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Functional Food Science

Non-thermal techniques for microbiological safety, nutritional preservation, and enhanced efficiency in dairy processing

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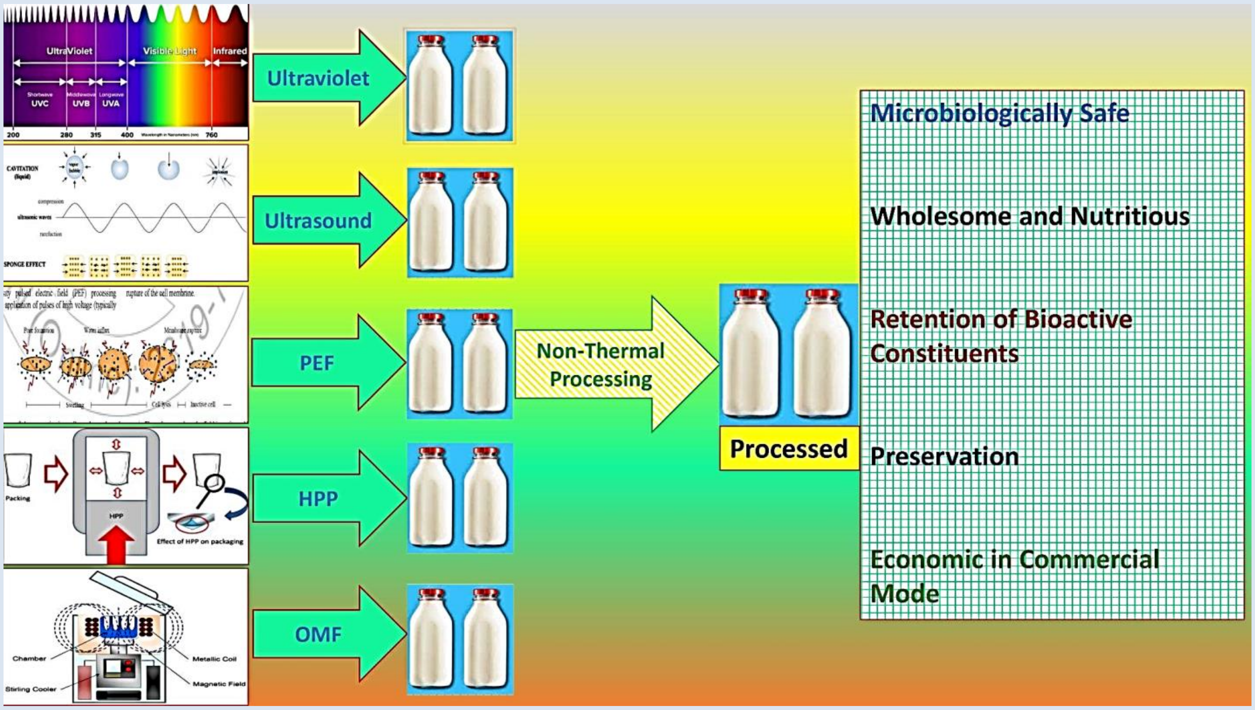
ABSTRACT

The ongoing era of adopted lifestyle among humans involves shortcuts in every aspect, and a similar trend can be observed in food processing and preservation, especially in dairy preparation. Thus, lots of non-thermal processes including high-pressure processing (HPP), ultrasound (US) treatment, pulsed electric fields (PEF), ultraviolet (UV) light and oscillating magnetic fields (OMF), etc. have been invented. By inactivating bacteria, these non-thermal processes keep food's sensory and nutritional quality while also preventing thermal degradation of food components near ambient temperatures. Non-thermal techniques can effectively preserve food's nutraceuticals and functional potential, while simultaneously ensuring microbiological safety and maintaining the integrity of its bioactive components. During the thermal processing, several heat-labile components of milk and milk products may be lost. In contrast, non-thermal processes are gentler compared to thermal treatments, making them preferred choices that avoid compromising nutritional value. It has been observed that non-thermal technology can increase processing efficiency and microstructure through constituent interactions, alteration of enzyme activity, and development of hypoallergenic products. However, in order to use thermal technologies profitably, the risk of operation, equipment cost, technological compatibility, and goal of processing the product must be thoroughly assessed prior to using these processes. Non-thermal processes can also be combined with other strategies like the addition of bio-preservatives or modified atmospheric packaging etc., to enhance the antimicrobial effects.

Keywords: Bioactive components; Bioactive compounds; Bio-preservatives; Dairy processing; Enzyme activity; Microbiological safety; Non-thermal processes; Nutritional quality.



INTRODUCTION

Nowadays, consumers are highly interested in food products that are safe, healthy, minimally processed and natural with a fresher taste. In order to attend to consumer demands, industries are trying to seek alternative technologies keeping in mind the freshness, safety, storage stability, nutritional profile, environment friendliness, appetizing qualities, affordability, personalization, and therapeutic aspects of their processed products [1]. This presents a significant challenge to the industry and demands for worldwide innovation and market competitiveness. These alternative technologies are usually considered "non-thermal" wherein, food is generally processed at ambient or slightly above ambient temperature, and heat generation is minimal [29]. Milk, which is considered to be the most perishable commodity, contains several heat sensitive flavorings, aroma compounds and vitamins that are often lost during heat treatments. Heat treatment also leads to certain undesirable changes in milk components like browning, off-flavoring, and denaturation of proteins. The quality of the product is governed by the function of both temperature and process exposure time [2].

Traditional thermal energy-based methods are losing popularity as non-thermal technologies gain prominence in food processing [3-5]. These innovative non-thermal techniques utilize electrical, electromagnetic forces, light, and mechanical forces instead of heat energy [6]. Unlike heat treatment, which has significant consequences for the environment, high costs, energy consumption, and water usage [7], emerging non-thermal technologies are characterized by their low-temperature operation. Hence, these technologies offer the potential to minimize these consequences to a greater extent [8]. This shift towards non-thermal methods reflects a growing emphasis on energy-efficient and sustainable approaches to food processing.

