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Review Article

Innovative thermal and non-thermal treatments for microorganism inactivation in plant milk

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ABSTRACT

The growing popularity of plant-based diets can be connected to the quick global expansion of plant-based dairy products. This review examines the classification of plant milk's fundamental components and outlines the principal processing stages involved in their manufacture. Particular attention is given to the influence of processing operations on product quality, stability, and safety. Microbial inactivation, a critical step in plant milk processing, is discussed alongside the conventional and emerging technologies used for this purpose. Current approaches include thermal methods and innovative technologies such as ohmic heating, microwave processing, high-pressure processing, ultrasonication, pulsed electric field treatment, and ultraviolet irradiation. The available literature indicates that these technologies can effectively improve microbiological safety while upholding sensory and nutritional qualities. Consumption of high-nutrient, plant-derived milk is anticipated to rise as new technologies develop globally, leading to the emergence of several plant-milk product categories.

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INTRODUCTION

A variety of food products created to meet evolving nutritional standards has been fueled by customers' desire for more nutritious food options. In parallel with these developments, functional and specialty beverages have become an important topic of interest for the food business as well as scholars. Beyond their traditional role in hydration, modern beverages are increasingly expected to provide additional nutritional and physiological benefits. Consequently, manufacturers have focused on developing products tailored to specific consumer needs and lifestyle preferences. Within this context, plant-based milk alternatives have attracted growing attention, particularly among individuals affected by cow's milk allergy, lactose intolerance, hypercholesterolemia, or concerns related to caloric intake [1].

Milk is a biological secretion produced by female mammals to nourish their offspring and is recognized as an important source of nutrients in the human diet [2]. Owing to its balanced composition of proteins, lipids,

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carbohydrates, vitamins, and minerals, milk is frequently regarded as a nutritionally valuable food [3]. Cow's milk has been consumed for centuries in many regions of the world and continues to play a significant role in daily nutrition [4]. However, dairy consumption may not be appropriate for all individuals. Conditions like cow's milk protein sensitivity and lactose intolerance may limit the intake of dairy and milk products. It has been estimated that nearly 75% of the global population experiences some degree of lactose intolerance [3]. This condition arises from inadequate small intestine lactase activity, reducing the body's capacity to digest lactose efficiently. As a result, in sensitive individuals, consuming milk and dairy products may cause gastrointestinal symptoms, including diarrhea, bloating, and stomach pain.

People prefer and consume plant milk alternatives for various health effects and lactose intolerance. For example, human cholesterol levels rise when animal products are consumed excessively. Cardiovascular illnesses are more likely to occur in this setting. Plant milk from grains, legumes, and seeds is preferred because it contains dietary fibre, vitamins, minerals, and antioxidants. These foods also fall into the category of both functional and nutrient-fortified foods [5]. Apart from the benefit of a plant-based diet in reducing the likelihood of cardiovascular disease, Pistollato et al. [6] reported that greater consumption of plant-based foods, including almonds and soybeans, may contribute to reducing the risk of neurodegenerative diseases such as Alzheimer's disease. Additionally, Sarni and Baroni [7] reported that the daily consumption of two glasses of cow's milk may increase D-galactose levels above 100 mg/kg, a condition suggested to be associated with the development of Parkinson's disease.

Plant milk alternatives have attracted attention because of their potential health benefits, particularly their antioxidant compounds and favorable fatty acid profiles, which may contribute to a lower risk of diabetes, cancer, cardiovascular diseases, and atherosclerosis [8]. However, plant-based milk products also present certain nutritional limitations, including lower protein content and reduced bioavailability of some vitamins and minerals. These limitations are largely associated with antinutritional factors and polyphenolic compounds that may interfere with nutrient absorption. Nevertheless, processing strategies such as nutrient fortification and fermentation have been proposed to improve the nutritional quality and bioavailability of plant milk products [9, 10].

Interest in plant-based dietary patterns emphasizing grains, legumes, seeds, nuts, fruits, and vegetables has increased substantially due to growing health awareness, environmental concerns, and animal welfare considerations [11, 12]. These developments have contributed to the wider adoption of dietary approaches such as lacto-vegetarianism, ovo-vegetarianism, and vegetarianism. A vegan follows a dietary and lifestyle pattern that excludes animal-derived products, including leather, wool, silk, and other animal-based materials [13]. As the number of individuals adopting vegan lifestyles continues to increase, the importance of vegan product categories within the food industry has also expanded [14].

Plant milk is frequently used as a beverage and an ingredient in various recipes. Consequently, significant interest has emerged in developing plant-based products as alternatives to conventional milk. In addition to direct consumption, these products are used in several applications, including nutritional supplements, infant foods, cream-based products, and fruit-based formulations. Many nations still lack unified standards and regulatory frameworks controlling the labeling. Some plant-based alternatives to milk are still developing. The Food and Drug Administration (FDA) classifies these products within the category of "imitation milk" or "imitation dairy products" because they share several sensory and physical characteristics with conventional milk, including similarities in appearance, texture, flavor, and overall product structure [15]. Under current European Union regulations, only specific plant-based goods, such as coconut and almond milk, may be marketed using the term "milk" [16–18].

