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Revolutionizing dairy: Exploring the potential of non-thermal processing methods for milk products: A review

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Abstract

Microorganisms in milk and dairy products are a major threat to the food and dairy industries. The application of nonthermal methods in the dairy industry has the potential to satisfy consumer demands for minimally processed and nutritious dairy products, while also offering a wide range of product innovation opportunities. The objective of this review is to provide a thorough overview of the use of non-thermal procedures in dairy and milk products, as well as how they affect the product's microbiological, and nutritional qualities. In addition, the impact of these procedures on the quality attributes of the product is also discussed.

Keywords: High-pressure processing, dairy products, nonthermal methods, pulsed electric field, microorganisms

1. Introduction

Milk and dairy products are a significant source of nutritious components for consumers (Pereira *et al.*, 2014; Ortega *et al.*, 2019) ^[1-2]. The food business has long been quite concerned about food scandals caused by bacterial contamination resulting in food poisoning occurrences, notably dairy products. The functionality and quality of many food components are negatively impacted by thermal treatment, despite the fact that it is currently the most popular process for producing goods and ensuring food safety (Bandla *et al.*, 2012; Liepa *et al.*, 2016; Silva *et al.*, 2020; Coolbear *et al.*, 2022; Huppertz and Nieuwenhuijse, 2022) ^[3-7].

According to recent studies (Silva *et al.*, 2020; Minj and Anand, 2020; Asaithambi *et al.*, 2021) ^[5, 8, 7] on nutrient-rich goods with established health advantages, new trends are emerging. Consumers are becoming more particular about the foods they buy these days. In order to meet consumer demands, the industrial sector is simultaneously looking for noninvasive processes that address quality aspects and nutritional degradation time (Valdramidis and Koutsoumanis, 2016; de Toledo Guimares *et al.*, 2018; Chakka *et al.*, 2021) ^[10, 11, 21].

Traditional techniques employing thermal energy are getting less popular and non-thermal technologies are emerging (Jermann *et al.*, 2015; Valdramidis and Koutsoumanis, 2016; Baboli *et al.*, 2020) ^[13, 10, 14]. Non-thermal technologies need electrical, electromagnetic, light, and mechanical forces rather than thermal energy (Rodriguez-Gonzalez *et al.*, 2015) ^[15]. In general, heat treatment is a less sustainable processing technique compared to innovative developing technologies owing to high costs, energy, and water consumption (Guimarães *et al.*, 2021) ^[16]. They are frequently referred to as approaches that are effective at ambient and sublethal temperatures (Cullen *et al.*, 2012) ^[147] without physically exposing the product to heat (Chacha *et al.*, 2021) ^[18].

Novel non-thermal approaches have demonstrated their efficacy in the microbial inactivation of milk and dairy products, while preserving their nutritional value and functionality (Shabbir *et al.*, 2020) ^[19]. This has drawn significant attention from researchers and dairy industry professionals. Non-thermal methods not only help retain the sensory and functional properties of dairy products but also hold promise for creating innovative and healthful dairy products. In fact, (de Toledo Guimares *et al.*, 2018) ^[11] have highlighted the potential of non-thermal methods for the development of novel and health-promoting dairy products. The application of such non-thermal approaches in the dairy industry has the potential to satisfy consumer demands for minimally processed and nutritious dairy products, while also offering a wide range of product innovation opportunities.

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The objective of this review is to provide a thorough overview of the use of nonthermal procedures in milk and milk products, as well as how they affect the product's microbiological, and nutritional qualities.

2. High- Pressure Processing (HPP)

High Pressure Processing uses high pressure, typically 100-600 MPa for up to 20 minutes, to kill harmful bacteria and extend the shelf life of liquid and solid goods (Silva and F.V.M., 2015) [20]. This non-thermal approach is not new and has long been used in a variety of non-food businesses (Show *et al.*, 2012) [21]. The use of HPP on food was first documented in the late nineteenth century. Although the commercialization of this non-thermal approach is new, as seen by the growth in the number of HPP units deployed worldwide, its use on foods has been explored for over 100 years (Sousa *et al.*, 2016) [22]. HPP has been shown to modify the properties of dietary proteins; however, this transformation is dependent on the power used, the time of treatment, and the temperature. It produces permanent alterations in the secondary, tertiary, and quaternary protein structures by primarily altering covalent bonds (Dhakal *et al.*, 2013) [23]. The following are the principles that describe the process's functionality:

- The principle of Le Chatelier (Naik *et al.*, 2013) [24].
- The Principle of Microscopic Ordering.
- The concept of isostatic equilibrium (Naik *et al.*, 2013; Huang *et al.*, 2020) [24-25].