Global adaptation to innovative technologies recently introduced in the field of food and dairy processing is facilitated by reported data from fast-paced research conducted in their laboratories. Ambient temperature processing advantageously benefits Asian processed foods to maintain their flavor and aroma after exposing them to these technologies viz; HPP, PEF, Cold plasma, and ultrasonication. Non-thermal technology is the least energy consuming with a wide scope of applications besides their intense aroma and flavor benefits which also aligns well with Western countries' foods. [9]. These technologies have improved not only the safety and quality of microorganisms but also a number of other applications, such as (a) more efficient processing, (b) diversification of components and products, including "tailored" functionality, (c) preserving heat-labile bioactives, (d) controlling of enzyme activity, (e) enhancing microstructure by interacting with components, and (f) hypoallergenic milk products [10].

Non-thermal approaches have been shown to be successful at deactivating bacteria while preserving the functionality and nutritional content of milk and its derivatives [11, 12]. According to research, they have a good effect on milk and milk product preservation [13, 14]. Furthermore, several studies on ultrasound in enzyme activation and inactivation highlight the potential of non-thermal technologies to provide new pathways for innovative and healthy dairy products, highlighting their significance in fostering innovation in the dairy industry [15].

Consumers today are increasingly conscious of the food products they buy, showing a preference for items that offer nutritional value and health benefits while being produced through environmentally friendly methods. Several investigations [16-20] support this emerging trend. Simultaneously, industries are actively investigating non-invasive processes that maintain product quality and prevent nutritional degradation over time, in order to meet consumers' evolving demands [21-24].

Non-thermal processing is the least harsh when bioactive and other phytoconstituents are concerned. These technologies also maintain the nutritional power for processed stuff as of low energy operation and at ambient temperature processing besides their environment-friendly usage. By utilizing little to no heat in this method, the microorganisms in food products can potentially be rendered inactive. In addition, NTP (Non-Thermal processing) is capable of handling all food types, including fruits, vegetables, legumes, spices, meat, and fish, to preserve their naturally occurring, heat-sensitive bioactive components [25] (table 1 presented comparison of major non-thermal technologies in dairy products).

Table 1. Comparison of major non-thermal technologies in dairy products

Process	Range of intensity	Applications	References
High-pressure processing (HHP)	400-600MPa	Milk	[4-7]
		Ice cream and butter	[90-96]
		Colostrum	[77, 78, 81, 82]
		Yogurt	[31]
		Cheese	[28, 30, 49, 76]
		Milk Beverages	[13]
Pulsed electric field	20-80Kv/cm for <1s	Milk, Yogurt andUHT (Ultra-High Temperature)	[14, 32, 50]
		Fruit Juice mixed with milk beverages	[53, 55, 60]
Ultrasound	20-100 Hz	Whole milk	[83, 93]
		UHT (Ultra-High Temperature)	[94, 97, 99]
		Yogurt, butter cream and WPC (Whey Protein Concentrate)	[15, 104, 108]
Oscillating Magnetic Field	1-100 OMF pulses of 5-500kHz, 0-50˚C for 25-100ms	Milk, beverages and other liquids	[129]

The review aims to provide an overview of the effects of these emerging non-thermal processes on the growing interest in nutritious and sustainably produced dairy products while emphasizing the need for innovative techniques to preserve microbial quality and nutritional content in response to consumer expectations.

UV Technology: light's non-ionizing nature means it lacks the ability to ionize atoms or molecules, making it a safer form of radiation. UV light is a type of non-ionizing irradiation with three distinct spectrums: UV-A (315 to 400 nm), UV-B (280 to 315 nm), and UV-C (200 to 280 nm). UV light is described as a non-toxic and environmentally friendly method that employs physical energy for various applications. This technique can be conducted using continuous or pulsed light modes, where the product being treated absorbs the light photons. UV light has been applied in food and dairy processing for microbial inactivation and preservation. By using UV light, it's possible to achieve microbial deactivation and other desired effects without introducing harmful chemicals or altering the product's chemical composition. Continuous or pulsed light modes highlight the versatility of the technique, allowing for diverse mechanisms and factors involved in UV light-based microbial inactivation, particularly in the context of dairy food and liquid processing [26].

Photochemical changes in DNA and RNA, photothermal effects with prolonged pulse durations boosting temperatures to kill bacterial cells, and photophysical damage to cell structures are among the processes. The efficiency of the treatment is affected by the type of bulb used, such as mercury lamps or pulsed light. Notably, pulsed light treatments are more effective than continuous UV light treatments [27].

The generation of light pulses is achieved through the impulse of electrical energy from xenon gas lamps. However, the treatment's efficacy is influenced by a myriad of external factors, including product surface area, opacity, and turbidity of the liquid. Higher opacity and greater turbidity result in reduced light penetration, necessitating higher doses for effective treatment, as seen in milk due to its colloidal and suspended solids content [28].

Additional factors affecting UV-C treatment effectiveness include light intensity, flow patterns, equipment geometry, temperature, time, number of passes, microorganism type, pH, water content, and absorbance coefficient of the medium. Moreover, intrinsic factors like growth phase, prior stresses, process conditions, and conditions after treatment significantly influence UV-C treatment outcomes [29]. Due to this intricate interplay of multiple parameters, comparing data from different studies on UV-C technology becomes challenging, underscoring the complexity of the process.

Mechanistic Insights: The sensitivity of microorganisms to UV-C treatment has been extensively studied, revealing distinct trends in resistance among different types of organisms which suggests that vegetative bacteria are the most sensitive to UV-C treatment, while yeasts exhibit greater resistance. Among the microorganisms, viruses and protozoa stand out as the most resistant, a resistance that is influenced by various factors [30].