COMPARISON OF ANIMAL MILK AND PLANT MILK

Plant- and animal-derived milks differ considerably with respect to their origin, nutritional composition, digestibility, environmental footprint, and sensory characteristics. While plant-based milk substitutes are made from a range of plant sources, such as soy, rice, and oats, animal milks are derived from mammals like cows, goats, and sheep. Important nutrients like calcium, riboflavin, vitamin B12, and high-quality protein are found in animal milk [19], but its lactose content may cause digestive discomfort in lactose-intolerant individuals [20]. On the other hand, the ingredients used to make plant-based milks affect their nutritional composition. Compared to most plant-based substitutes, soy milk has a higher protein content, whereas almond- and rice-based beverages typically provide lower protein levels. Because plant-based milk products sometimes have low levels of naturally occurring calcium and vitamin B12, fortification is commonly used to improve their nutritional value [21]. Furthermore, plant-based milks may support cardiovascular health because they typically have less saturated fat and are naturally cholesterol-free [22]. From a sustainability perspective, their production is often associated with lower energy and water use and lower greenhouse gas emissions compared with conventional dairy farming [23]. Consumer acceptance is significantly influenced by sensory attributes, including flavor and scent, which are heavily dependent on the plant source utilized.

In addition to having a lower glycemic index than animal milk, plant-based milk substitutes often provide fiber and a variety of bioactive phytochemicals, including phenolic acids, flavonoids, stilbenes, lignans, condensed and hydrolyzable tannins, proanthocyanidins, carotenoids,

alkaloids, phytates, terpenes, and phytoestrogens are among them. However, compared with animal milk, their protein content is often lower, and other essential nutrients are present at lower concentrations or have reduced bioavailability. For example, it's possible that certain nutrients, like iron, calcium, iodine, vitamin D, and vital amino acids, aren't as abundant as they are in dairy milk. long-term reliance on beverages made from insufficiently fortified plant-based ingredients may make vulnerable groups—children in particular—more susceptible to malnutrition. In order to overcome these limitations, some studies have explored combining plant- and animal-derived milks in a single food matrix to develop functional products with enhanced nutritional profiles [17, 24].

Many parts of the world have seen an increase in issues such as limited access to conventional milk, milk protein allergy, lactose intolerance, and the need for alternative sources of specific nutrients and bioactive compounds. Initially dominated by soybean-based beverages, this market segment has diversified considerably and now includes products derived from oats, almonds, coconuts, hemp seeds, and cocoa. In addition, plant milk production generally requires lower energy input per unit of product than conventional dairy milk, whereas its composition can be modified to satisfy [18, 25, 26].

Classification and Production of Plant Milk

Plant-based milk alternatives represent an expanding segment of the beverage industry and are increasingly consumed worldwide because of changing dietary preferences and lifestyle choices [4]. The idea of plant-derived milk beverages is not new and dates back to historical documents from the thirteenth century, even though the dramatic increase in demand for these goods over the past few years. Plant milk is derived from plants, for example, legumes, oilseeds, cereals, or pseudocereals, that function and look like cow's milk. Production generally involves dispersing plant materials, including cereals, legumes, seeds, and nuts, in water followed by homogenization [27]. These processing techniques yield drinks with particle sizes typically between 5 and 20 μm, giving them a texture and appearance similar to regular milk. Based on their source materials, plant milks can be classified into four primary categories [18], as illustrated in Figure 1.

Although specific processing conditions vary among plant materials, the main stages involved in plant milk manufacture are largely comparable across different raw material groups [28]. The principal stages involved in plant milk production are illustrated in Figure 2. Generally, plant milk is produced by a series of steps: peeling the shell, adding

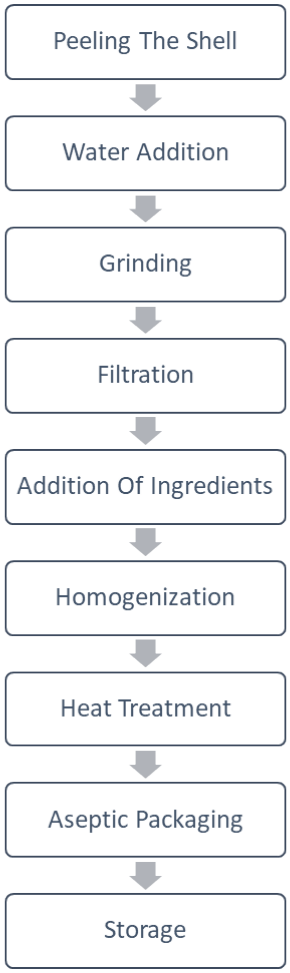

Figure 2. Basic process steps in plant milk.