2.1 Applications in the dairy industry

(Huppertz and Nieuwenhuijse, 2022) [7] noted the following features in their detailed review on the impact of high pressure on milk contents and properties:

- Denaturation of whey proteins, notably β -lactoglobulin
- Lowering of the freezing point of water
- Changes in mineral balance
- Crystallization and increased solid fat content of milk fat
- Breakdown of casein micelles, resulting in enhanced casein solubility.

2.2. Impact on microorganisms

L. innocua, *E. coli*, *S. aureus*, *L. monocytogenes*, *Bacillus* spores, and other microorganisms, as well as the various properties of these microorganisms in milk, were among the microorganisms that were inactivated as a result of pressure and temperature applied to them. HPP played a significant role in this process. *Clostridium sporogenes* PA3679 and *Bacillus stearothermophilus* ATCC 7953 were reduced by 0.67 log after HPP treatment (300 MPa, 84 °C on skim milk) (Pinho *et al.*, 2011) [26]. *L. monocytogenes* ATCC 19115, and *Staphylococcus aureus* ATCC 25923 were decreased by 6-log and 8-log respectively after HPP treatment (400 MPa, 21 to 31 °C, and 0 to 50 min) was applied to human milk (Viazis *et al.*, 2008) [27].

According to (Strakos *et al.*, 2019) [28], *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp. in milk were reduced by more than 5 logs at pressure of 600 MPa for three minutes. Additionally, compared to pasteurized milk, the particular application decreased TVC, Enterobacteriaceae, LAB, and *Pseudomonas* spp. and increased the microbiological shelf life of milk by 7 days. Despite being effective for microbial reduction, the particular technique has little to no effect on spores when employed alone (Mjica-Paz

et al., 2011; Penchalaraju and Shireesha, 2013; Balasubramaniam *et al.*, 2015; Stratakos *et al.*, 2019; Huang *et al.*, 2020) [29-31 28, 25]. While other research (Penchalaraju and Shireesha, 2013) [30] note that different temperatures can be used to accomplish spore inactivation (Shao *et al.*, 2010; Gao *et al.*, 2011; Balasubramaniam *et al.*, 2015) [32, 33, 31]. The numerous layers and low water activity of the cortex may be to blame for the strong resilience of spores to pressure (Parekh *et al.*, 2017) [34].

3. Pulsed Electric Fields (PEF)

Pulsed Electric Field (PEF) has garnered attention as a promising technique for eradicating microorganisms, particularly in liquid food products (Pal, 2017) [35]. PEF demonstrates significant potential in effectively eliminating both harmful pathogens and spoilage-causing microbes, as well as enzymes responsible for the deterioration of food quality, all while preserving consumer preferences (Alirezalu *et al.*, 2020) [36]. This innovative method boasts a distinct advantage by offering high-quality food and surpassing traditional thermal processing methods, as it minimizes detrimental alterations to nutritional content, quality, sensory characteristics, and physical attributes of food (Syed *et al.*, 2017) [37].

The inactivation of specific enzymes and microbes through PEF is attributed to the process of electroporation and the breakdown of cell membranes due to dielectric effects (Sharma *et al.*, 2014a) [38]. Several factors influence this process, including the number of pulses, electric field intensity, pulse width, flow rate, and shape. Additionally, parameters such as temperature, conductivity, and physiological characteristics of microbes can also impact the efficacy of the PEF treatment (Sharma *et al.*, 2014b) [39]. The fundamental working principle of PEF revolves around the application of high-intensity electric fields, administered in the form of short pulses lasting mere microseconds, typically ranging from 10-80 kV/cm. Processing time can be calculated by multiplying the actual number of pulses by the effective pulse duration. As the electric field permeates the liquid food sample, a current flow, distributing itself evenly among all points, facilitated by the presence of charged molecules. Following the treatment, it is crucial to aseptically package the food and maintain cold storage conditions to ensure an extended shelf life (Pal, 2017) [35].