Further investigation into vegetative bacteria reveals an intriguing pattern. Gram-positive bacteria display a higher resistance to UV-C inactivation compared to their Gram-negative counterparts. This phenomenon could potentially be attributed to the presence of an outer layer of glycoproteins and liposaccharides [31]. Additionally, a study on the varying UV resistance between different bacterial species, viz., *Listeria monocytogenes*, a Gram-positive bacterium, demonstrates greater resistance to UV light than *Escherichia coli*, a Gram-negative bacterium [32].

Spore-forming bacteria, represented by *Bacillus cereus* endospores, exhibit an interesting dynamic in UV-C resistance and found that UV-C light effectively targets *Bacillus cereus* endospores; however, these endospores remain more resistant to UV-C treatment compared to their vegetative counterparts [33]. This enhanced resistance can be attributed to the protective coating that endospores possess, while UV-C treatment has the potential to kill bacteria, post-treatment precautions are critical [34]. The importance of monitoring microbial survival and development after UV-C therapy, via photo-reactivation is possible. According to the same study, measurement by classic plate count methods may be insufficient due to the presence of viable but non-culturable bacteria [35].

The importance of process efficiency indicators becomes clear when it comes to assuring microbial inactivation and food safety using UV-C systems. In a similar vein to heat pasteurization, such as alkaline phosphatase, to assess the efficacy of UV-C treatment [36]. Furthermore, long-term food safety issues include conducting shelf-life tests and diving into the examination of injured cells and their potential healing processes after treatment.

The application of non-thermal technologies, such as UV-C treatment, has demonstrated notable benefits in the dairy food industry, particularly in enhancing the vitamin content of certain products. A compelling instance of this effect is observed in UV-C-treated milk, where the vitamin D3 content is significantly increased. The European Food Safety Authority (EFSA) acknowledges that while UV-treated milk closely resembles non-UV-treated milk in most aspects, vitamin D3 content is notably higher in the former [37]. This enrichment in vitamin D3 is a result of the UV-C treatment process, which imparts a significant amount of vitamin into the milk.

However, the efficacy of UV-C treatment on vitamins within milk is subject to multiple factors. These include light intensity, initial vitamin content in the milk, and the number of passages through the UV-C system, all of which influence the impact of UV-C treatment on vitamin levels. The intensity of UV-C light can significantly affect the nutritional quality of the treated products and found that using higher fluence could lead to the degradation of Vitamin C in milk, highlighting the importance of optimizing treatment conditions [38-39].

In cheese production, UV-C treatment poses unique challenges due to its limited penetration into solid foods. The surface-level inactivation of bacteria is demonstrated in UV-C treated Fiord latte cheese, where microbial populations remain consistent after extended shelf life [40-42], indicating that UV-C treatment primarily affects the surface layers of cheese, potentially leading to differential microbial inactivation and changes in organoleptic properties.

The effectiveness of UV-C treatment varies based on the type of cheese and the specific microorganisms present and found that UV-C treatment effectively targets *Listeria monocytogenes* and *Penicillium roqueforti* in both packed and unpacked hard American cheeses [43]. However, *Penicillium roqueforti* demonstrated higher resistance to UV light. Under certain conditions, such as extreme exposure, lipid peroxidation and changes in color were observed. The organoleptic profile of UV-C treated foods is notably influenced by factors such as equipment, dosage, exposure, and passages. Different UV lamps, such as high-intensity pulsed lamps, can lead to distinct outcomes, including protein precipitation in milk [44]. UV-C treatment can also result in changes in cheese quality, including off-flavors and alterations in volatile compounds [45, 46] highlighting the presence of off-flavors due to specific compounds and a "burnt" flavor in treated cheese samples.

High-Pressure Processing (HPP): High-pressure processing (HPP) is a crucial non-thermal process in which pressure is applied to liquid or solid foods with or without packaging, often between 400 and 1000 MPa (1–20 min), to inactivate microorganisms and make them shelf-stable. HPP is used for food-borne microorganisms, for example, when milk is exposed to 650 MPa of pressure for 10 minutes considerable decrease in the number of live microorganisms is observed [47]. There were about 19 HPP commercial plants in the food industry and this had grown to about 112 units with a total value of over US $2 billion [48-50].

HPP primarily affects the non-covalent bonding (hydrogen, ionic, and hydrophobic bonds). High molecular weight components are susceptible because their tertiary structure plays a crucial role in determining their functionality, but low molecular weight compounds, which are responsible for food's nutritional and sensory qualities, are unaffected by HPP [51, 52]. The effect of high pressure on milk components has been given in table 2.

Table2. The effect of high pressure on milk constituents

Pressure	Changes	References
101.32-295MPa	1. The Casein Micelles are disintegrated into smaller particles. 2. An increase of caseins and calcium phosphate levels in the serum phase of milk 3. Decrease in both non-casein nitrogen and serum nitrogen fractions 4. Irreversible denaturation of whey proteins 5. Casein micelles native confirmations do not alter through minimal pressurization but on severity of treatment through pressure they get denatured. 6. β-lactoglobulin (β-lg) recognized to be pressure-sensitive while α-lactalbumin (α-la) as pressure resistant protein fraction.	[24]
300MPa	7. As per the gathered data, shelf life could be enhanced for milk if subjected to pressurization of 400-500MPa range for 15 min and 3 min respectively for pasteurized milk to 10 days at 10˚C.	[21, 75]
>295Mpa	Irreversible denaturation of β- lactoglobulin	[25]
500 Mpa	Denatures lactalbumin and immunoglobulins.	[9, 24]
690MPa at 10-30 min.	Improved hardness, surface hydrophobicity, solubility, gelation, and emulsifying properties of whey protein	[26, 95]
100-500MPa	Aggregation and disaggregation /disintegration of fat globules - improved the stability of milk treated at 25 and 50° C and cream separation of butter at 4^0C; No effect on FFA – avoid off-flavour	[27]
100–400 MPa for 10–60 min at 25 °C	No effect on Maillard reaction or lactose isomerisation	[7, 28]
400 MPa for 30 min at 25 °C	Vitamins, amino acids, simple sugars and flavour compounds remain unaffected.	[6]
300-600 MPa, 5 min	Retention of an essential vitamin B12 retain their level in HHP treated milk in competition with thermal treatment subjected milk, to 10% losses as of UV-C exposure.	[115]

