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Recent developments in non-thermal processing techniques to improve the quality and safety of milk and milk products

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ABSTRACT

This review has highlighted about the different types of non-thermal processing techniques and their recent development in field of dairy processing. The findings underscore the importance of low temperature processing in nutritional, physical and sensory qualities of dairy products, which have significant implications for new generation market requirement for minimally processed food. Despite the progress made, several gaps may remain, particularly in their feasibility in economical and legal terms including its efficacy to reduce microbial load in comparison to traditional methods of heat treatment. Future research should focus on cost-effective solution for both small and large producers, with minimizing preconceived notion of general consumers about non-thermally processed food and its health effects, which will be crucial for advancing these novel technologies to solve in real world issues for providing minimally processed, fresh like dairy products. Ultimately, the continued exploration in this topic promises to yield valuable result in field of food industries and processing.

1. INTRODUCTION

Milk is a secretion of healthy mammals' mammary gland, whose primary natural function is nutrition of the young containing lactose, protein, fat, vitamin and mineral. It serves as a superb dietary source of both essential and trace nutrients. Milk from especially cows, buffaloes, goats and sheep, has been consumed routinely for decades by human to meet their nutritional requirements throughout life either as such or in the form of a range of dairy products (Kelly & Meena, 2020; Walstra et al., 2005). It is essentially an emulsion of fat and protein in water, containing dissolved sugars, minerals, and vitamins, though their proportions can vary significantly between species and even within the same species. On average, milk is composed of 87.1% water, 4.6% lactose, 3.3% protein, 4% fat, 0.7% minerals, and 0.1% vitamins (Walstra et al., 2005).

Milk is a nutrient-rich, perishable product with limited shelf life due to the presence of spoilage microorganisms (Shabbir et al., 2020). Milk is nutritious not only for mammals but for numerous microorganisms. These microorganisms are primarily bacteria, but some molds and yeasts can also grow in milk (Walstra et al., 2005). While healthy mammals produce nearly sterile milk, contamination can occur during milking or processing. Unfortunately, milk acts as an ideal medium of growth for microbes. Without preservation, pathogenic and spoilage microorganisms multiply in milk, reducing shelf-life and potentially making it unsafe for general consumption. so, this perishable product require timely processing to maintain best quality (Kelly & Meena, 2020).

Thermal processing methods like pasteurization and sterilization, which are commercially available, enhance both the safety and shelf life by microbial reduction of the pathogenic and spoilage microorganisms and enzyme inactivation in dairy (Guimaraes et al., 2019). Thermal processing apart from high temperature short time (HTST) pasteurization and thermization can cause both beneficial and detrimental changes in milk components, changes occur in term of nutritional value, color, and flavor of milk (Aprea & Mullan, 2022). These changes may result from whey/ casein protein complexes formation, casein micelles dissociation then aggregation, conversion of lactose into organic acids, and the oxidation of lipids (Pegu & Arya, 2023). For instance, heating milk at temperatures above 100°C reduces its nutritional quality by lowering vitamin content, causing milk proteins to denature, and giving it a cooked flavour and brown color. The severity of these changes in milk depends on the time-temperature combinations used during processing. However, novel thermal processes can help reduce or limit the extent of these negative effects to some degree (Kelly & Meena, 2020). Taking in account the sensory or nutritional changes, non-thermal technologies like high-pressure processing (HPP), ultrasound, ultraviolet irradiation, supercritical carbon dioxide technology (SC-CO_2), pulsed electric field (PEF) and cold plasma have been developed to effectively inactivate both pathogenic and spoilage microbes (Shabbir et al., 2020).

Consumers today are highly conscious and concerned about the food quality they consume. As a result, consumers are increasingly preferring and demanding "natural," minimally processed, and fresh-like foods. They now view convenient, nutritious, fresh, safe and long-lasting foods as their right (Kelly & Meena, 2020). Consequently, alternative technologies are explored for applications in food, either as replacements or combination with heat treatments (Pegu & Arya, 2023). New processing techniques are essential for delivering fresh, nutritious, and safe foods with improved storage stability (Alegbeleye et al., 2018). Therefore, non-thermal methods are becoming an appealing approach to produce both safe and nutritious dairy product, without compromising their nutritional value (Ahmad et al., 2019).

Non-thermal food processing technologies offer promising approaches to balancing microbiological and chemical safety, as well as sensory and nutritional properties. Taking all factors into account, this review paper offers an overview of non-thermal processing methods to improve the quality and safety of milk and milk products. Furthermore, this review paper outlines the potential applications and advantages of these technologies as alternatives to traditional processing method.

2. NON THREMAL TECHNIQUE IN MILK PROCESSING

Non-thermal processing of dairy products entails treating them at ambient temperatures to maintain superior quality and sensory attributes, as well as retain more nutritional components and enhance safety aspects (Kelly & Meena, 2020). These innovative preservation technologies enable the reduction of heat's adverse effects on food quality, are environment friendly and reduce processing costs (Ismail, 2021).

2.1 High Pressure Processing (HPP)

HPP (high-pressure processing) or HHP (high hydrostatic pressure) is a processing technique that employs high pressures, with or without external heating, to achieve microbial inactivation or alter food characteristics (Ismail, 2021). The application of this non-thermal technique has been long employed in various non-food industries; Food preservation by HPP was first documented in the late nineteenth century. However, the commercialization and application in foods have been studied for over a century (Shabbir et al., 2020). During HPP processing, pressure is transmitted to food sample very rapidly and uniformly thus, pressure application is not dependent on the size and shape of the product which provides homogenous processing of irregular shaped food products (Evrendilek, 2014).

