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Invited Review Article

Effect of non-thermal processing on nutritional and functional properties of milk and milk products- A review

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Abstract

Challenges persist in the thermal treatment of milk and milk products as functionality and quality of food constituents are compromised due to heat treatment. Alternative technologies, which are energy efficient, provide scope for automation and environment friendly, are the need of the hour. Development of non-thermal technologies such as high pressure processing, pulse electric field, ultrasound, membrane filtration, cold plasma etc. has widened the scope of research in dairy processing to meet the consumer expectations in terms of health and wellness products. Many such technologies are successfully adopted in the processing of milk and milk products. However, many challenges lie in ensuring food safety, as the mechanism of action of these non-thermal technologies on different dairy constituents needs to be properly understood. Apart from this, process adoptability is low due to high initial investment. With the wider adoption of these technologies and increased sales, it is expected that the cost on machineries will come down. There is a need to understand the basic principle of these technologies and their effect on milk constituents so that they can be used to control the factors responsible for nutritional and microbial spoilage. This review offers a systematic description of how non-thermal technologies affect the nutritional quality and microbial safety of milk and milk products.

Keywords: HPP, Membrane filtration, Milk, PEF, US

Highlights

- This review highlights the application of non-thermal technologies in milk and milk products processing.
- The basic principles of non-thermal technologies are discussed.
- The challenges in adopting these technologies are discussed.
- This article will provide valuable information on the pros and cons of non-thermal technologies used in the dairy industry.

INTRODUCTION

Milk and milk products have been a vital source of energy for human beings since their inception. However, they are characterized by perishability in nature due to higher moisture content and are susceptible to microbial growth. Milk and milk products are full of important nutrients, like proteins, vitamins, minerals, and fats. Ensuring the retention of these nutritious qualities is crucial for upholding the standard and health-promoting attributes of dairy products. Conventional heat treatment processes such as pasteurization and sterilization are used to ensure microbial safety of the milk and milk products and shelf-life extension. Heat desiccation is a severe heat treatment process to concentrate the milk to produce different Indian milk products. So, the methods adopted in developing milk products cause drastic loss of nutritional value and changes in organoleptic qualities (Coolbear *et al.*, 2022).

Organic and minimally processed meals are becoming more and more popular in the growing area of nutritional foods. The increasing focus on food safety and health considerations has led to the growing significance of non-thermal processing methods in the realm of minimally processed foods. Sustainable non-thermal technologies are emerging as an intriguing alternative for dairy industries to develop safer, healthier and nutritious milk and milk products (Ahmad *et al.*, 2019). These non-thermal processing adopt a temperature range close to ambient temperature unlike thermal treatments (65 to 150°C). Though there is a resurgence of interest in adopting non-thermal technologies, several aspects of process parameters and the product's intrinsic qualities need to be looked into while standardizing a non-thermal process. Moreover, a single non-thermal process may not give the required lethality or other quality parameter expected as that of thermal one. So, a combination of