Impact on Food Structure and Functionality: The utilization of high-pressure processing (HPP) at 600MPa for aduration of 3 minutes has demonstrated substantial efficacy in reducing microbial populations in milk [53] and found that major pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella spp*. were reduced by more than 5 log cycles. Furthermore, when compared to pasteurized milk, this specific HPP application resulted in a significant decrease in total viable count (TVC), *Enterobacteriaceae*, lactic acid bacteria (LAB), and *Pseudomonas spp*., extending the microbiological shelf-life of milk by 7 days.

While this particular HPP process proves to be effective for microbial reduction, it is important to note its limitations concerning spore inactivation. Multiple studies, [54-58] observed no effect or only a limited effect on spores when HPP is used as a sole treatment. This is particularly significant for certain spore-forming microorganisms that exhibit higher resistance. However, the literature presents differing perspectives on the inactivation of spores using HPP and [59-61] suggests that spore inactivation is achievable with HPP under specific conditions. Temperature is highlighted as a crucial variable influencing the effectiveness of spore inactivation during HPP, as noted by [62].

The resilience of spores to pressure treatment can be attributed to their complex structure and low water activity within the cortex layer [63]. This complexity emphasizes multiple protective actions of spores, which in turn contributes to their enhanced resistance to external stressors like high pressure.

High-pressure processing (HPP) has been explored for its impact on various components of food products, with distinct effects observed on different molecular compounds. HPP treatment appears to have little effect on low molecular weight chemicals such as vitamins and volatile substances [64-68].

HPP is suitable for fermented probiotic products in some situations [69-71] and HPP treatment of milk-based beverages has been found to improve calcium bioavailability over thermally treated counterparts [72].

The application of HPP extends beyond microbial safety, showing preservation benefits in human milk. HPP preserves adipokines and growth factors and increases levels of lactoferrin, and Immunoglobulin G (IgG) compared to traditional pasteurization [73]. Additionally, HPP can influence allergenicity, altering proteins like beta-lactoglobulin in bovine milk by increasing antigenicity [74, 75].

Cheese production is more advantageous if exposed through HHP processing, in terms of more sensory appealability and hence much adaptation through commercial means. The beneficial traits depicted in them as coagulation time, ripening, and curd strength [76]. It is experimentally proven that HPP improves coagulation and cheese ripening characteristics, reduces syneresis in yogurt, and slows down ice cream melting. Increased firmness in HPP yogurts could be attributed to enhanced interaction surfaces [77]. Control of microorganisms in milk by high-pressure application is depicted in Table 3.

The mild effect of HPP on product components contributes to extended shelf life. HPP has been observed to prolong shelf life in cheeses and influence enzymatic activity and casein micelle structure, accelerating ripening [78-81]. Casein micelles treated with HPP exhibited similar sizes to those in raw milk, contrasting with pasteurized milk [82]. Table 3 explained the effect of HPP in controlling microorganisms in milk and dairy products.

HPP's influence on protein structure is complex. While some studies suggest no impact on protein primary or secondary structure [83, 84] others propose that higher pressures can lead to irreversible denaturation of proteins and disruption of casein micelles [85-87]. Denaturation of α-lactalbumin and bovine serum albumin in skim milk is suggested only at pressures exceeding 400MPa [88].

Research on cheeses made from fresh pasteurized cow milk indicates that HPP increases resistance to deformation, lowers fractur ability, and affects color [89]. Additionally, it was found that pressurized cheeses were firmer, exhibited increased yellow color, and decreased lightness during storage. Preferences were similar for fresh and shelf-life-aged cheeses.

Ultrasonic Waves: Powerful ultrasonic waves generate force when they strike the food's surface. These forces pass through the food products either as a shear wave (parallel to the surface) or a compressive wave (which hits the surface perpendicularly). As both waves are attenuated, they move through food causing shear disruption, free radical production, localized heating due to rapid changes in pressure or temperature, cavitations (the formation of thousands of bubbles in liquid foods that collapse and then generate micro-current), and the thinning of cell membranes.

Table 3. Control of microorganisms in milk by high pressure application [33]

Foods	Pressure (MPa)	Time (min)	Organism (s)
Whole milk	680	10	Natural microflora, *Listeria monocytogens*
Skimmilk	400	30	Aerobic bacteria *Psychrotrophs, Pseudomonas, Enterobacteriaceae*
Ewes milk	200-500	5-15	*Listeria innocula* 910 CECT

Impact on Microbial Ecology: Pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*and total coliform count [90] were inactivated in sonication treated milk. Thermo-sonication, which combines heat and ultrasound, was successfully used to reduce the D-value of *E. coli* K12DH5 UHT (Ultra-High Temperature) and *Listeria monocytogenes* (Skim milk) from 2.1 minutes to 0.3 minutes and 77 seconds to 33 seconds, respectively at 60^0C [91].

D-value of *Listeria monocytogenes* was successfully reduced by mano-sonication (pressure plus ultrasound) treatment of skim milk to 1.5 minutes at 200 kPa while mano-thermosonification (pressure plus heat plus sonication) increased its lethality toward Listeria cells [92] and discovered that the yield and activity of chymosin extraction improved following the treatment that reduced the processing time (20 kHz and 25°C for 80 minutes) [93].