2.1.1 Principle and Mechanism

The idea behind HPP process can be explained using two main principles: Le Chatelier's principle and isostatic/pascal's principle (Kelly & Meena, 2020). Le Chatelier's principle pertains to changes in temperature, pressure, and volume in a system at equilibrium. When a force is applied, a new equilibrium is established, leading to structural changes and modifications associated with reduced volume (Allai et al., 2023). Food products are hermetically sealed and placed in airtight, thermally insulated vessel. Ultra-high pressure is then applied through a liquid medium, resulting in a uniform pasteurization effect. When milk undergoes HPP at 100, 300, and 500 MPa, water compresses by 4%, 10%, and 15%, respectively. Such compression leads to adiabatic heating, where the temperature of water rises by 2-3°C for every 100 MPa increase in applied pressure (Kelly & Meena, 2020).

This technology relies on three key parameters for process optimization: time, temperature, and pressure. It induces irreversible alterations in the secondary, tertiary, and quaternary structures of proteins, primarily impacting covalent bonds (Shabbir et al., 2020). Additionally, the product's intrinsic composition and parameters significantly influence the effectiveness of the processing (Allai et al., 2023). For inactivating microorganisms or enzymes, extending shelf life, and altering physicochemical qualities, HPP (100–1000 MPa) is used as cold pasteurization method in various food applications as alternative to thermal processing. HPP can be used to process both liquid and solid foods whether packaged or open, and using or not using extra barriers of preservatives (Roobab et al., 2023).

2.1.2 Effects of HPP on quality and safety

HPP of milk at pressures >400 MPa results in the inactivation of pathogenic and spoilage microbes through various mechanisms, including the inhibition of protein synthesis, reduction in ribosome numbers, leakage of cellular elements, denaturation of essential enzymes and changes in plasma membrane permeability. Generally, pressures >200 MPa at ambient temperature HPP induces bacterial cell inactivation. At 400-600 MPa and ambient temperature, it destroys vegetative cells of yeast, fungi and bacteria although pressure-immune strains may still persist (Singh et al., 2019). The effectiveness of HPP in microbial destruction rely on various factors, including the processing condition, physiological state of the microbe, characteristics of the food. Yeast and molds have lower pressure resistance compared to bacteria, with their vegetative forms being more pressure-sensitive than their spores (Kelly & Meena, 2020).

HPP induces changes in milk constituents and attributes, resulting in HPP milk that retains a color similar to raw milk, with vitamins and amino acids remaining unaffected, while

1 unit drop in pH of milk is observed at 1000 MPa of pressure change. Additionally, the milk's freezing point drops to -8°C and -22°C at 100 MPa and 210 MPa, respectively, while its viscosity increases due to denaturation of whey proteins and breaking of casein micelles (Kelly & Meena, 2020; Singh et al., 2019).Treating milk at 400 MPa/15 minutes or at 600 MPa/3 minutes can produce product similar to pasteurized milk by competently eliminating pathogenic and spoilage microbes.

Different studies are done on HPP effect on milk processing where, recently in study of (Stratakos et al., 2019) raw milk is externally inoculated with pathogens such as *Escherichia coli, Salmonella, and L. monocytogenes*, and then treated at 400–600 MPa for 1-5 minutes, showed 5 log CFU/mL decrease in bacterial levels. Besides its safety benefits, HPP milk also has an extended shelf life, lasting one week longer than pasteurized milk (Stratakos *et al.*, 2019). Razali et al. (2021) studies on goat milk preservation at 200-600 MPa for (5-15) min for <40°C resulted improved quality in terms of TSS, pH, viscosity and *S.aureus* reduced by 7 log CFU/ml. Kapoor et al. (2021) studies on use of HPP in Indian cottage cheese at 500 MPa/15min resulted in improved cheese yield, extended shelf life, and enhanced quality attributes, similarly studies conducted by Munir et al. (2020) on cheddar cheese made from milk processed at 400 MPa/15min <40 °C shows increase in cheese nutritional profile as well as bioactive compounds. Inácio et al. (2022) studies on ewe milk cheese at 121 MPa/30 min <40 °C shows Improved curd yield > 9% with beneficial cheese microbiota.

2.2 Pulsed Electric Fields (PEF)

PEF processing uses short electrical pulses using electrodes to eliminate microbes and deactivate enzymes in various foods. PEF is one of the emerging techniques for the inactivation of microorganisms especially in liquid. It has the potential of producing foods with excellent sensory and nutritional quality and extended shelf life and safety (Pal, 2017). The primary components of a PEF system are high voltage pulse generator, a control system, treatment chambers, and a fluid handling system. The product to be processed is placed in either a static or continuous design treatment chamber (Evrendilek, 2014). Earlier Fetterman (1928) had a process patented called "Electropure" for milk pasteurization by killing *Mycobacterium tuberculosis* and *E.coli* through the flow of current. New researches are conducted to elucidate the mechanisms involved in enzyme inactivation and microbial destruction, along with the optimization of process criteria, aim to achieve better nutrient retention and required sensory quality while enhancing product safety in PEF (Upadhyay et al., 2019).

2.2.1 Principle and Mechanism

The principle of PEF involves applying high electric field short pulses (1–10 ms), generated at high voltage (5–20 kV) to food placed between electrodes with a 0.1–1 cm treatment gap. These pulses last from microseconds to milliseconds, with electric field intensities ranging from 10 to 80 kV/cm (Upadhyay et al., 2019). Processing time can be calculated by multiplying the number of pulses by the effective pulse duration. When the electric field is applied, current flows in the liquid food sample and is transferred to every point due to the charged molecules. After treatment, the food must be packed aseptically and stored in cold conditions to achieve longer shelf life (Pal, 2017; Shabbir et al., 2020). Short pulse application of high voltage leads to the electric field generation causing microbial and enzyme inactivation in food, electroporation and dielectric breakdown of their microbial cell membranes (Kelly & Meena, 2020). This process is affected by factors like pulse width, number of pulses, flow rate, electric field intensity, and shape, temperature, conductivity and physiological parameters of microbes (Shabbir et al., 2020).