3.1 Application in Dairy industry

PEF application (at 35 kV/cm, with a pulse width of 3 μ s and duration of 9 μ s) on raw skim milk exhibited no significant differences in protein content, color, moisture, and pH (Michalac *et al.*, 2003) [40]. Moreover, when PEF treatment (at 35 kV/cm, with a pulse width of 2.3 μ s) was applied immediately after high temperature short time (HTST) pasteurization at 65 °C for less than 10 seconds, it remarkably extended the shelf life of milk up to 78 days when stored at 4 °C (Sepulveda-Ahumada, 2003) [41]. In a study focusing on bovine immunoglobulin (IgG) enriched soymilk, PEF treatment at a dosage of 41 kV/cm for 54 μ s did not affect the activity of bovine IgG, while significantly reducing the initial microbial flora by 5.3 logarithmic units (Li *et al.*, 2003) [42]. Notably, sensory attributes of dairy products subjected to PEF treatment were found to be comparable to thermally treated products, and they received favorable acceptance from consumers (Sobrin-López & Martin-Belloso, 2008) [43].

3.2 Impact on microorganisms

Bacteria on exposure to pulse electric field are believed to be rendered inactive through the breakdown of their cell walls and membranes by high-voltage impulses. Research has demonstrated the efficacy of PEF in targeting various microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *L. monocytogenes*, and *S. cerevisiae* (Cserhalmi *et al.*, 2002) [44]. Initial investigations in this area have shown inhibitory effects on microorganisms through the application of high voltages ranging from 3000-4000V (Bendicho *et al.*, 2002) [45].

In the case of UHT skimmed milk, a 2 log reduction in *B. cereus* vegetative cells was achieved by applying a 90 μ s treatment at an electric field intensity of 35 kV/cm. Similarly, in UHT milk, a 3 log reduction in *B. stearothermophilus* was observed at 50 °C through a 210 μ s treatment at an electric field intensity of 60 kV/cm. According to (Walkling-Ribeiro *et al.*, 2011) [46], the microbial load of bovine skim milk, inoculated with native milk microorganisms, experienced reductions of 2.0, 2.1, 2.3, and 2.5 log₁₀ CFU/mL. These reductions were achieved by applying specific electric field strengths and treatment durations: 16 kV/cm for 2105 μ s, 20 kV/cm for 1454 μ s, 30 kV/cm for 983 μ s, and 42 kV/cm for 612 μ s. This effect was achieved using monopolar exponential decay pulses with a pulse width of 1.5 μ s. The temperature of the milk during the PEF process ranged from 16 °C at the inlet of the PEF chamber to 40-49 °C at the outlet. After PEF treatment, samples were cooled to 10 °C for analysis. In comparison, thermal pasteurization (at 75 °C for 24 seconds) conducted in the same study resulted in a microbial inactivation of 4.6 log₁₀ CFU/ml in the milk microflora.

4. Ultraviolet Light (UV)

UV light, a non-ionizing form of irradiation, consists of three spectrums: UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (200-280 nm) (Shabbir *et al.*, 2020) [19]. It is considered a non-toxic and environmentally friendly technique that utilizes physical energy (Delorme *et al.*, 2020). The procedure can be conducted with continuous or pulsed light, where the product absorbs the light photons (Rodriguez-Gonzalez *et al.*, 2015) [15].

The mechanism of UV light treatment can occur through various processes. One is photochemical, where chemical changes in DNA and RNA take place. Another is photothermal, which occurs when long-duration pulses increase the temperature and inactivate bacterial cells. Additionally, photophysical effects can occur, causing damage to the cell structure (Can *et al.*, 2014) [48]. Different types of lamps, such as mercury lamps and pulsed light, can be used for UV treatment (Rodriguez-Gonzalez *et al.*, 2015) [15]. According to (Can *et al.*, 2014) [48], pulsed light treatments are more effective than continuous UV light. These lamps generate pulses by compressing electrical energy delivered by a xenon gas lamp (Can *et al.*, 2014; Singh *et al.*, 2021) [48-49].

4.1 Application in Dairy industry

UV light treatment for pasteurization faces a significant challenge in milk due to its turbidity. Turbidity, caused by suspended and colloidal solids, reduces microbial inactivation by hindering the penetration of UV light. This opacity of milk poses a challenge in achieving effective pasteurization.

However, advancements in UV reactors have introduced two strategies to enhance UV light penetration in milk, opening new possibilities for the food and dairy industries.