This can offer more chymosin to the cheese-making industry. When milk containing chymosin was subjected to the manothermosonication process, the coagulation time did not change [94] and the liberation of enzymes from *Pseudomonas fluorescens* during milk storage under refrigerated conditions by manothermosonication (110-140^0C, 650 KPa) treatment could also lessen the quality problem [95].

Nutritionally, the sonication is attributed to elevated antioxidant capacity as of cavitation's effect on the proteins and hence its disruption among its quaternary and tertiary structures [96]. Ultrasound is responsible for the denaturation of protein by the effect of free radicals on disulphur bonds [97]. Furthermore, ultrasound-treated milk's viscosity decreases from 1.9 CP to 1.77 CP, and due to the homogenization-like impact on milk fat [98], the pH of sonicated milk also reduces significantly due to the hydrolysis of phosphoric esters and activation of some chemical reactions as well. Cavitations might be the cause of this. More homogenous aggregation of smaller fat globules carrying out change in light reflection is an additional advantage to the milk exposed to ultrasound-assisted cavitation that dwells in reduction of fat globule size. In milk that had been subjected to ultrasound treatment, the cavitations also reduced the size of the fat globules and caused a more homogeneous aggregation of the globules and changing the light reflection. Additionally, the milk's lightness (L*) and redness (a*) values were raised, which led the milk to be white in appearance [45].

When evaluated to heat-treated milk or conventionally pasteurized products, milk beverages that have undergone sonification display better color, homogeneity, appearance, and storage stability due to inactivation of protease and lipase from Bacillus spores.

Compared to traditional/conventional processes, the ultrasound treatment milk showed 20% higher lactose breakdown [99] and reported that sonication induced for homogenization of human milk, reduce fat globules and maintain a longer stable emulsion and less losses of nutrients (mainly immunoglobulin A and G) in final products in comparison to previously pasteurized, homogenized, frozen or lyophilized human milk used for feeding newborn babies.

By increasing the acid in the yogurt, the ultrasound shortened the fermentation process. This might be caused by the release of microbial intracellular enzymes, which then led to a rise in the enzyme-galactosidase activity as a result of cavitation [100]. The whey syneresis in yogurt was reduced during transportation and/or storage by mano-thermosonicating the milk (12 s, 20 kHz, 2 kg pressure, and 40°C). Because the components (fat and serum) of the casein micelles network interacted better with one another, the sonicated yoghurt also displayed better viscosity, WHC (water holding capacity), structure, and rheological parameters like penetration, relaxation forces, texture profile analysis parameters, flow behavior index, yields stress, storage modulus (G'), and loss modulus (G") than control yogurt [101].

In cheese preparation, superior curd firmness was reported in sonication applied cheese milk. This may be because the curd firms more quickly. In addition, the activity of associated enzymes chymosin, pepsin and other related enzymes speeds up by use of ultrasound treatment and thus may be responsible for firming [102]. Ultrasound was also used to get “cell-free extract” of *Lactococcus Lactis* subsp. *Cremoris.* The extraction of peptides and amino acids becomes faster and easier, so the flavor of the resulting cheese is improved [103]. The product "queso fresco" (a typical Hispanic cheese product) was found to have a whiter color, hardness, higher WHC, higher yield, and ultimately better consumer acceptance.

Pluses Pulse Electric Field (PEF) Technology: Pulse electric field (PEF) is one of the non-thermal techniques that employs short to high pulses of electricity to inactivate microbes and bring out the least destructive impact on food quality. It is regarded as the most effective technique for food quality attributes when compared to conventional thermal treatments since it significantly lowers the adverse impact on the sensory, physical and nutritive attributes of food [104]. The food is exposed to high-voltage pulses of high intensity (20-80 kV/cm) and short duration (1-100 µs) that are applied between the electrodes[51]. The high field intensities are attained by accumulating a significant quantity of energy from a DC power source in a capacitor bank (a collection of capacitors), which is subsequently released in the form of high-voltage pulses to generate an electric field in the food [105].

Several events lead to the inactivation of bacteria when food is exposed to electrical high-intensity pulses such as (a) Electric breakdown- The natural potential difference exists between membranes [106]. As the voltage increases (>10mV), the reduction in cell membrane thickness also increases (PD α field strength). When the voltage reached 1 V, a breakdown of membranes occurred, resulting in the formation of transmembrane pores. (b) Electroporation is the phenomenon in which a cell's lipid bilayer and protein membranes are momentarily made unstable by high voltage electric field pulses. Due to the loss in permeability, small molecules can pass through the plasma membranes, which leads to swelling and ultimately the cell membranes rupture. PEF is performed at temperatures that are ambient, slightly above ambient [107].

Tailored Approaches for Specific Food Matrices: One of the liquid foods that is believed to be the most electrically conductive in PEF technology is milk. Electric pulse fields extend the shelf life of pasteurized liquids by deactivating native bacteria and enables pasteurization at lower temperature [108]. The electric pulse field deactivates natural microflora and allows for lower pasteurization temperature while improving the shelf life of pasteurized liquids [109]. When milk and dairy products, like skim milk, SMUF and yoghurt etc. are exposed to high intensities pluses, several pathogens experience a log reduction in number.

It has been reported that there was a 90% inactivation of plasmin (30kV/cm-2µs at 15^0C) from bovine milk treated in SMUF, 96% of alkaline phosphatase (18.8 kV/cm-2µs at 22^0C) and 60% protease and lipase from raw milk [110]. Some references suggest that PEF alone may not be as effective in inactivating some bacteria as mild heat combined with PEF [111]. From a nutritional standpoint, no changes in water-soluble vitamins (riboflavin, thiamin, and ascorbic acid) or fat-soluble vitamins (cholecalciferol and tocopherol) were seen after PEF treatment (18.3 to 27.1 kv/cm-400 s) [112].