2.2.2 Effects of PEF on quality and safety

PEF led to improvement in the preservation of milk with minimal adverse effects on food attributes as PEF excels in eliminating microbes while preserving the nutritional value and sensory attributes of raw food, like texture, flavor, color. Although numerous studies highlight the significance of PEF in low-temperature milk processing, they report approximately 3 log cycles of microbial reduction, with limited impact on certain microbial and endogenous enzymes. Enzyme inactivation is also reported in PEF treated milk in varying levels of inactivation based on the treatment intensity and the specific enzyme involved. Therefore, enhancing the effectiveness of PEF on both microbes and enzymes remains a critical and challenging aspect of PEF milk processing (Pegu & Arya, 2023).

Compared with heat pasteurization, a high-intensity PEF has lower impact on acidity, pH, or mineral level in milk, but reduction in size of casein micelles and viscosity in addition to energy saving and better microorganism inactivation (Pegu & Arya, 2023) hence, PEF could potentially serve as pasteurization alternative. However, when used together, they result in greater microbial death and extended self-life due to the synergistic effect. In the future, PEF could enhance product quality to meet consumer demand for fresh-like food, including milk product (Kelly & Meena, 2020) but, there is still need for more commercial exploitation of this technology. As a novel technique related to the effect of PEF processing on milk and milk products and their nutritional properties and sensorial quality such as flavor of milk is scarce in the literature. Besides, up to the present moment, no technical–economic feasibility studies have been found regarding using high-intensity electric pulses in milk and milk products (Cavalcanti et al., 2023; Shabbir et al., 2020; Yang et al., 2021).

According to Yang et al. (2021) studies on raw bovine milk with different milk fat globules (MFG) sizes processed at 25 °C, 16 kV/cm; 30 µs; 100 mL/min fattyacids (FA) increased with increased long-chain FAs in raw milk and milk with large MFGs with a slight decrease in the fat content. While, in term of proteins 29% and 15% decrease in α-La and β-Lg respectively (in small MFGs of milk) with higher small (<40 kDa) protein aggregates was also reported (Yang *et al.*, 2021). While Mohamad et al. (2021) studies on raw goat milk processed at 20, 30 and 40 kV/cm; 30 °C, 5 and 10 µs; 2.5 L/h resulted reduction in total saturated fatty acids (Fa) and total poly unsaturated fatty acids (PUFA) with increase in total mono unsaturated fatty acids (MUFA). Authors proposed that PEF induces milk fat globule membrane disruption, which can alter enzymatic activity related to lipid metabolism, effecting molecular structure of fatty acids, hence breakdown of certain Fa and PUFA while promoting the formation of MUFA. Authors also reported significant increase in iron and non-significant increase in chromium, nickel, and manganese, authors concluded that the electrochemical reaction occurring during PEF treatment may have caused the metal components from the PEF electrode to dissolve (Mohamad et al., 2021). Similarly studies by Sharma et al. (2016) on bovine whole milk processed at 55 °C, 20 and 26 kV/cm; 34 µs; 4.2 mL/s demonstrates increase in fat crystal conversion from α into β -crystal with increment in the hydrophobic protein surface. Authors concluded PEF alters the interactions between milk fat globule membrane proteins and skim milk proteins, which may influence fat crystallization behavior. Reduction in milk protein denaturation with xanthine oxidase denaturation ~13% less after PEF treatments compared with the thermal treatments was also reported by (Sharma et al., 2016). PEF treatment of 10% liquid whey protein solution (20–40°C, 50 kV/cm; 3–8 µs; 5 L/h) by Schottroff et al. (2019) shows Stable IgM and IgG with no changes in vitamins A and C. Results also showed an inactivation of 6.51 $\log_{10}$ cycles of *L. innocua* with limited or no impact on the concentration of selected heat sensitive bioactives from their initial values before PEF treatment (Schottroff et al., 2019).

2.3 Ultrasound Cavitation

Ultrasound (US) is a term for sound waves above human auditory range of 20 kHz-10 MHz. It is one of the common non-thermal processing methods because it is environment friendly, safe/ nontoxic with wide range of applications in food industry. It is applied in food industry because of its ability to improve functional, physical and chemical properties of various food items. US can be categorized by frequency range into power ultrasound (20-100 kHz), high frequency ultrasound (20 kHz-2 MHz), and diagnostic ultrasound (>1 MHz) (Shabbir et al., 2020). Applications involving food and proteins often use power ultrasound with intensities 1-1000 W cm^{-2}. Ultrasonic systems can be categorized into two types: non-contact and contact. Both are designed and operated similarly, where electrical power generators provide excitation signal then piezoelectric transducer converts electric energy to mechanical vibration. System transmits wave produced by vibration to propagation medium, air in a non-contact or liquid in contact system (Pegu & Arya, 2023).

2.3.1 Principle and Mechanism

The optimal frequency range of 20–40 kHz results in most potent cavitation. This cavitation, induced by ultrasound, is crucial for physical changes and destructive effect on bacterial cells in food. Additionally, ultrasound generates localized pressures as high as 100 MPa and temperature around 5000K. Temperature is controlled dissipating excess heat generated during ultrasound by circulating cold water through the cooling coil in treatment chamber and pressure monitored by a manometer (Evrendilek, 2014). The bactericidal effect of low-frequency, high power US is associated with cavitation. Mechanical vibration, microstreaming and cavitation are three physical phenomena of low frequency ultrasonication (Kelly & Meena, 2020). The cavitation phenomenon involves the formation of nucleus, the expansion, then subsequent collapse of bubble filled with vapor or gas in liquids. Cavitation initiates a series of compression and rarefaction cycles, producing vibrations, acoustic streaming, then cavitation. Microbubble expand through rectified diffusion and bubble coalescence in solution. Upon reaching their maximum size, these bubbles collapse violently, creating physical and chemical effects that rupture big aggregates through shockwave formation and turbulent motion (Pegu & Arya, 2023).