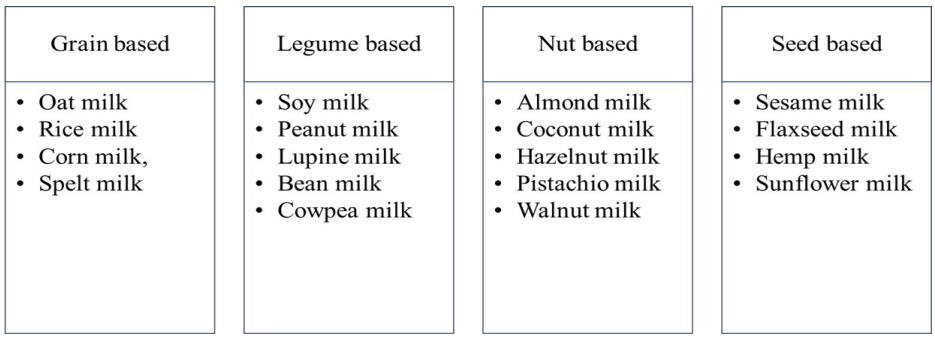

Figure 1. Classification of plant milks according to their raw materials.

water, grinding, filtering, adding additives, homogenizing, heating, aseptic packaging, and storing.

Both the yield of soluble extract and the procedure of making plant milk are greatly influenced by the properties of the plant raw material. As a result, preliminary processing operations may vary depending on the source material used. Nuts and seeds are commonly subjected to shell removal, whereas grains and legumes are typically soaked in hot water and subsequently dried. Additional pretreatments, including chemical peeling or roasting with acidic or alkaline solutions, may be applied to increase process efficiency, eliminate unwanted chemicals, and improve emulsion stability [18]. These operations also promote softening and hydration of the raw materials. During soaking, changes in starch composition may occur, and certain contaminants, such as mold-associated toxins, can migrate into the soaking water [29]. Previous studies have evaluated the use of alkaline soaking solutions for sesame seeds [30], soybeans [31], and peanuts [32].

Grinding is a critical operation in plant milk production because it reduces particle size and encourages the plant matrix's soluble components to be released. Wet milling is generally preferred over dry grinding because it is more compatible with subsequent processing operations and can improve overall process efficiency. This approach has been extensively implemented in plant milks derived from soybeans, coconuts, cashews, hazelnuts, hemp seeds, almonds, walnuts, peanuts, and beans [33–36]. Several processing variables, including grinding time, feed rate, pH, temperature, and the water-to-raw-material ratio, affect the final product's characteristics. Among these variables, the concentration and composition of the final beverage are significantly influenced by the amount of additional water [37]. The addition of water during grinding can facilitate subsequent blanching operations and reduce processing times. Furthermore, blanching may help reduce microbial populations and enzymatic activity in the product [38].

The bleaching step in plant milk production, particularly for oat milk, uses enzymes such as amylases to enhance product quality [18]. This process improves color, flavor, and stability while reducing bitterness and preventing gelation. It exemplifies innovation in the plant milk industry, aiming to meet growing consumers' demand [39]. However, manufacturers must balance the benefits of enzymatic treatments with maintaining nutritional value and addressing preferences for less processed products [26].

Following the enzymatic treatment stage, the liquid fraction is separated from the insoluble material through filtration. During this operation, insoluble particles originating from the ground plant matrix are removed, allowing the recovery of the water-soluble extract. Different filtration materials have been employed for this purpose, including double-layer cheesecloth, muslin cloth (25 μm), filter paper, and sieves or membrane filters with pore sizes ranging from 4 to 180 μm [30, 34, 35, 40, 41]. In addition to conventional filtration techniques, Naziri et al. [42] successfully produced plant-based milks from corn, sesame, and hazelnut using ultrafiltration technology.

To increase stability and enhance the nutritional composition of plant milk formulations, a variety of additions can be used. Proteins, minerals, and vitamins are commonly included to enhance the final product's nutrient composition [24]. To enhance physical stability, ingredients such as acacia gum, gellan gum, and sunflower lecithin are frequently utilized. Ascorbic acid may also be added as an antioxidant to limit oxidative changes during storage. Flavor-adjusting ingredients, including cane sugar and sea salt, can be incorporated depending on product requirements. Makinde and Adebile [43] and Manzoor et al. [44] reported the use of sugar syrup in almond- and cashew-based beverages. The protein, vitamin, and mineral composition of plant milks plays an important role in consumer acceptance. Higher protein levels may be achieved by increasing the proportion of raw materials or combining ingredients with complementary nutritional properties. In addition, fortification is commonly used to improve the levels of minerals, including calcium and vitamins A, B1, B2, B12, D2, and E [18].

The homogenization process reduces the size of suspended particles in plant milk while causing minimal changes to viscosity and protein characteristics, contributing to improved physical stability [41]. It is possible to do homogenization at low, high, or extremely high pressures. Product consistency, visibility, and brightness are often improved by increasing homogenization pressure [36, 45, 46]. However, viscosity and protein stability are not markedly influenced by this operation [45]. Particle size can be further reduced under ultra-high-pressure conditions [46]. Although repeated homogenization cycles may increase product yield, temperature elevation can occur because of the applied pressure [47, 48]. Gul et al. (2018) reported that homogenization may increase product temperature by approximately 5–10°C [47]. Consumers generally prefer plant-based milks with appearance, stability, rheological characteristics, and compositional characteristics similar to those of traditional milk [18, 49]. Technologies including ultrasonication, pulsed electric field treatment, ohmic heating, and high- or ultra-high-pressure homogenization have attracted increasing attention as approaches for improving the stability of plant milk products [30].