Pulsed Electric Fields (PEF) technology offers a promising approach for food processing, as it minimally impacts food components while maintaining a fresh taste and preserving valuable compounds. PEF's effects on vitamins have been investigated in whey protein isolate (WPI) formulations [113] and found that PEF processing (32 kV/cm, pulse width of 3 s, inlet temperature 40°C, pH 4-7) did not result in substantial vitamin A and vitamin C losses. Elution profiles from High-Performance Liquid Chromatography (HPLC) revealed that these vitamins were chemically unaltered throughout PEF treatment. In the same investigation, PEF treatment had effect on immunoglobulins (IgM and IgG) in WPI formulations.

In the context of goat milk, a study by Mohamad et al. (2020) indicated that PEF treatment (20–40 kV/cm, monopolar square wave pulses of 5 and 10 µs treatment time, pulse width of 8 µs) led to reductions in total saturated fatty acids (SFAs) and total polyunsaturated fatty acids (PUFAs), along with an increase in total monounsaturated fatty acids (MUFAs). This shift was attributed to fat disintegration and triacylglycerol breakdown from the fat globule membrane, coupled with oxidative degradation facilitated by heterogeneous metal catalysis. The authors suggested the need for further investigation to comprehensively understand the underlying mechanisms.

The effect of PEF treatment on the sensory qualities of dairy liquids is still being studied. Few studies have been conducted on this subject in recent years [114] and investigated the volatile profile of milk after PEF treatment (15-30 kV/cm for 800 s, bipolar square-wave pulse width of 2 s, pulse repetition rate of 200Hz, and temperature 40°C). They discovered a rise in aldehydes without changing the methyl ketones, but heat treatment (75°C for 15 seconds) increased both substances. There were no discernible variations in the makeup of alcohols, acids, and lactones among raw, heat-treated, and PEF-treated milk, except for specific compounds like 2(5H)-furanone and an unidentified "fatty and waxy" odorant, which were present in PEF-treated milk.

Similarly, color analysis of PEF-treated milk conducted by McAuley et al. (2016) (30 kV/cm for 22 µs) demonstrated marginal changes in lightness. These studies collectively indicate that PEF technology has a minor impact on volatile compounds and color properties, suggesting that PEF-treated products may retain a sensory profile closely resembling that of raw or pasteurized milk. The microbial inactivation in milk and dairy products with PEF is given in Table 4.

Membrane Technology: In the modern era, membrane processing plays a vital role in the dairy industry for clarification, concentration, along with separation of particular milk ingredients from milk and milk by products. The figure illustrates four distinct kinds of membrane filtration processes with different properties commonly used in the dairy industry i.e. microfiltration (MF), nanofiltration (NF), ultrafiltration (UF), and reverse osmosis (RO)[115]. Fractionation of milk can be done by membrane separation technology based on milk components size. By lowering production costs and establishing new sources of income, membrane technology contributes in the improvement of economies of the dairy. The dairy sector has used membrane technologies in a variety of areas including the cheese industry, shelf-life extension of milk, whey and milk protein processing, and separation of milk fat and desalting or demineralization of milk by products [116]. Table 4 explained the various microbial inactivation strategies in milk and dairy products.

Table 4. Microbial inactivation in milk and dairy products with PEF [16, 45]

Target microorganism	**Medium**	**Processing conditions**	**Log Reduction**	**References**
Saccharomyces cerevisiae	Yogurt	1.8 V/µm, 55ºC, batch system	3	[59, 121]
Escherichia coli	Milk	3.3 V/µm, 43ºC, 35 pulses, batch system	3	[59, 121]
Escherichia coli	Skim milk	4.0 V/µm, 15ºC, 3µs, 64 pulses	3	[50, 55, 61]
Escherichia coli	SMUF*	2.5 V/µm, 25ºC, 20pulses, batch system	3	[67, 85, 92, 119]
Salmonella Dublin	Milk	3.67 V/µm, 63ºC,36 µs, 40 pulses,batch system	4	[59, 121]
Lactobacillus brevis	Yogurt	1.8 V/µm, 50ºC, batch system	2	[59, 121]
Bacillus subtilis	SMUF	1.6 V/µm, monopolar, 180 µs,13 pulses, batch system	4.5	[23]
Pseudomonas fragi	Milk	9.0 V/µm, 1 µs, batch system	4.5	[62]
***Staphylococcus aureus* ATCC 6538**	SMUF	30ºC, 1.6 V/µm,200-300 µs, 60pulses batch system	3.4	[63]

Reverse osmosis is a membrane filtration process that uses an extremely dense membrane and is powered by high pressure. It concentrates the total solids by allowing only water to pass through the membrane film. Reverse osmosis is usually used in the dairy sector to pre-concentrate, concentrate, or reduce the volume of milk and whey, recover milk solids, and recycle water. Monovalent ions can flow through the membrane structure of nanofiltration, a moderate to high-pressure operated membrane filtration process. The membrane is mostly responsible for the retention of divalent ions [117]. The key uses of nanofiltration in the dairy sector include the volume decrement of whey, partial demineralization of whey and whey powders, lactose-free milk, and the purification of CIP solutions. A membrane having a medium open structure is used in the moderate pressure-driven ultrafiltration process. The majority of dissolved and some non-dissolved components can pass through it, but the membrane retains bigger components like protein and fat [118].

Whey protein concentration (WPC), milk protein concentration, standardization, and to enhance the yield of fermented milk products are all common uses of UF in the dairy sector. A membrane filtering method termed microfiltration uses an open-structured membrane and low pressure. While the majority of non-dissolved components are retained by the membrane, it permits dissolved components to pass. Microfiltration is frequently used in the dairy sector to reduce bacteria and spores, lactose reduction, remove fat from milk and whey, and to standardize casein and protein.