The use of ultrasound to inactivate microbes was first reported in the late 1920s. However, due to its limited lethal effect on both pathogenic and spoilage microorganisms, the use of ultrasound is not commonly used as a sterilization method in the food industry. Intensities higher than 1 W/cm² with frequencies between 18-100 kHz are employed in milk homogenization, inactivation of bacteria and enzyme (chymosin) with β-galactosidase extraction. Factors like pressure, temperature, amplitude of wave, exposure time, volume and composition of processed food, effect efficiency of US processing. Since, US cannot affect a large area on a food surface; it might not efficiently destroy all resistant spores and bacteria. Microbial inactivation level depends on factors such as cell morphology, viscosity, pH. It is proposed that the target of ultrasonic damage might be the cytoplasmic membrane. It is suggested that the cytoplasmic membrane could be the target of ultrasonic damage (Evrendilek, 2014).

2.3.2 Effects of ultrasound on quality and safety

Shockwave from US can interact with cell structures, mechanically disrupting cell membranes and allowing intracellular molecules to be extracted more quickly. It serves as an alternative to traditional homogenizers to reduce fat globule size. Consequently, US processing and preservation of food is a practical option for processes like pasteurization, emulsification, homogenization, and mixing, it also results in greater homogeneity and significant energy savings. Milk gels and yogurt produced from milk treated with ultrasound exhibits superior physical and textural characteristics, such as cohesiveness and firmness. Additionally, it has improved water-holding capacity, high viscosity, and reduced syneresis (Ismail, 2021). When used as a pre-treatment sonication induces conformational changes in milk proteins by the weakening of hydrophobic and electrostatic interactions. shear forces generated by US processing can hydrolyze proteins, releasing bioactive peptides. In cheese made from sonicated milk, fat retention increases due to the milk fat homogenization. The shear forces /turbulence generated in ultrasound break fat globules into much smaller ones, which gets stabilized by milk proteins and entrapped in the protein network during the coagulation in cheese making (Munir et al., 2020).

Ultrasound treatment experiment performed by Hernández-Falcón et al. (2018) at 20 kHz, (10-15) min, 95% amplitude on milk exhibited similar characteristics and microbiological quality to pasteurized milk. US samples had similar color to the control and stable emulsion with preserved physicochemical and microbiological quality of milk. Researchers called US as an effective method to reduce levels of aflatoxin M1 (Hernández-Falcón et al., 2018). Study by Munir et al. (2020) on cheddar cheese made from ultrasonicated milk to specific energy 41 J/g, 20 kHz, during ripening reported high fat percentage and increased soluble nitrogen. Same study shows increased proteolysis, antioxidant and Angiotensin-I converting enzyme inhibitory activities due to conformational changes in proteins resulting from cavitation. Therefore, US can have the potential to get adapted in industries to produce nutritionally improved milk product. (Almanza-Rubio et al., 2016) reported an increased retention of fat and yield of cheese, reduced size of milk-fat globules in cream cheese made with sonicated milk at power 50 W, ≤30 min, 35-50 °C. Furthermore, the sonication of the milk improved the thermostability with better textural and rheological properties of cream cheeses.

Guimaraes et al. (2019) studies on prebiotic soursop whey beverage produced and processed using high-intensity ultrasound varying the power (0, 200, 400 and 600 W) reports that US technology induced beneficial effects, increase in phenolic content, improved antioxidant and anti-hypertensive activity with reduced unwanted minerals with some negative changes, include degradation of the ascorbic acid, decrease in certain mineral and production of specific volatile compounds. These results highlight the feasibility of using ultrasound in manufacturing functional dairy products with therapeutic properties is promising. However, further research is needed to minimize the negative effects of ultrasound and to conduct sensory analysis with consumer. Monteiro et al., (2018) found similar result after treating chocolate whey beverage with ultrasound, achieving 82.4% ACE inhibition with an energy density of 3 KJ/cm^3. These findings indicate that ultrasound acts at a molecular level, extracting and releasing bioactive peptides and phenolic compounds with antioxidant and anti-hypertensive properties, thereby enhancing the product's health benefits. Gholamhosseinpour et al. (2020) study on effects of ultrasound, microwave, and heat treatment of milk on *L. acidophilus* viability resulted that ultrasonicated microwaved (300 W, 24 kHz, 50% amplitude, 30 min) milk had higher antioxidant and anticancer activities. Study also demonstrated increased radical scavenging capacity, a-amylase inhibition, peptide concentration and exopolysaccharide content in US microwaved milk than those of the other treated milks. Study by Potoroko et al. (2018) showed that reconstitution of dry milk with use of ultrasound(22 ± 1.65 kHz, 60–120 W/L) improves further accumulation of biologically active compounds and rises the nutritional quality as well as the viscosity in curd of the fermented product.

2.4 Cold Plasma

Free electrons, reactive radicals, highly reactive intermediate species, negatively and positively charged ions, ultraviolet photons, and neutral molecules and atoms make up plasma, a fully or partially ionised state of matter (Sarangapani et al., 2015). Plasma can be categorized into two types: non-thermal plasma (NTP), also known as cold plasma, and thermal plasma. Cold plasma (CP) is produced at temperatures ranging from 30 to 60°C, either at atmospheric pressure or in a vacuum. It requires less energy to generate, has electron temperatures significantly higher than the surrounding gas temperature, and does not achieve local thermodynamic equilibrium (Thirumdas et al., 2015).

Cold plasma treatment offers several benefits, such as rapid processing, simple equipment operation, the ability to work at room temperature, and preserving sensory attributes, and functional property of food without relying on solvents. This makes it a valuable tool for applications in the food industry (Umair et al., 2022). The cold plasma method is ideal for sterilizing a variety of liquid food products due to its ultra-rapid process, operation at room temperature (making it suitable for heat-sensitive products), cost efficiency, and eco-friendly nature (Aslan, 2016).