The first approach involves creating a laminar flow of milk, forming a thin film on a UV-irradiated surface, allowing for complete light penetration through the milk. The second approach utilizes a turbulent flow of milk, bringing all liquid components in close proximity to UV-exposed surfaces. This reduces the required path length and facilitates better UV light penetration in milk (Datta *et al.*, 2015) [50]. These strategies have paved the way for the application of UV technology in pasteurization processes.

Studies investigating the impact of UV processing on the quality of whole milk have shown minimal changes in various parameters. Viscosity, color, pH, and soluble solid contents of milk remained largely unchanged. The pH of UV-treated milk ranged from 6.66 to 6.70, with an average viscosity of 2.00 ± 0.01 mPa s. The color change (ΔE^*) was minimal, ranging from 0 to 0.5, and the soluble solid content was $12.78 \pm 0.10\%$ (g/g) when whole milk was pasteurized and treated with UV at a dose of 10 mJ/cm² for a duration of 12 to 235 minutes (Orlowska *et al.*, 2013) [51]. These findings indicate that UV treatment had negligible effects on the measured quality attributes of the milk.

4.2 Impact on microorganisms

(Reinemann *et al.*, 2006) [52] demonstrated that treating raw cow milk with a UV dose of 1.5 J/mL using two different versions of reactors resulted in a 3-log reduction in natural microflora. (Bandla *et al.*, 2012) [3] investigated the efficiency of UV reactors (Dean flow) and their impact on the inactivation of *B. cereus* endospores and *E. coli* W1485 in various types of milk, including raw cow milk, commercially processed skim milk, and soymilk.

By utilizing a reactor (Dean flow) with a diameter of 1.6 mm and a UV dose of 0.05 J/mL, significant reductions in *E. coli* W1485 were observed, with over a 7-log reduction in skimmed milk and soymilk, and a 4-log reduction in raw cow milk. However, due to the limited UV transmission in raw cow milk compared to soymilk and skimmed milk, a higher UV dose was recommended for raw cow milk.

The treatment of UV poses challenges in milk and dairy products compared to fruit juices, as these products tend to contain higher levels of spoilage and pathogenic microorganisms. However, (Gupta, 2011) [53] found that in sweet, acid, and brine whey, the total bacterial count was reduced by 7 logs, suggesting the potential use of UV treatment in brine and whey for dairy product processing.

5. Ultrasound (US)

Ultrasound (US) technology is a widely utilized non-thermal processing method in the food industry due to its environmentally friendly, non-toxic, and gentle nature. It offers a broad range of applications (Shanmugam *et al.*, 2012) [54]. While the destructive effects of US on microorganisms have been known for a century, its implementation in the food industry to control and enhance microbial activities is relatively recent. Early research by (Harvey and Loomis, 1929) [55] demonstrated the significant bactericidal effect of US on luminous bacteria in aqueous environments (Ojha *et al.*, 2017) [56].

In the case of milk, the driving force behind ultrasound technology is the phenomenon of acoustic cavitation, which

occurs when ultrasonic waves pass through liquid milk. With successive cycles, cavities grow in size, leading to the formation of acoustic cavitation bubbles. These bubbles rapidly collapse in a violent manner within a very short timeframe (On the millisecond scale), driven by the attractive forces between molecules in the food system (Chandrajith *et al.*, 2018) [57]. Ultrasound operates through three distinct mechanisms:

- Pressure gradients created by the cavitation bubbles' collapse inside or close to the cells causing mechanical damage to the cell wall.
- Micro-streaming takes place within the cell.
- The breakdown of the cell wall structure as a result of chemical compounds formed during cavitation (Hernández-Hernández *et al.*, 2019) [58].

5.1 Application in Dairy industry

It has been employed for milk homogenization (Al-Hilphy *et al.*, 2012) [59], and innovative dairy products with distinct physicochemical and functional features may be generated using ultra-sonication alone or in conjunction with several standard homogenization procedures. (Jin *et al.*, 2014) [60] demonstrated that in situ ultrasonication enhanced crossflow ultrafiltration of skim milk. One of its uses in the dairy sector is microbial inactivation using sonication. The efficacy of microbial inactivation in response to ultrasound relies on the type of bacteria targeted. Gram-positive bacteria have a thick and securely adhering peptidoglycan cell wall layer that is sonication resistant (Chemat *et al.*, 2011) [61]. Gram-positive bacteria are more susceptible than Gram-negative microorganisms in general, but spores are more resistant than vegetative cells (Halpin *et al.*, 2013) [62].