Thermal processing is generally applied as the final preservation stage in plant milk manufacturing. Pasteurization remains one of the most frequently used approaches for improving microbiological stability and reducing enzymatic activity. Conventional thermal treatments include ultra-high-temperature (UHT) sterilization and pasteurization [50, 51]. In addition to these established methods, several emerging technologies have been investigated for plant milk stabilization, including ultrasonication, high-pressure processing, microwave treatment, pulsed electric field (PEF) treatment, ohmic heating, supercritical carbon dioxide processing, and ultraviolet (UV) irradiation [52].

Conventional heat treatments are highly effective for microbial control and therefore remain widely utilized in commercial food production [53]. However, prolonged exposure to elevated temperatures may affect nutritional and sensory quality through protein denaturation, vitamin degradation, and changes in flavor characteristics [52]. To address these limitations, alternative processing technologies have been explored as potential means of improving microbial safety while preserving product quality [54].

Despite these technologies' potential benefits, there are still a number of practical obstacles to their industrial application, such as equipment costs, process complexity, and regulatory considerations [55]. Consequently, the selection of an appropriate stabilization strategy should take into account both product characteristics and targeted quality requirements. Although the number of studies investigating these technologies in plant milk systems continues to increase, available information remains relatively limited [56]. Following processing, aseptic packaging and refrigerated storage at approximately 4°C significantly preserve product stability and increase shelf life.

Microorganism Inactivation in Plant Milks

Plant-based milk alternatives contain nutrients that can support microbial growth when appropriate preservation measures are not implemented. Microbial development during storage may adversely affect these items' physicochemical qualities and sensory attributes. Consequently, effective processing technologies play an essential role in plant milk manufacturing. Thermal processing is widely applied to control spoilage and pathogenic microorganisms, thereby extending shelf life and improving product stability [57]. Besides enhancing microbiological safety, thermal processing may also contribute to increased total solids and the development of desirable flavor. However, exposure to excessive heat can adversely affect product quality through vitamin degradation, amino acid losses, non-enzymatic browning reactions, and undesirable flavor formation [58]. Therefore, processing conditions should be carefully optimized to achieve adequate microbial control while preserving product quality.

Conventional Thermal Treatments

In the manufacture of plant milk products, pasteurization and ultra-high-temperature (UHT) processing remain among the most widely adopted preservation strategies to ensure product durability and improve shelf life. Conventional thermal treatments include pasteurization, in-container sterilization, and UHT processing, each designed to reduce microbial populations and enhance product safety. In-container sterilization is typically performed at 121°C for 15–20 min to achieve commercial sterility, whereas UHT treatment involves short exposure periods at temperatures between 135 and 150°C [58]. During UHT processing, heat may be delivered either directly via steam infusion or injection, or indirectly through plate or tubular heat exchangers. Following thermal treatment, products are generally packaged under aseptic conditions to maintain microbiological stability. While UHT-treated and sterilized products can remain stable for extended periods at ambient temperature, pasteurized products typically require refrigerated storage.

Han [59] evaluated the effects of pasteurization and sterilization on soymilk production. They reported that pasteurized (60°C/30 min) soy milk could remain stable at ambient conditions for 3 days without noticeable quality loss, and that sterilized (120°C/5 min) soy milk remained stable under ambient conditions for 1 year. Whereas thermal treatments can be preferred and more easily applied to durable products such as soy milk and peanut milk, their use is limited by the high starch content in products such as oat milk and rice milk.

Bartula et al. [60] investigated the behavior of foodborne pathogens and spore-forming bacteria in commercial plant-derived substitutes for milk stored under refrigerated and ambient conditions (4, 8, and 20°C). The study included coconut-, almond-, and cashew-based beverages as well as UHT-treated cow's milk. Samples were inoculated with *Listeria monocytogenes, Salmonella enterica, Paenibacillus spp.*, and *Bacillus subtilis* at an initial level of approximately 1×10^3 CFU/mL. The findings indicated that the tested microorganisms generally exhibited faster growth in plant-based beverages than in cow's milk. Increased growth of *L. monocytogenes* was observed at both 8 and 20°C, whereas *Salmonella* and *Paenibacillus* showed more pronounced growth at 20°C. In contrast, *B. subtilis* displayed comparable growth behavior in almond milk and cow's milk under the same storage conditions. No statistically significant differences were detected in the growth responses of *Listeria* and *Salmonella* at 4 and 8°C ($p>0.05$). These observations suggest that plant-based milk alternatives may require particular attention to controlling pathogens associated with listeriosis and salmonellosis.

The plant milk industry must ensure product quality and safety. Pasteurization is frequently employed to improve product stability and storage life by reducing populations of spoilage and pathogenic microorganisms. Thus, plant milks are processed to provide consumers with pleasing sensory qualities while also increasing microbiological stability and reducing perishability [61, 62].

When pasteurization and sterilization processes are carried out as thermal treatments, undesirable physical, chemical, sensory, and nutritional conditions arise. To eliminate these negativities, the tendency towards non-thermal or innovative thermal treatments is increasing [63]. Using these innovative technologies, microorganisms and enzymes can be rendered inactive without compromising or adversely affecting the food products' nutritional value. Accordingly, ohmic heating, microwave heating, high-pressure processing, ultrasonication, pulsed electric field treatment, and ultraviolet irradiation have gained increasing attention as alternative processing technologies in plant

milk production. While the microbial load is reduced with these innovative technologies, the process variables that preserve the nutritional content of plant milks must be well adjusted and regulated.