2.4.1 Principle and mechanism

Cold plasma is generated when gas is exposed to high energy levels, with electrical energy being the most practical and efficient source. The collision processes within cold plasma result in extremely small particle sizes, requiring a continuous energy supply for its use in various applications in different processes (Hertwig et al., 2018). Cold plasma consists of a fully or partially ionized gaseous mixture, including free radicals, electrons, photons, positive and negative ions, as well as excited or non-excited molecules (Mandal et al., 2018). Its generation typically occurs under low-pressure or atmospheric pressure conditions, making it particularly useful in the food processing industry.

2.4.2 Effects of cold plasma on quality and safety

Korachi et al., (2015) investigated the treatment of raw milk over a 3-minute duration. They observed no significant impact on the lipid content within this time frame. However, extending the cold plasma treatment beyond 3 minutes led to a reduction in polyunsaturated fatty acids (PUFA) and an increase in saturated fatty acids (SFA) due to the oxidative degradation of lipids by reactive species generated during the treatment. Coutinho et al. (2019) examined how cold plasma processing time influences shear properties, particle size, and milk proteins. Chocolate milk exposed to cold plasma for 10 minutes showed no significant changes in its properties and had minimal impact on protein structure. However, a longer treatment time of 15 minutes caused protein denaturation and altered the flowability of the chocolate milk.

Reactive oxygen species and reactive nitrogen species interact to produce strong oxidative effects on double bonds in the microbial cell's lipid bilayer, which impairs the movement of macromolecules both inside and outside the cell and contributes to plasma's antimicrobial properties (Jermann et al., 2015). These reactive species are arguably the most significant contributors to pathogen inactivation (Phan et al., 2017). The species of the target microorganism, input power, treatment duration, gas composition, and food composition are some of the variables that affect the antibacterial effectiveness of cold plasma technology in dairy products.

The initial concentration of microorganisms in food is a crucial factor in assessing how well cold plasma processing works. Because more microorganisms group cells together, making it harder for the active chemicals in plasma to reach cells, a higher initial concentration reduces the inactivation impact of the cold plasma. In order for the microbial load to drop to appropriate levels, this parameter should be taken into account while choosing the cold plasma apparatus' process settings (Liao et al., 2017). The raw materials used in processing also affect how well cold plasma works. The surface of solid goods, such cheeses, is typically the only area treated.

2.5 UV-C Radiation

UV light radiation is the electromagnetic spectrum in between visible ray and X-rays that has wavelengths between 100 and 400 nm (Delorme *et al*., 2020). UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm), and vacuum-UV (100–200 nm) are the four primary UV ray types it emits (Figure 1).

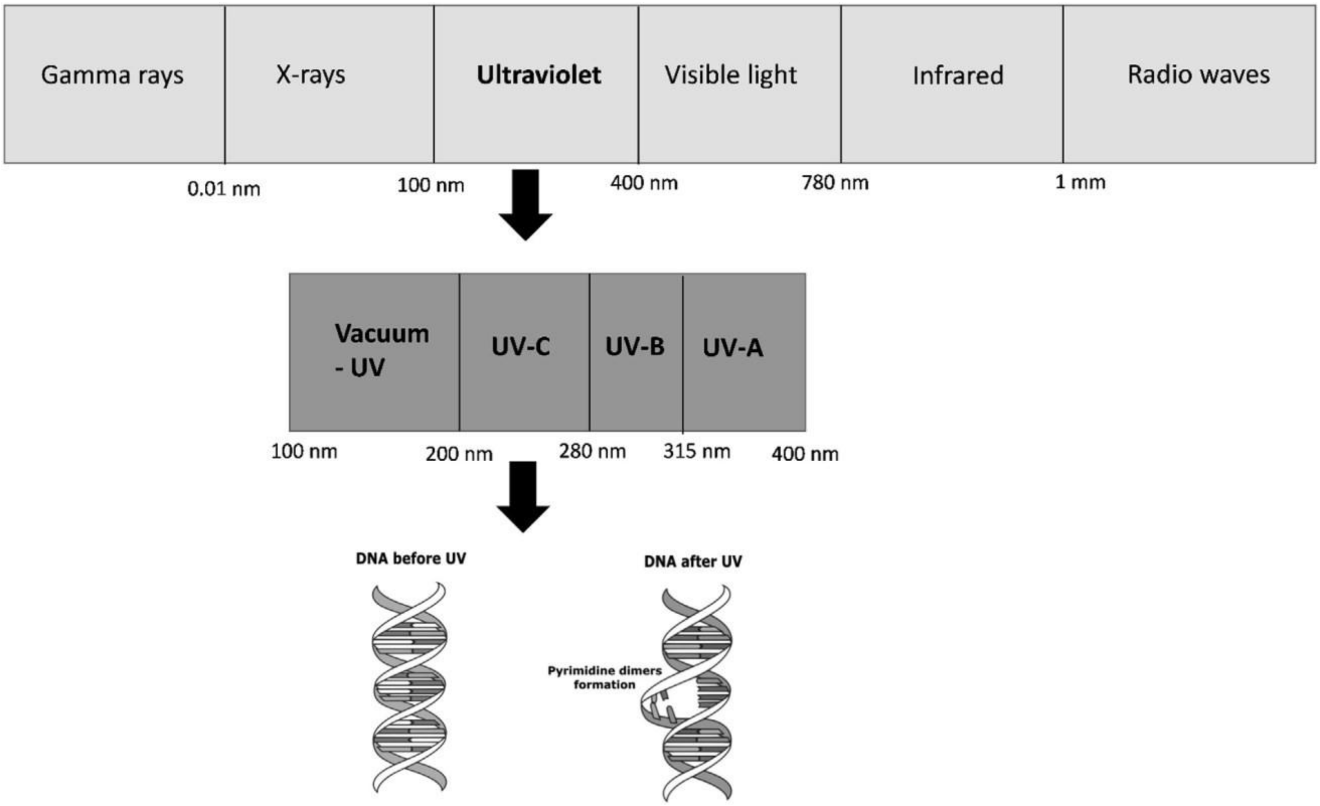

Figure 1
UV rays, their wavelengths, and the process by which microbes are rendered inactive. (Delorme et al., 2020)

