing, Supervision, Conceptualization. **Márcio Carochi:** Writing – review & editing, Supervision, Conceptualization.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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