Innovative Thermal Treatments

Numerous processing techniques have been investigated to guarantee product stability and safety while preserving food's nutritional value. Ohmic and microwave heating are the most preferred and applied technologies. Table 1 summarizes the studies conducted in the literature [64-77].

Ohmic heating is an electrothermal processing technique that produces internal heat by passing an electrical current straight through the food material. This mechanism enables rapid, relatively uniform heating, particularly in liquid food systems, thereby improving heat-transfer efficiency. [78]. Numerous studies have looked into the use of ohmic heat in food treatment, especially pasteurization as well as sterilizing procedures. [79]. The effectiveness of this technology is associated with the combined action of electrical current and temperature, which can contribute to both enzymatic and microbial inactivation. Plant-derived milk products benefit from these effects since they improve microbiological stability and retain quality throughout storage. [68, 80, 81]. Unlike conventional heating systems, ohmic heating provides volumetric heat generation throughout the product, reducing temperature gradients and minimizing localized overheating. This method has attracted interest as an alternate processing approach to improve the safety and preservation of plant-based milk products.

Studies on soy milk have shown that ohmic heating can achieve urease inactivation with an effectiveness comparable to conventional thermal processing. In addition, enzymatic inactivation performance has been reported to improve as the operating frequency increases [81]. Despite these advantages, excessive heat generation during processing may promote protein coagulation. Elevated temperatures reached during ohmic treatment of soy-based milk alternatives have also been associated with undesirable changes in sensory attributes and nutritional quality [82]. According to Tiravibulsin et al. (2021), ohmic-assisted sterilization achieved complete inactivation of Clostridium sporogenes spores (>5 log CFU/mL) in coconut milk when applied at 121.1°C for 5 min. Compared with conventional sterilization, this process provided a shorter overall treatment time. In addition to its effectiveness in microbial inactivation, the application was associated with reduced formation of undesirable odors while preserving the product's visual characteristics. These findings indicate that ohmic-assisted sterilization may offer an effective alternative for the processing of coconut milk [68].

The most popular and favored technique for thermal processing liquid foods is continuous-flow microwave heating, an alternative to traditional heating methods. Dipole rotation and ionic conduction are responsible for heating food in microwave ovens. Since volumetric energy transfer causes temperature to rise more quickly, processing times are shortened, and energy is conserved [83]. Unlike conventional thermal systems, microwave processing generates heat directly within the product and does not require an external heat-transfer medium. In addition, microwave technology has been associated with reduced energy and water consumption as well as improved processing efficiency.

Microwave pasteurization enables rapid heat transfer and can achieve the desired processing temperature in less time than many conventional heating systems. Reduced processing time may preserve the dietary and sensual characteristics of foods throughout preservation. During microwave treatment, electromagnetic energy is absorbed directly by food components, resulting in volumetric heating and a more uniform temperature distribution than conventional surface-to-center heat transfer. Using microwave energy to process food can reduce total processing time [84]. Microwave processing yields positive results in reducing enzymatic spoilage, which is as important as microbial growth in plant milk. Studies have examined the trypsin-inhibitory effects of microwave processing in soy milk. The results showed that this method achieved trypsin inhibition and affected protein structures [85–87].

Microwave pasteurization (360 W/38 s) and conventional pasteurization (70°C/465-480 s) were compared for their ability to inactivate Salmonella enteritidis in almond, soy, and oat milk samples [69]. Microwave treatment resulted in decreases of 5.39, 5.77, and 6.62 log concentrations of CFU in soy, almond, and oat milk. Compared with classical pasteurization, microwave pasteurization showed similar inhibition of microorganisms, indicating it constitutes an alternative pasteurization method.

Innovative Non-thermal Treatments

High-pressure process (HPP), commonly known as high hydrostatic pressure (HHP), is a non-thermal, preservation a food method that exposes foods to higher pressure levels for a set period. During processing, packed products are placed in a pressure chamber and treated with a pressure-transmitting liquid, usually water. HPP enhances microbiological safety while preserving nutritive and sensorial properties of foods. [88, 89]. Prior researches have also demonstrated that pressure treatment combined with moderate heat can enhance the inactivation of bacterial spores [90–94]. HPP technology is a viable solution for maintaining microbiological stability in plant-based milk products. [1, 70–72] (Table 1).