2.5.1 Principle and Mechanism

Ultraviolet radiation has emerged as promising novel food processing technologies, with significant commercialisation ability (Jermann et al., 2015; Morales-de la Peña et al., 2019). Earlier, UV was exclusively used for solid foods, but FDA and the United States Department of Agriculture have allowed its use in liquids as an alternative to heat pasteurisation (Vasuja & Kumar, 2018). UV-C ray is referred as germicides since they kill microorganisms, including bacteria, protozoa, algae, fungi and virus (Shin et al., 2016). The microbial destruction by the action of UV light occurs through several mechanisms depending on the wavelengths applied. The effect of ultraviolet light causes microbial inactivation via many mechanisms that vary depending on wavelengths used in the therapy. Inactivation can occur indirectly or directly by absorption of light that strikes microbial cell's DNA. The primary principle of UV radiation inactivation of bacteria is direct impact of lesions interfering with replication of DNA (Brem et al., 2017). UV light in the germicidal range (UV-C) from 200-280 nm, is being investigated as alternative to thermal treatment for inactivating pathogens and improving shelf life and safety of skim milk (Cappozzo et al., 2015).

2.5.2 Effects of UV-C radiation on quality and safety

According to Kharnitov et al., (2019) there are no significant variations between heat and UV-C treated milk in terms of macro- (protein, fat, total solids) and micro-components. Photooxidation of milk protein due to UV-C irradiation has two major consequences: protein aggregation and unfolding (Singh & Huppertz, 2019). There was no significant change in protein or fatty acid components of milk treated at 253.7 nm, dose range 5-102 mJ cm^{-2}, milk layer (400 µm) (Kharnitov et al., 2019). But, drastic UV treatment may cause changes in the sensory or physicochemical characteristic of products, such as colour changes, protein oxidation, occurring due to UV light absorption by amino acids or the use of photosensitisers such as riboflavin, and pH reduction (Keklik et al., 2019). In addition, several authors found a rise in aldehydes and hydrocarbons, which are volatile chemicals produced by amino acid breakdown and lipid oxidation (Fernández et al., 2016).

It is cost-effective to install, operate, and maintain ultraviolet radiation; it can also be used in conjunction with other processing procedures to provide extra or combined results.

2.6 Supercritical Carbon Dioxide

A super critical fluid is any substance, the thermodynamic state of pressure and temperature of which is above the critical point in phase equilibrium (Amaral et al., 2017). It has gas-like low viscosity, intermediate diffusivity, and liquid-like high density, making it a good solvent (Knez et al., 2014). Supercritical CO_2 (SC-CO_2) is by far the most commonly utilised fluid in food processing since it is universally recognised as safe (GRAS), ecologically friendly, economical, widely available in high purity, and simple to remove (Silva et al., 2020; Smigic et al., 2019). However, the major characteristics that make SC-CO_2 suited for meeting new product development trends are linked to its critical point. CO_2 exhibits critical qualities at high pressure and temperature, which are very easy to accomplish; this property makes it appealing for the industrial processing of heat-sensitive items such as vitamins, flavours, natural colours, and antioxidant chemicals (Silva et al., 2018; Figure 2).