5.2 Impact on microorganisms

Sonication treatment has been studied for its impact on microbial reduction in various types of milk. (Cameron *et al.*, 2009) [63] found that sonication treatment at 20 kHz for 10 minutes at 750 W resulted in a 5.34-log reduction in *E. coli* and a 2.07-log reduction in *L. monocytogenes* in raw pasteurized milk. Similarly, a 5.64-log reduction in *P. fluorescens* was observed at 6 minutes under the same ultrasonic conditions, indicating the high sensitivity of microbes to ultrasound treatment. In raw whole cow's milk with 4% fat, (Herceg *et al.*, 2012) [64] observed a 3.1-log reduction in *E. coli* after sonication treatment at 20 kHz, 120 μm for 12 minutes at 60 °C. (Matak *et al.*, 2005) [65] reported that sonication treatment at $15.8 \pm 1.6 \text{ mJ/cm}^2$ for 18 seconds resulted in a reduction of *L. monocytogenes* to 107 CFU/mL in goat milk. In UHT milk, ultrasound treatment at 20 kHz and 60 °C resulted in a 0.3 min D value for *L. monocytogenes* (Earnshaw *et al.*, 1995) [66]. Additionally, (García *et al.*, 1989) [67] observed a 70-49% reduction rate in *B. subtilis* and a 2.5-3-log reduction in *Salmonella Typhimurium* in skim milk after sonication treatment at 20 kHz, 150 W, and 100 °C temperature.

6. Membrane filtration (MF)

Membrane filtration has been used in the dairy sector since the 1960s (Ribeiro *et al.*, 2010; Kumar *et al.*, 2013) [68, 71] and works by separating substances by preventing some (Retentate) and allowing others (permeate) to pass through. Hydrostatic pressure (Transmembrane pressure) forces the liquid product across a membrane (Skrzypek and Burger,

2010; Kumar *et al.*, 2013; Shabbir *et al.*, 2020) [10, 71, 19]. Microfiltration (MF), Reverse Osmosis (RO), Ultrafiltration (UF), and Nanofiltration (NF) membranes are distinguished by the size of their pores and the point at which they are shut off (Ribeiro *et al.*, 2010; Kumar *et al.*, 2013) [68, 71].

The particles moving through the membrane during MF are 0.2-2 μm in size, and the pressure is substantially lower than in UF (Kumar *et al.*, 2013) [71]. Only water and low molecular weight chemicals may flow through semipermeable membranes used in UF, with a cut off of 10,000 MW (Molecular Weight). RO is a high-pressure technique in which only low-molecular-weight liquids may enter through the pores. By setting a cut off of 100 MW, NF may be assumed to be a form of RO, allowing only monovalent ions to pass through the membranes. The fraction that is held is known as "retentate" or "concentrate," whereas the part that goes through the membrane is known as "permeate" (Kumar *et al.*, 2013) [71].

Due to their inexpensive cost and low fouling percentage, cellulose acetate membranes are often employed (Shabbir *et al.*, 2020) [19]. According to (Leeb *et al.*, 2014) [72], cross flow electro membranes are renowned for being inexpensive and simple to scale up. According to (Kumar *et al.*, 2013) [71], the application in the dairy sector is mostly focused on whey purification/processing, demineralization, extending the shelf life of milk, and fat/protein separation. Since the majority of the whey/brine is waste, whey filtration is crucial for environmental protection. Minerals and other dangerous substances are prevented from being discharged into the environment via filtering (Kumar *et al.*, 2013) [71]. The use of MF to decrease somatic cells in milk (Ribeiro *et al.*, 2010; Wang *et al.*, 2019) [68, 73] and the use of RO to produce condensed milk (Skrzypek and Burger, 2010) [70] are two other instances.

6.1 Application in the dairy industry

Membrane filtration (MF) has many uses in the dairy sector, according to (Khanal, 2014) [74], including casein concentration (milk fractionation), fat separation, and bacterial and spore elimination. It may be used in the food sector to clear food products and separate suspended particles with diameters ranging from 0.10 to 5 μm . MF is used to increase the shelf life of milk by lowering microbial load and removing spores while retaining sensory attributes (Khanal, 2014) [74]. Because of their low cost and resistance to fouling, cellulose acetate membranes are frequently employed in the dairy sector.