Soy milk was subjected to high hydrostatic pressure treatment at two pressure levels, namely 200 and 300 MPa, in the study reported by Cruz et al [70]. The total number of aerobic and mesophilic bacteria were found to have decreased by 2.42 and 4.24 log CFU/ml, respectively. Total aerobic and mesophilic bacterial populations decreased

Table 1. Research on ensuring microbial safety in plant milk with innovative technologies

Innovative Technology	Inactivation methods and process conditions	Target microorganism	Product	Results	References
Ohmic Heating	**Ohmic heating** Frequency: 10 kHz; Time: 40, 45, 50, and 55 s.; Temperature: 50, 55, 60, 65, 70, and 80°C; sodium lactate solution	• *S. Typhimurium,* • *E. coli* O157:H7, • *S. aureus* • *L. monocytogenes,*	Soy milk	• Ohmic heating has demonstrated considerable potential as an alternative approach for ensuring microbial safety. • An increase in the sodium lactate content caused a decrease in pathogens. • Lethal damage to microbial cell structures was observed in samples containing 3% sodium lactate.	[67]
Ohmic Heating	**Ohmic heating** Pressure: 1.5 bar; voltage: 600 V current: 80A; frequency: 50Hz sodium sulfate solution; 121.1°C/ 2 min **Sterilization** Temperature: 121.1°C/ time: 2 min	• *Clostridium sporogenes* • *Clostridium perfringens*	Coconut milk	• Ohmic-assisted sterilization at 121.1°C for 5 min achieved complete inactivation of C. sporogenes spores at levels exceeding 5 log CFU/mL.	[68]
Microwave Application	**Microwave** Microwave power: 360 W; time: 38 s **Pasteurization** Temperature: 70°C; time: 465-480 s	• *Salmonella enteritidis*	Almond milk Soy milk Oat Milk	• Microbial inactivation was achieved in all plant-based dairy products to a level that can demonstrated the potential to replace conventional thermal pasteurization.	[69]
High-pressure processing (HPP)	**High-pressure processing (HPP)** Pressure: 200-300 MPa	• Total mesophilic aerobic bacteria • Enterobacteria • Total mesophilic aerobic spores	Soy milk	• Counts of mesophilic aerobic bacteria decreased as the applied pressure increased. • Enterobacteria remained below the detection limit in both applications.	[70]
High-pressure processing (HPP)	**High-pressure processing (HPP)** Pressure: 200-300 MPa Temperature: 55, 65, and 75°C **Pasteurization** Temperature: 95°C/ time: 30 s **Sterilization** Temperature: 142°C/ Time: 6 s	• *Bacillus cereus* • Total mesophilic aerobic bacteria • Total mesophilic aerobic spores • Enterobacteria • Mold and yeast • *Staphylococcus aureus* • Salmonella species • Coliform	Soy milk	• The sterility obtained in the UHT process was achieved with the HHP method.	[71]
High-pressure processing (HPP)	**High-pressure processing (HPP)** Pressure: 200 and 300 MPa Temperature: 55, 65, and 75°C **Pasteurization** Temperature: 90°C/ time: 90 s **Sterilization** Temperature: 142°C/ time: 6 s	• Total mesophilic aerobic bacteria • Total mesophilic aerobic spores • Yeast and mold • *Enterobacteriaceae* • *Micrococcaceae* • *Bacillus cereus*	Almond milk	• After pasteurization, sterilization, and HPP processes, microbial counts were determined below the detection level.	[1]

Table 1. Research on ensuring microbial safety in plant milk with innovative technologies (continued)

Innovative Technology	Inactivation methods and process conditions	Target microorganism	Product	Results	References
High-pressure processing (HPP)	**High-pressure processing (HPP)** Pressure: 200-300 MPa Temperature: 55-75°C **Pasteurization** Temperature: 95°C/ time: 30 s **Sterilization** Temperature: 142°C/ time: 6 s	• Total mesophilic aerobic bacteria • Total mesophilic aerobic spores	Soy milk Almond milk	• The HPP process provided a higher reduction in microorganisms than pasteurization. • Almond and soy milks treated with HPP exhibited superior colloidal stability compared with samples subjected to conventional thermal processing.	[72]
Ultrasonic Application	**Ultrasonic Application** Power: %20-80; time: 2-8 min Pulse: 2-6 s	• *Escherichia coli O157:H7* • *Listeria monocytogenes*	Almond milk	• It has been found that ultrasonic application can cause non-lethal damage to pathogens.	[65]
Ultrasonic Application	**Ultrasonic Application** Power: 300 W; frequency: 20 kHz time: 0, 2.5, and 5 min	• Total mesophilic aerobic bacteria • *Enterobacteriaceae*	Almond milk	• No microbial growth was detected in ultrasonically treated almond milk throughout the storage period.	[73]
Ultrasonic Application	**Ultrasonic Application** Power: 600 W; Frequency: 40 kHz; temperature: 30, 45 and 60°C; time: 10, 20, 30, and 40 min **Pasteurization** Temperature: 90°C/ time: 60 s	• Total mesophilic aerobic bacteria • Yeast and mold	Almond milk	• Processing at 60°C led to reductions of ≥5 log in total plate counts and ≥4 log in yeast and mold populations. • In ultrasonic application, the process applied to microorganisms pierces the cell membranes, causing the intracellular matrix to be expelled and forming free radicals, eventually inhibiting the organisms.	[74]
Pulse Electric Field (PEF)	**Pulse Electric Field (PEF)** Temperature ≤35°C; electric field power: 7, 14, 21, and 28 kV/cm; Accent frequency: 1 kHz; emphasis width: 40 µs, process time: 200 µs **Pasteurization** Temperature: 90°C/ time: 60 s	• Total mesophilic aerobic bacteria • *Escherichia coli,* • Yeast and mold	Almond milk	• The microbial load in almond milk decreased effectively with thermal treatment and PEF (28 kV/cm) applications. • PEF treatment applied at 28 kV/cm produced a level of microbial stability comparable to that obtained with thermal processing.	[75]
Pasteurization in UV-C Reactor	**Pasteurization in UV-C Reactor** 2-10 loop **Pasteurization** Temperature: 63°C/ time: 30 min	• *Escherichia coli* K-12 (ATCC 25253)	Oat milk	• UV-C reactor treatment of oat milk resulted in a 3.22 log CFU/mL reduction in *E. coli* K-12.	[76]
Pasteurization in UV-C Reactor	**Pasteurization in UV-C Reactor** Flow rate: 25-100 ml/min Tube length: 240-1376 cm **Pasteurization** Temperature: 72°C/ Duration: 32 s	• *Escherichia Coli* • *Bacillus cereus*	Soy milk	• UV-C processing effectively reduced *E. coli* cells and *B. cereus* spores in soy milk while maintaining product quality.	[77]