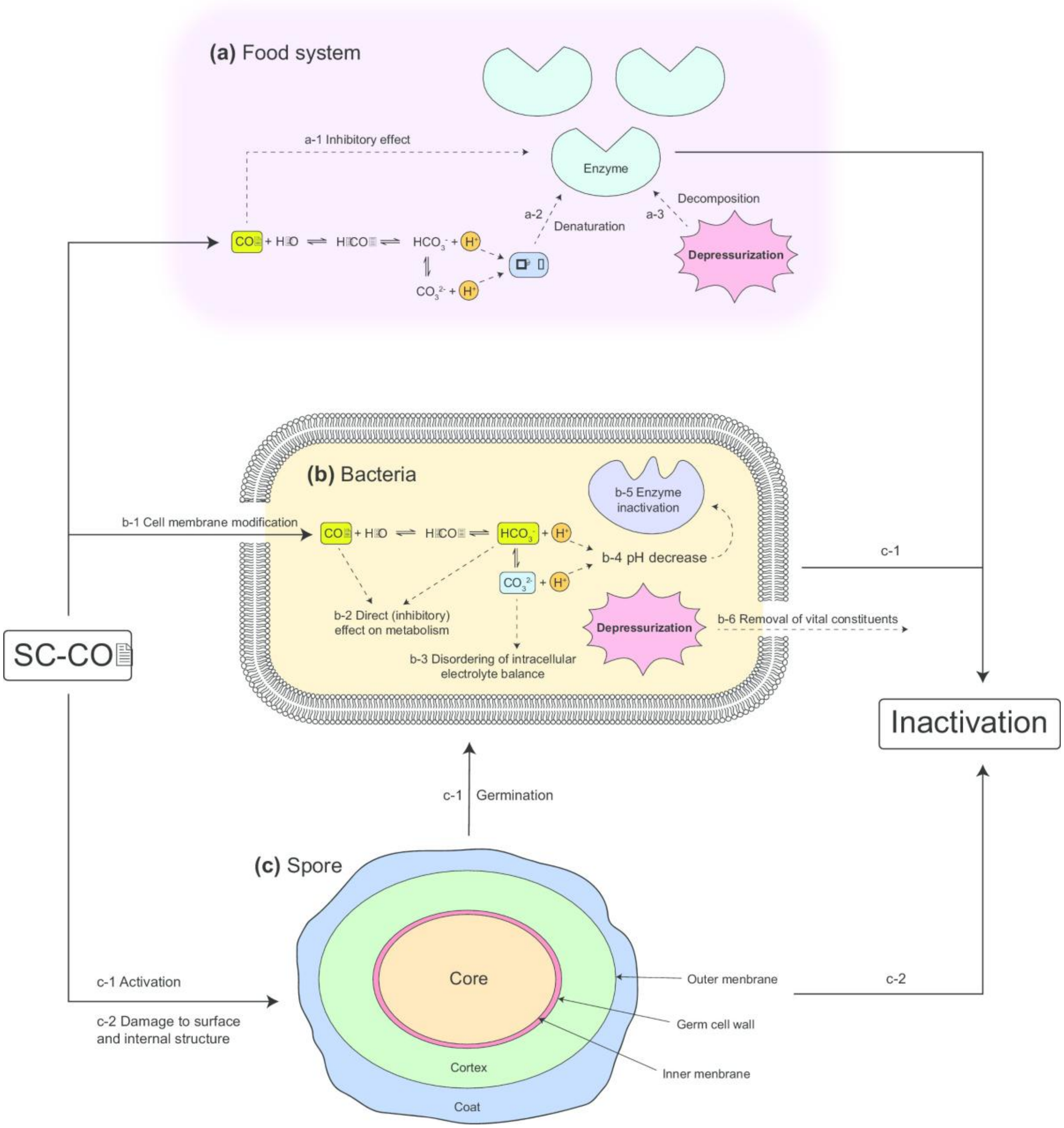

Figure 2

Diagrammatic representation of the inactivation effect of SC-CO2 on (a) enzymes, (b) bacteria, (c) spores. (Wang et al., 2021)

Table 1
Effects of supercritical carbon dioxide (SC-CO_2) technology in physicochemical, sensory and quality parameters of foods.

Foods	Evaluated parameters	Applied conditions	Comparative treatments	Main results	References
Mongolian cheese	Texture profiles, volatile compounds and sensory analysis during storage	SC-CO_2at 10, 15 and 20 Mpa, 40, 50 and 60 °C	Sample untreated	SC-CO_2 could maintain texture stability during storage, decreased the contents of volatile compounds (acids and esters), avoiding off-flavors. No effects on the sensory properties of cheese.	(Feng et al., 2023)
Milk	Alkaline phosphatase	SC-CO_2at8- 18 Mpa, 30-70 °C, 10-30 min	-	Enzyme inactivation of 94.5% was reached at 70 °C, 8 Mpa, for 30 min.	(Ceni et al., 2016)
Whey-grape juice	pH, titrable acidity, total soluble solids, phenolic compounds, anthocyanin, DPPH, ACE activity and volatile compounds	SC-CO_2at 14, 16 and 18 Mpa, 35 °C, 10 min	Thermal processing at 72 °C for 15 s and sample untreated	pH, titrable acidity, soluble solids were similar. Ketones and acid compounds were identified in the treated products.	(Amaral et al., 2018)
	Color, particle size, rheology tests, physical stability and sensory acceptance	SC-CO_2at 14, 16 and 18 Mpa, 35 °C, 10 min	Thermal processing at 72 °C for 15 s and sample untreated	Changes in color parameters, but resulting in no visual change. No effect on apparent viscosity. Lower particle diameter and consistency index. No changes in all sensory attributes.	(Amaral et al., 2018)
Soursop whey beverage enriched with inulin	pH, soluble solids,rheological behaviour, color parameters and phase separation kinetics	SC-CO_2at 10 and 20 Mpa, 35 and 55 °C, 20 min	-	No changes in physicochemical and color parameters. The temperature and CO_2volume ratio showed effect on microstructure, rheological behaviour and kinetic stability.	(Silva et al., 2019)

2.6.1 Principle and Mechanism

Carbon dioxide possesses both liquid and gas properties when kept above the critical point at high pressure (7.38 MPa) and temperature (31.1 °C). This can inhibit oxidative enzymes and cause cell rupture in bacteria (Verma & Srivastav, 2018). The negligible surface tension and lower viscosity of carbon dioxide facilitate the penetration in the complex microbial cell structure leading to cell rupture (Zhou et al., 2015). Carbon dioxide's insignificant surface tension and low viscosity allow it to penetrate the complicated microbial cell structure, causing cell rupture. However, detailed answers for how SC-CO_2 inactivates spores are unknown. As shown in Fig. 2, there are two main explanations: "the germination and inactivation mechanisms" based on kinetic studies and "the structure damage and inactivation mechanism" based on morphological and molecular spore investigations (Bi et al., 2016).

2.6.2 Effects of SC-$C0_2$ on quality and safety

The SC-CO_2 processing factors pressure, temperature, length time, compression/decompression cycles, and water content all have a significant impact on microbial inactivation. SC-CO_2 has emerged as a viable inactivation technique for bacterial spores, with a 6-log reduction attainable (Soares et al., 2019). SC-CO_2 alone requires rather high temperatures (60-90°C) for successful spore death, however modest amounts of additives such as H_2O_2, peracetic acid (PAA), or water allow for spore killing at lower temperatures and pressures(Rao, Wang, et al., 2016; Setlow, 2016). For example, Setlow et al., (2016) used SC-CO_2 plus 112 ppm PAA at 35 °C to produce more than 6-log decrease of *Bacillus subtilis* spores in 15 minutes, and they also discovered that dry spores were more resistant than wet spores.