While membrane processing was first used in the late 1960s to separate milk components, specifically cream and skim milk, using polymeric filters with pore sizes ranging from 0.2 to 10 μm , confirmed that 2 μm ceramic membranes were successfully used to obtain skim milk that was virtually fat free. Membrane processing is now widely used in whey and cheese production (Gésan-Guiziou, 2010) [76].

6.2 Impact on microorganisms

(Fritsch and Moraru, 2008) [77] studied the effectiveness of MF in removing bacteria, spores, and somatic cells from skim milk at low temperatures. Following the application of MF treatment (pore size of 1.4 μm at 6 °C), they were unable to identify any bacteria in permeate from skim milk, which had an initial count of 5.25 and 2.15-log CFU/mL of vegetative bacteria and spores, respectively, while somatic cell count

was decreased to 3.0-log. (Gosch *et al.*, 2014) [78] processed colostrum and skim milk using 0.8 and 1.4 m MF (tubular ceramic ISOFLUX membranes). Microbial eradication using a 0.8 m MF membrane was more efficient than >5.4 log decrease in total viable count and >3.5 log reduction in count utilizing a 1.4 m hole size membrane. However, both forms of MF decreased total viable counts in skim milk to more than 2.3 log CFU/mL. They also utilized 0.14 and 0.2 m MF and found that the permeate from each of these membranes was practically devoid of microorganisms (1.0-log CFU/mL). A 1.4 m ceramic membrane MF treatment was used to test the efficacy of membrane filtration in eliminating germs and spores from milk (Caplan and Barbano, 2013) [79].

The bacterial count was decreased to 4.13-log cycles in skim milk processed through 1.4 m MF at 51 °C, whereas the spore count was determined to be 1.0-log. Depending on the starting count and shape of the bacteria, (Daufin *et al.*, 2001) [80] reported a microbiological decrease of 2.1 to 3.1-log CFU/mL when milk was passed through 1.4 m MF, whereas (Gésan-Guiziou, 2010) [76] reported a 2-3-log reduction when employing a ceramic membrane with 1.4m (pore size). However, owing to their small pore distribution size, Sterilox membranes (Pall-Exekia Company) have a substantially higher effectiveness and may decrease microbial load by 5 to 6-log and 3 to 4-log CFU/mL when employing 0.8 and 1.4 m MF, respectively.

(Elwell and Barbano, 2006) [81] investigated the quality and storage stability of skim milk after MF using ceramic membranes with pore sizes of 1.4 m and found a 3.79-log reduction in the bacterial count as well as an undetectable spore count from initial counts of 2-log CFU/mL in raw milk. Another research found that filtering skim milk via a 1.4 m pore size membrane at 50 °C resulted in >3.5-log reductions in bacterial count and retention of all somatic cells. When compared to 0.5 m membrane processing, the bacterial reduction rose to 2-3 log when the smaller pore size membrane was applied (Saboyainsta and Maubois, 2000) [82]. When skim milk was treated with a 1.4 m membrane, (Trouvé *et al.*, 1991) [83] showed >4.5-log decreases in spore-forming bacteria. (Brans *et al.*, 2004) [84] studied the usage of a 0.5 m micro-sieve, an advanced form of membrane filter, in another investigation. This membrane has a narrow pore size distribution and can operate at low transmembrane pressure, achieving a 6.6-log decrease in *B. subtilis* inoculated in SMUF.

7. Cold Plasma (CP)

Cold Plasma is also known as the fourth state of matter because of the increasing internal energy of the substance when it transitions from solid to liquid to gas and finally to an ionized form of gas (Plasma). Cold plasma is formed when a gas is exposed to high energies, and electric energy is regarded as the most convenient source of energy. Because of the collision process, the particle size within CP is extremely tiny, requiring constant energy for its utilization (Hertwig *et al.*, 2018) [85] in various processes. (Mandal *et al.*, 2018) [86] define CP as a totally or partly ionized gaseous combination of free radicals, electrons, photons, positive ions, negative ions, excited or non-excited molecules. For the creation of plasma, which has applications in the food processing sector, low-pressure conditions or atmospheric pressure are used. Furthermore, CP production uses the majority of its energy in the form of electrons rather than heating the whole gas

system. As a result, gas molecules are kept at room temperature and are known as "cold plasma" or Atmospheric cold plasma (ACP), which is suited for foods where thermal processing is undesirable (Stoica *et al.*, 2014; Mishra *et al.*, 2016) [87-88].