by 2.42 and 4.24 log CFU/mL, respectively, as a result of the therapy. Additionally, Enterobacteriaceae remained less than the detection limit in both pressure conditions, although overall spore counts decreased by around 2 log CFU/mL. Comparable microbiological stability to that achieved by UHT processing was obtained at 300 MPa and 75°C. Overall, HHP treatments demonstrated greater microbial inactivation than conventional pasteurization. Furthermore, HHP was associated with lower oxidation levels while maintaining substantial inactivation of trypsin inhibitors [71].

Microbial populations in almond milk were reduced to levels under the detectable level (-0.5 log CFU/mL) after pasteurization, sterilization, and high-pressure processing procedures. During HPP treatment, temperature increases may result from adiabatic heating and mechanical forces generated within the system, including turbulence, shear stress, and cavitation. These processing conditions are considered important contributors to microbial inactivation and overall product stabilization [1].

Ultrasonication has attracted considerable interest as an alternative processing technology in the food industry. In the food sector, ultrasonication has attracted a lot of interest as an alternative processing technology. The cavitation phenomena produced during ultrasonic treatment are the main cause of this technique's anti-microbial effect. Ultrasonic waves, typically operating at frequencies above 20 kHz, create localized mechanical and physical effects that can damage microbial cells [95, 96]. Ultrasonic processing has been shown in earlier research to be an efficient way to lower microbial populations. Additionally, using it in conjunction with thermal treatments may reduce processing times while minimizing unfavorable alterations to food's nutritional value. The ultrasonic frequency used to inactivate microorganisms is generally between 20 and 100 kHz [96, 97]. The microbial inactivation mechanism typically depends on the cavitation force [98]. When ultrasonic treatment is used with other techniques, the efficacy of inactivating microorganisms is boosted [99, 100]. Ultrasonic treatment effectiveness relates to the targeted microorganisms. Combining ultrasonic processing with other preservation techniques, such as thermal and pressure-based treatments, can increase its efficacy, especially for spore management. Nevertheless, more research is needed to assess the practical application of integrated treatment strategies in food systems and optimise conditions for the process. Ultrasonic uses in plant milk have been the subject of numerous investigations. [65, 101–106].

Listeria monocytogenes and *E. coli* O157:H7 growing behaviors were affected by the ultrasonic treatment of almond milk. Application decreased the growth rate of E. coli O157:H7 and postponed the growth of L. monocytogenes. Although complete pathogen elimination was not achieved, ultrasonication contributed to a measurable reduction in microbial populations [65]. Furthermore, microbiological analyses indicated that no detectable microbial growth occurred during the storage of ultrasonically treated almond milk [73].

In a study comparing pasteurization and ultrasonic treatment (60°C) in almond milk, similar results were obtained for microbial inactivation. With both methods, a log reduction of more than 5 in the total number of plaques and more than 4 in the number of yeast and molds was detected [74]. Ultrasonic treatment's antimicrobial action is linked to structural damage to microbial cells, which can lead to internal component leakage and the production of reactive species that aid in microbial inactivation.

Short-duration, high-intensity electrical pulses are applied to food systems using pulsed electric field (PEF) technology, a non-thermal preservation method that lowers microbial activity. The antimicrobial effect of PEF is primarily associated with the disruption of cell membrane integrity through electroporation [107]. Because the treatment can be applied within a very short time while minimizing thermal damage, it has attracted considerable interest for the production of minimally processed foods [107, 108].

PEF systems are appropriate for liquid, semi-liquid, and some solid food products since they usually operate at electric field levels of 20 to 80 kV/cm with pulse durations of 1 to 100 μs [107,108]. The qualities of the treated food matrix, microbial attributes, and processing conditions all affect how well microbial inactivation Works [107, 108-110]. Although PEF has been investigated as an alternative to conventional thermal pasteurization, current evidence indicates that its performance can be enhanced when combined with thermal treatments, particularly in applications requiring high levels of microbial reduction [109–111].