Integration With Sustainable Practice: Tetra Pak International SA in Switzerland has successfully used the Bactocatch system to remove microbials from skim milk using a 1.4m cross-flow transmembrane ceramic membrane [119, 120]. Furthermore, IsofluxTM ceramic membranes have shown success in creating Extended Shelf Life (ESL) milk in conjunction with heat, facilitating spore removal while keeping a steady flux, allowing them to be extensively used.

Membrane filtration has already been shown to be useful in the manufacture of ESL milk, [121] as well as in the separation of dairy proteins, which yield helpful peptides when hydrolyzed. This mechanism has the potential to diminish enzymatic activity. Despite these advances, increased fractionation efficiency and higher throughput are still required.

Skrzypek and Burger (2010) observed that when paired with heat treatment, IsofluxTM ceramic membranes are well-suited for ESL milk production. When stored at 4-6°C alongside pasteurization, microfiltration (MF) can reduce the temperature of UHT milk by 20°C, potentially increasing its shelf life to 30 days [110]. Protein retention and commercialization are made possible by MF.

Balancing the imperatives of safety and nutritional quality for ESL milk poses a significant challenge. While maintaining a consistent flux is challenging, membranes with a pore size of 1.4µm can ensure continuous flux, prevent the rejection of valuable compounds such as proteins, and achieve microbial reduction. However, a limitation arises due to the similarity in size between fat and microbes, leading to rapid fouling and diminished system performance.

The utilization of brine obtained through microfiltration (MF) has demonstrated its potential in producing higher quality ripening cheeses when compared to brine derived from heating processes [122] and conducting triangle tests involving MF and non-MF skim milk to discern differences during shelf-life. Results indicated that, after 5 days of refrigeration, random panelists were unable to identify any disparities, but after 24 days of refrigeration, significant differences emerged. These differences were attributed to a "lighter taste," "less sweet" profile, and reduced creaminess in the MF milk samples. The authors reasoned that these observations were influenced by the decreased solid content in the MF milk. Interestingly, trained panelists did not detect any variations over the 24-day shelf-life period [123] which emphasized that the integration of membrane technologies in the dairy industry led to products of enhanced quality, paved the way for innovative advancements, and highlighted the improvement of sensory characteristics in milk due to membrane filtration processes. This collective body of research underscores the potential of membrane technologies, particularly MF, to contribute to the enhancement of dairy product quality and sensory attributes [124].

Oscillating Magnetic Field: An environmentally responsible way to extend the shelf life of milk and dairy products is through the use of magnetic fields in dairy manufacturing operations. Milk can get contaminated by a variety of bacteria both during and after milking, even though the udder is sterilized. For example, mastitis can be caused by *staphylococcus aureus*, *streptococcus uberis*, and *coliform spp*. [125]. Because bacteria that cause food to spoil are inhibited by magnetic fields, they have lately been used to eradicate pathogens. Microorganisms in non-conductive environments may be killed or rendered inactive by applying high-intensity magnetic fields at a reasonable frequency. Bacteria in milk and food are eliminated by an oscillating magnetic field. In non-conductive environments, high-intensity magnetic fields with a reasonable frequency can be used to either kill or inactivate microorganisms. Bacteria in milk and food are eliminated by relatively brief treatments in the form of pulses when subjected to an oscillating magnetic field; this prevents a discernible temperature rise in the finished goods.

Magnetic Field Application in Dairy Processing Operations: Meals, whether solid or liquid, can be stabilized with oscillating magnetic fields. Liquid meals can be pushed through a conduit, while solid meals are stored with OMF by being sealed in a plastic bag. The product is exposed to 1 to 100 pulses in an OMF at frequencies between 5 and 500 kHz, at 0 to 50 °C, for a total exposure time between 25 and 100 ms. Barbosa-Cánovas et al. (1998) found that higher frequencies tend to heat food and are less effective at inactivating microorganisms.

Milk and milk products can be pasteurized or treated in other ways. At a field intensity of 12 tesla, the treatment of milk reduced the number of bacteria from 25000 to 970 cfu/ml. Additionally, magnetic milk gives tired individuals energy and strength [126]. Milk can be steadily magnetized by immersing a magnetic device in a container of milk and leaving it there for four to six hours. The resulting magnified milk has several uses, including improving milk quality, promoting sexuality, healing ailments, and preserving food quality by minimizing heat damage.

It has been demonstrated that food microorganisms inactivate when exposed to OMFs at intensities greater than 2 t [127]. The number of microorganisms was reduced by two log cycles by a single 5-50 t pulse at a frequency of 5-500 kHz. Food can be pasteurized using this method by magnetizing the sample and placing it in a magnetic field.

Risks to workers' health at work include (i) milk contaminated with *streptococcus thermophilus*; (ii) yogurt contaminated with *Saccharomyces cerevisiae*; (iii) orange juice contaminated with *S. cerevisiae*; and (iv) bacterial spores in brown 'n serve roll dough. These investigations also revealed that the temperature rises during the magnetic field is essentially non-existent (maximum 2°C) and that the decrease in microorganisms occurs in a range of 1.4 to 3.6 log cycles. A magnetic field strength of between 2 and 100 teslas would work just as well as the optimal range of 5 to 50 tesla. Less magnetic strength is often required to destroy germs when materials that are contaminated have higher electrical conductivity.

Foods with low electrical conductivity need to be oscillated 10-100 times in order to achieve complete disinfection. Effective liquid and powder separation of iron and stainless-steel particles. Compared to powders, smaller quantities can be removed from liquids like milk, butter, or whey. A metal detector cannot pick up on the little metal particles found in milk powder [128].

The creation and application of magnetic separation techniques that make use of minute magnetic particles have seen a recent upsurge in interest. The assessed magnetic methods for the purification and separation of proteins and peptides includes magnetic resonance imaging (MRI): Due to its non-invasive and non-perturbing characteristics, MRI is a special instrument for in-situ and real-time measurements, which makes it appropriate for food quality monitoring.