7.1 Application in Dairy industry:

There have been few investigations on cold plasma therapy in the milk and dairy sectors, although it is largely employed in the chemical, polymer, and medical industries for microbe inactivation.

7.2 Impact on microorganisms

Salmonella, *L. monocytogenes*, and *B. cereus* were reduced in skim milk using a concentrated high-intensity electric field (CHIEF) treatment, according to research done by Ruan in 2007 [89]. By exposing the skim milk to 35–40 kV with an exit temperature below 60 °C, they were able to reduce the amounts of Salmonella, *L. monocytogenes*, and *B. cereus* by 2.95 log, 2.74 log, and 0.18 log, respectively. In another experiment, they achieved decreases of 5.55-log, 4.36-log, and 4.73-log for Salmonella, *E. coli*, and *L. monocytogenes*, respectively, in skim milk utilizing double pass CHIEF treatment with 35–40 kV at an exit temperature below 60 °C. The effects of low-temperature plasma therapy (AC power supply 9 kV, 20 min, below 35 °C) on *E. coli* ATCC 25922 in various kinds of milk were examined by (Korachi and Aslan, 2011) [90] and (Korachi *et al.*, 2010) [91]. They discovered that *E. coli* ATCC 25922 was decreased in semi-skimmed milk by 3.40 log, in whole milk by 3.63 log, and in skimmed milk by 3.34 log. Additionally, in whole, semi-skimmed, and skim milk that was kept at 4 °C for 42 days, the inactivation rates of plasma on *E. coli*, Salmonella typhimurium, and *S. aureus* were assessed. The counts of *E. coli*, *S. typhimurium*, and *S. aureus* were decreased to 3.63-log, 2.00-log, and 2.62-log CFU/mL, respectively, after plasma treatment with 20 kV.

8. Combined Treatments

It has been shown that combining two separate nonthermal approaches yields better results for the elimination of pathogens than any strategy used alone. *E. coli* O157:H7 was reduced by 5.0 logs when HPP and heat were added to UHT milk (400 MPa, 50 °C for 15 min.) (Patterson and Kilpatrick, 1998) [92]. Bacterial vegetative cells had the highest resistance to HPP between 20 and 30 °C, but at lower and higher temperatures, microorganisms were considerably more susceptible to HPP. When pressure and heat are combined, bacterial vegetative cells exhibit less resistance to HPP, even at non-lethal temperatures. With this combination, pathogenic and spoilage bacteria may be inactivated (>6-log cycles) at pressures or periods that are much lower than those needed at room temperature.

In UHT milk, *L. monocytogenes* did not become inactive at 200 MPa up to 45 °C, but at 200 MPa, 55 °C, and 15 min, a 6-log drop in cell count was recorded (Simpson and Gilmour, 1997) [93]. Salmonella species, *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 all shown varying degrees of resistance at 25 °C with 345 MPa, but at 50 °C, these variations were significantly reduced (Alpas *et al.*, 1999) [94]. According to (Smelt, 1998) [95], the kinetics of HPP's inactivation of the majority of vegetative cells at low temperatures often exhibit an initial exponential rate followed by a clear tailing. When HPP and heat are present, this trace vanishes (Kalchayanand

et al., 1998) ^[96].

When milk with 4% fat was subjected to 20 kHz frequency for 4–8 min and 120 m with a temperature of 60 °C, (Herceg *et al.*, 2012) ^[64] found that *S. aureus* decreased by 1-log and *E. coli* decreased by 1-log. They also found that these reductions occurred when milk was subjected to thermosonication (20 kHz, 2.78 min and 60 °C). There are several advantages to ultrasound therapy at lethal or sub-lethal temperatures (ultrasound-assisted thermal processing), and it has been shown to be a successful method for extending the shelf life of goods. By improving a product's look, flavor, and texture over how it would typically be handled with heat, it may improve its quality while using less money and energy. Along with its impact on the cavitation phenomena, the temperature sensitivity of microorganisms during sonication may also be a factor.