The antimicrobial effect of pulsed electric field (PEF) processing is generally attributed to electroporation of microbial cell membranes. When the applied electric field exceeds a critical threshold, permanent structural changes may occur in the membrane, increasing its permeability and promoting the leakage of intracellular components, which can ultimately result in cell death [108, 109]. Previous studies have reported the effectiveness of PEF against various microorganisms, including Escherichia coli, Listeria innocua, Staphylococcus aureus, Enterobacteriaceae, and Pseudomonas fluorescens[110, 112, 113]. Siemer et al. [111] reported that combining thermal processing with PEF treatments can effectively inactivate spores, with PEF being the dominant factor at higher energy inputs. It should be emphasized here that better results can be achieved when combining PEF with heat. In comparing the pasteurization process and PEF (28 kV/cm) application in almond milk, they reported that PEF reduced the microbial load. According to Manzoor et al. [75], The study found that PEF PEF processing performed at 28 kV/cm offered comparable microbiological stability to thermal treatment over the 28-day storage period.

Ultraviolet (UV) irradiation is a non-thermal preservation technology that utilizes electromagnetic radiation within the wavelength range of 100–400 nm. Based on

wavelength, UV radiation is commonly categorized into four spectral regions: vacuum-UV (100–200 nm), UV-C (200–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm). Among these regions, UV-C has received particular attention in food processing applications because of its strong antimicrobial activity. In most industrial applications, UV radiation is generated using mercury-based lamp systems that provide continuous emission [114].

The antimicrobial action of UV light is primarily associated with damage to microbial genetic material. UV radiation is widely used for the decontamination of surfaces, equipment, and air environments [115]. UV processing is inexpensive, leaves no chemical residue behind, and generates minimal heat [116]. The effectiveness of UV treatment largely depends on the ability of UV radiation to penetrate the treated medium. Successful microbial inactivation relies on sufficient exposure of target microorganisms to UV photons. UV application rooms need to be constructed to inactivate organisms that cause microbial spoilage in liquid food products [114]. Spores are not significantly affected by UV radiation. However, UV exposure may enhance the effectiveness of subsequent thermal processing by increasing the sensitivity of bacterial spores [117]. Research investigating UV inactivation mechanisms has consistently identified DNA damage as the principal factor responsible for microbial inactivation. Applications of UV treatment are particularly common in liquid food systems, including fruit beverages, dairy-based products, honey, and liquid egg products [114, 118].

Thermal pasteurization and UV-C reactor treatment have been compared for stabilizing oat milk [76]. After ten passes through the UV-C system (cumulative dose: 72.34 J/mL), *E. coli* K-12 populations were reduced by 3.22 log CFU/mL. However, this reduction remained below the FDA-established 5-log target [119]. In soy milk, UV-C processing in a 1.6 mm reactor resulted in reductions of up to 5.6 log CFU/mL for E. coli and 3.29 log CFU/mL for *B. cereus* spores. These results suggest that UV-C irradiation can substantially decrease microbial populations while preserving the quality attributes of soy milk [77].

The large-scale implementation of novel processing technologies in plant milk production is influenced by a range of technological, economic, and operational considerations. The substantial investment required for specialized equipment may hinder adoption, particularly among small and medium-sized manufacturers [112]. In addition, the successful operation of these technologies often depends on sophisticated monitoring and control systems, which can complicate process standardization [120]. Inadequate control of processing conditions may affect product consistency and microbiological reliability [55]. Beyond technical performance, environmental impact, energy demand, and long-term sustainability should also be considered when evaluating these Technologies [121]. Therefore, wider industrial adoption requires a balanced assessment of economic feasibility, technical performance, regulatory acceptance, and practical applicability. Furthermore, evaluating the performance of emerging technologies in different plant milk matrices is important because variations in composition, viscosity, and structural characteristics may influence processing outcomes. Since plant milks such as almond, soy, oat, and coconut differ in composition, viscosity, and stability, the same technological process does not yield similar results across all types of milk. Therefore, it is necessary to determine special process conditions for each plant milk. In this way, both product quality and maximum efficiency can be preserved by the applied technology. In this regard, systematic and comparative studies on different types of milk will increase knowledge in the field.

CONCLUSION

Healthier food alternatives are becoming more popular due to shifting consumer preferences and greater nutrition awareness. In addition, the market for plant milk has grown due to the rising prevalence of lactose intolerance and the adoption of vegetarian and vegan diets. This growing demand has encouraged further research into plant milk processing and the creation of new goods.

Understanding the main production stages of plant milk and their influence on product quality is essential for manufacturing high-quality products. In addition, effective control of spoilage and pathogenic microorganisms remains a key requirement for ensuring product safety. This review examined innovative thermal and non-thermal technologies applied to improve microbial safety in plant-based milk products. The available literature indicates that these technologies can contribute not only to microbial inactivation but also to the preservation of nutritional and sensory qualities. To improve processing conditions for various plant milk matrices and to enable the broader industrial application of developing technologies, more research is required. Because the physicochemical characteristics and composition of plant milks vary with their raw materials, process parameters should be carefully tailored to each product type. Continued technological developments are expected to support the production of plant-based milk beverages with improved quality, safety, and consumer acceptance. Plant milks will likely become more significant both as stand-alone drinks and as ingredients in new food products as interest in plant-based diets grows.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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