SUSTAINABILITY AND ACCEPTANCE:

Effective communication and accurate terminology play a pivotal role in shaping consumer perceptions and acceptance of novel non-thermal technologies in the dairy industry. Educating consumers about the benefits of these technologies is paramount, as they often perceive them as risky or overestimated. This perception leads to resistance and reluctance to pay more for non-thermal products compared to traditional ones. Additionally, consumers tend to reject novel non-thermal products due to a lack of familiarity [73].

Studies have indicated that transparent labeling and clear information about novel non-thermal processes can enhance consumer intention to purchase these products. Simplified, concise labels that highlight the advantages of the technologies are preferred by consumers and can contribute to their acceptance. However, there remains cautiousness among consumers regarding unfamiliar processing terms, which may act as a barrier to adoption.

The choice of terminology can significantly influence consumer perceptions. For instance, "Micro Electric Pulse" was associated with more neutral reactions compared to "Pulsed Electric Field," despite describing the same technology [116]. Moreover, consumers' age and attitudes toward unconventional treatments can impact their acceptance of non-thermal technologies.

Consumer acceptance of UV-C-treated dairy products is influenced by the information provided on labels. For example, a study showed that providing explanations about the benefits of UV light treatment increased consumer acceptance, highlighting the importance of informative labeling [117]. Similarly, highlighting the preservation of high nutritional value and safety in High-Pressure Processing (HPP) products can lead to higher consumer acceptance due to perceived naturalness and premium quality [118].

Consumer willingness to purchase and pay more for non-thermal products is often driven by perceived safety, added value, and premium quality. HPP, for instance, has shown significant potential for consumer acceptance due to its preservation of nutritional value and safety attributes. Cold plasma processing also demonstrated that maintaining consumer acceptance is possible despite changes in product consistency, emphasizing the feasibility of commercializing plasma-treated products [118].

FUTURE THIRST/ CHALLENGES:

In recent years, numerous studies have explored novel non-thermal processing technologies in the realm of dairy production. The next crucial phase involves the scaling up of these processes to eliminate their limitations and to ensure alignment with consumer preferences. The industrialization of these technologies necessitates the establishment of efficacy and safety parameters. Industries must comprehend the underlying mechanisms of action, pinpoint critical control points within the production line, assure product quality, and integrate risk and cost analyses to facilitate the upscaling process. It's essential for industries to strive for cost-effectiveness and gather data specific to the technology in comparison to traditional thermal treatments. For instance, conventional thermal processes can be readily monitored by assessing milk enzymatic activity, such as alkaline phosphatase activity, which serves as an indicator of pasteurization adequacy. Similarly, novel non-thermal technologies require the development of rapid assessment indicators to ensure product safety and efficacy. However, the challenge with these novel technologies lies in their dependency on various product parameters that influence processing outcomes. Factors like product surface properties, opacity, turbidity, light intensity, microbial species, and other variables are crucial for end-product safety. As a result, specific studies are essential for each technology-product combination to define the necessary conditions for product safety. Furthermore, optimizing product penetration and efficiency is vital to counterbalance the high initial investment costs. The efficacy of High-Intensity Ultrasound (HIUS) technology also hinges on precise processing parameters. Currently, there is a lack of consensus on presenting these parameters, hindering cross-study comparison. Further research should aim to establish processing parameters for different dairy products and determine the inactivation kinetics of various pathogens. The integration of quantitative risk analysis models is a promising direction for future research to enhance food quality management. Scaling up Pulsed Electric Fields (PEF) equipment and processes presents a challenge due to extra capital expenses. Balancing these costs against premium-priced products is necessary. Additionally, laboratory-scale trials should be compared to results from industrial-scale operations to ensure reliability and feasibility. The same challenge applies to technologies like High-Pressure Processing (HPP), Cold Plasma, and Ultrasound. In parallel, consumer education is pivotal for the acceptance of products derived from novel technologies. Consumers often exhibit skepticism toward unfamiliar terms like "irradiation." Hence, creating awareness and familiarity with these technologies becomes a crucial step toward gaining consumer trust and acceptance.

CONCLUSION:

Non-thermal methodologies, such as high-pressure processing (HPP), pulsed electric field (PEF), ultrasound (US), and ultraviolet (UV) irradiation, present innovative approaches to bolster microbiological safety, uphold nutritional excellence, and enhance operational efficiency within dairy processing. These cutting-edge technologies are proficient in deactivating microorganisms effectively, ensuring food safety while safeguarding the nutritional value of dairy items. By embracing non-thermal practices, dairy manufacturers can prolong product shelf life, diminish spoilage, and reduce nutrient depletion, thereby enriching the overall quality of the merchandise. The amalgamation of non-thermal techniques with edible coatings can further heighten the safety, quality, and shelf life of dairy goods, providing a sustainable avenue for food processing. Grasping the mechanisms and variables of these technologies is imperative for optimizing their utilization in dairy processing, thereby charting the course for safer, superior-quality dairy products. The exploration of innovative non-thermal processing technologies within the dairy sector signifies a thrilling frontier with substantial potential for breakthroughs. Nevertheless, the transition of these technologies from laboratory settings to large-scale industrial applications necessitates surmounting various obstacles. This necessitates establishing efficacy and safety criteria, comprehending the underlying mechanisms of operation, pinpointing crucial control points, ensuring product quality, and integrating risk and cost assessments into the upscaling process. Additionally, the formulation of prompt evaluation indicators is vital to guarantee the safety and effectiveness of products processed using novel technologies. Variables such as product characteristics and microbial strains must be thoroughly examined to define the requisite conditions for product safety.

Ultimately, while the expedition towards industrializing innovative non-thermal processing technologies in dairy production may pose challenges, it also presents vast opportunities for enhancing food safety, quality, and innovation in response to evolving consumer demands.

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