Current research on how SC-CO_2 technology affects the physicochemical, technical, and sensory qualities of meals is shown in Table 1. Additionally, the SC-CO_2 is recyclable and reusable, which lowers waste production and environmental issues. Because of its high diffusion in solids and dense liquids, as well as its tuneable density that can be increased by varying temperature and pressure, this solvent has attractive qualities for application in the food business (Prado & Rostagno, 2022). All things considered, the supercritical dioxide carbon technology has shown itself to be a viable choice for preserving the dairy product's nutritional value and sensory appeal while deactivating crucial enzymes for the dairy sector. To fully use supercritical CO_2 in the dairy sector, more research is required to clarify its effects on the primary intrinsic indicators parameters involved in the processing and quality of dairy items (Amaral et al., 2017).

3. CHALLENGES AND LIMITATIONS

Current advancements in non-thermal processing techniques for dairy products have gained significant attention due to their potential to enhance quality and safety while minimizing the adverse effects associated with traditional thermal treatments. However, these innovations are not without challenges and limitations, which must be critically assessed to optimize their application in the dairy industry.

One of the primary challenges associated with non-thermal processing techniques is the variability in their effectiveness against different microbial strains. For instance, while high-pressure processing (HPP) has demonstrated efficacy in reducing microbial loads, its effectiveness can vary based on the type of microorganism present in the milk. This variability can complicate the standardization of processing protocols across different dairy products and production environments. Moreover, the economic feasibility of implementing non-thermal technologies poses a significant barrier to their widespread adoption. Techniques such as PEF and cold plasma processing require specialized equipment and energy inputs that can elevate operational costs compared to conventional thermal methods. While PEF has been shown to improve the safety and quality of milk, the initial investment in technology and ongoing energy costs can be prohibitive for smaller dairy operations (Alirezalu et al., 2020; Vashisht et al., 2024). Additionally, the return on investment may not be immediately apparent, as consumer acceptance of products processed by these novel methods can take time to develop. Another critical limitation is the potential impact of non-thermal processing on the perceptible experience of dairy products. Therefore, it is essential to balance the microbiological safety benefits of non-thermal treatments with their effects on sensory characteristics.

The regulatory landscape also presents challenges for the adoption of non-thermal processing technologies. Many countries have established stringent regulations governing the safety and labelling of dairy products, which can complicate the introduction of new processing methods. For instance, while non-thermal techniques like HPP and UV treatment show promise in enhancing milk safety, regulatory bodies may require extensive safety and efficacy data before approving these methods for commercial use (Liu et al., 2022; Soni et al., 2021). This regulatory scrutiny can delay the implementation of innovative technologies and increase the burden on dairy producers seeking to comply with evolving standards. Furthermore, the preservation of bioactive compounds during non-thermal processing remains a significant concern. While these techniques are designed to minimize the degradation of nutrients and bioactive components, studies have shown that some methods can still lead to losses in essential vitamins and proteins (Pitino et al., 2019). For example, high-pressure processing has been associated with the retention of certain bioactive compounds, but the extent of this retention can vary based on processing parameters and the specific composition of the milk (Wesolowska et al., 2018). Thus, ongoing research is needed to optimize processing conditions that maximize the preservation of nutritional quality while ensuring safety. In addition to these challenges, the scalability of non-thermal processing technologies is a crucial consideration.

The perception and acceptance of non-thermal processed products by consumers can also significantly influence market success. Consumers may be hesitant to embrace innovative technologies that they are unfamiliar with, despite a growing demand for minimally processed and clean-label products. For consumers to be aware of the benefits of non-thermal processing and to trust the safety and quality of these products, education and marketing strategies are vital (Chughtai et al., 2021).

4. FUTURE TRENDS AND OPPORTUNITIES

The future of non-thermal processing techniques in the dairy industry, particularly for milk and milk products, is promising, with several innovative methods emerging to enhance quality and safety while maintaining nutritional integrity. As consumer demand for minimally processed foods continues to rise, non-thermal technologies are positioned to meet these expectations by offering alternatives that preserve the sensory and nutritional qualities of dairy products.

One of the most significant trends is the increasing application of Pulsed Electric Fields (PEF) in milk processing. This dual approach not only enhances the safety profile of milk but also aligns with consumer preferences for products that retain their natural characteristics (Vashisht et al., 2024). Ultrasonication is another emerging technology that has gained traction in the dairy sector. This method utilizes high-frequency sound waves to improve the shelf-life of milk by inactivating pathogens and spoilage microorganisms while preserving the nutritional and bioactive properties of the milk (Hazra et al., 2021). Research indicates that ultrasonication can enhance the radical scavenging activity of milk, suggesting potential health benefits alongside improved safety (Hazra et al., 2021).

As the technology matures, its integration into existing dairy processing lines could become more feasible, offering a cost-effective solution for small and large producers alike. Moreover, the development of innovative systems such as moderate electric field (MEF) technology presents new opportunities for non-thermal pasteurization. This technological integration could lead to more precise control over processing conditions, ultimately improving the safety and quality of milk products. Furthermore, the continuous improvement of high-pressure processing (HPP) techniques is expected to play a crucial role in the future of dairy processing. HPP has been recognized for its ability to maintain the nutritional and sensory qualities of milk while effectively inactivating pathogens (Wesolowska et al., 2018).

5. CONCLUSION

Innovation and adaptation to consumer demands for quality and safety will be the hallmarks of non-thermal processing techniques in the dairy industry in the future. The integration of technologies such as PEF, ultrasonication, MEF holds significant potential for enhancing the quality and safety of milk and milk products. Adoption of these techniques is driven by growing consumer demand for minimally processed, high-quality dairy products. However, challenges remain, including the scalability of these methods, regulatory approval, and cost-effectiveness for large-scale applications. Continued research and optimization of these technologies are essential for their broader implementation, paving the way for safer and more sustainable dairy processing practices.

CONFLICTS OF INTEREST

The authors declare that they do not have any conflict of interest.

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