The inactivation effect results from pressure fluctuations that occur during cavitation, which also cause the temperature to rise. Disruption also increases membrane fluidity, which weakens intermolecular forces (Russell, 2002) ^[97]. First discovered by (Garcia *et al.*, 1989) ^[67], bacterial cells receive sonication therapy before becoming extremely sensitive to heat treatment. *S. aureus* was decreased in UHT milk to 6.0-log after 500 MPa, 5 min at 50 °C, whereas 1.0-log in numbers was attained with either treatment after a single treatment. It has been claimed that pressure may be used with warmth for improved spore inactivation. Temperature elevation accelerated the destruction of spores (*B. subtilis* and *C. sporogenes*) (Stewart *et al.*, 2000) ^[98]. At high temperatures, HPP may effectively render spores (*B. stearothermophilus* inactive) (Ananta *et al.*, 2001) ^[99]. A single sonication treatment showed little impact, whereas thermosonication in glycerol decreased the population of spores in milk from 40 to 79% to 63 to 73% (1-log cycle CFU/mL). In distilled water, the decrease varies from 70 to 99.9% (3-log cycle CFU/mL). The thermosonication impact was significantly reduced when the treatment temperature rose to 100 °C. Under the test circumstances, 70 °C was the ideal temperature for the maximal spore inactivation.

9. Conclusion

Novel non-thermal technologies offer the ability to eliminate microorganisms in milk and dairy products. These techniques have the advantage of causing less damage to the nutritional components of milk compared to thermal methods, while also extending the shelf life of the products. The primary non-thermal approaches for decontaminating milk and its derivatives include high-pressure processing (HPP), pulsed electric field (PEF) treatment, sonication, thermosonication, and various other methods. Combining these technologies has shown promising results in milk processing. By utilizing these approaches, pathogenic and spoilage microorganisms can be inactivated, while minimizing the deterioration of nutritional quality in milk and its products. Consequently, these techniques are expected to be widely implemented in the dairy and food industries for large-scale processing operations in the future.

11. Future prospects

In recent years, there has been a strong emphasis on studying innovative processes, with the next step being to scale these processes up while resolving their limits and achieving customer approval. To do this, the effectiveness and safety

criteria of these methods must be established. Industries must comprehend the processes of action, identify crucial control points in the manufacturing process, maintain quality, and execute risk and cost analysis. It is critical that the industry prioritizes cost-effectiveness and has precise data on these procedures in comparison to typical thermal treatment techniques.

Thermal processes, for example, may be readily checked by detecting changes in milk enzyme activity, such as alkaline phosphatase activity, which indicates a lack of pasteurization. To enable the creation of safe goods, similar quick indicators for emerging non-thermal technologies must be devised. These emerging technologies, however, provide a difficulty since their mode of action is dependent on product factors such as surface qualities, opacity, turbidity, light intensity and dosage, and microbe type. As a result, specialized studies for each system and product are needed to identify the essential parameters for assuring product safety. Furthermore, increasing product penetration and efficiency is critical to offsetting the high investment costs associated with these technologies.

Processing factors that impact the acoustic intensity or total acoustic energy absorbed by the food also influence the effects of High-Intensity Ultrasound (HIUS) technology. However, there is presently no agreement on how to describe and quantify these factors, making comparing findings across research problematic. Further study should concentrate on defining processing settings for each kind of dairy product as well as knowing the inactivation kinetics of various pathogens in various dairy products utilizing ultrasound. The long-term objective of this study is to create quantitative risk analysis models that can be incorporated into food quality control systems.

It is vital to balance the higher capital expenditures of commercial equipment and procedures with the manufacture of premium-priced goods when it comes to Pulsed Electric Field (PEF) processing and other technologies such as High-Pressure Processing (HPP), Cold Plasma, and Ultrasound. In addition, data from laboratory-scale experiments should be compared to findings from industrial-scale operations, taking into account changes in uniformity and circumstances. Upscaling PEF equipment for dairy applications is still a difficulty that needs further research.

Consumer awareness and acceptability, in addition to technical factors, are critical to the economic viability of goods produced from these revolutionary technologies. However, there have been few research on public acceptability of these methods, and customers are generally wary of foreign words like irradiation. As a result, efforts should be made to acquaint customers with these words and to educate them on the benefits of non-thermal technology.

In conclusion, future research should concentrate on production cost efficiency, food safety by addressing spore inactivation, and properly educating consumers about the advantages of non-thermal methods to encourage their adoption.

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