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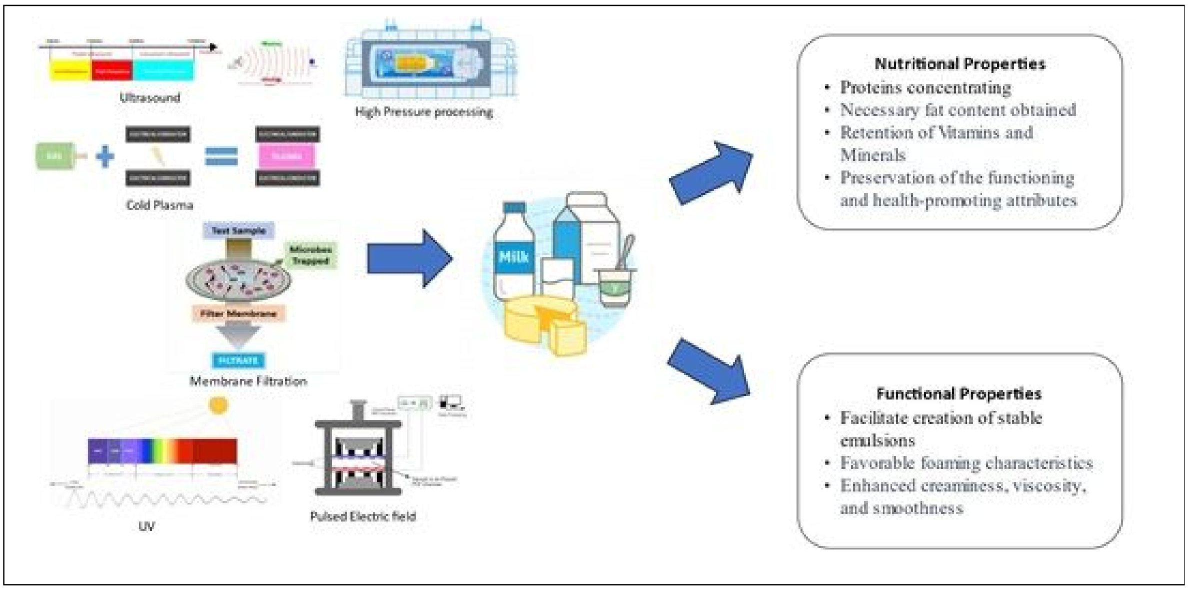

Fig. 1. Effect of non-thermal technologies on properties of milk and milk products

thermal and non-thermal operations would be an option (Toepfl *et al.*, 2006).

The goal of this review is to provide an overview of the effect of non-thermal processing on the nutritional, organoleptic and microbial quality of milk and milk products. Furthermore, the challenges in adopting these technologies and future scope are discussed elaborately. The concept diagram of this article is presented in Fig. 1.

Non-thermal process technologies and their effect on milk products High pressure processing (HPP):

Principle of HPP- High-pressure processing (HPP) is considered to be a very promising technique for enhancing the preservation and preparation of food items, leading to better functional and microbiological qualities. HPP is a novel food processing technique that does not rely on heat energy. In this approach, food products are exposed to an elevated pressure range of 100-800 MPa. HPP, sometimes referred to as high hydrostatic processing (HHP) or ultra-high-pressure processing (UHHP) or isostatic processing, is classified as a kind of cold processing technology due to the utilization of ambient temperature in the majority of the processing procedures (Farr, 1990). This technique enhances food safety by effectively eliminating microorganisms responsible for foodborne illnesses and spoilage, while preserving the freshness of the food. This particular category of food items has the ability to be stored for an extended duration while maintaining optimal quality.

There are two fundamental principles that are applicable in HP (high pressure) processes, namely Le Chatelier's principle and the Isostatic principle. According to the Le Chatelier's principle, 'if a change in conditions is imposed on a system in equilibrium, the system will react to counteract that change and restore the equilibrium'. The Isostatic principle states that the hydrostatic pressure process is not influenced by the volume of the sample. Consequently, pressure may be evenly and quickly delivered to the whole sample without the presence of pressure gradients. As a result, the size and shape of the product are inconsequential. However, there is another concept that is associated with HPP, the principle of microscopic ordering. According to this concept, under constant temperature conditions, an elevation in pressure leads to an augmentation in the degree of molecular ordering inside a certain material. Hence, it can be shown that pressure and temperature have opposing influences on molecule structure and chemical processes (Naik *et al.*, 2013). HPP units comprises of a cylindrical pressure vessel, pressure-generating system which includes pumps or intensifiers, pressure-transmitting medium, temperature control device and material handling systems (San Martín-González *et al.*, 2006). The pressures associated with the preparation of food items fall within a certain range. The range of pressure is often expressed as 100-1000 megapascals (MPa), which is equivalent to 1000-10,000 bar. In the present day, there exists a range of HPP units that are readily accessible and obtainable. The product capacities range from 35 to 687 litres. The essential factors for the therapy of HPP. Ideally, it is desirable for the material to possess a tensile strength below 420MPa, as this characteristic serves to reduce the initial investment cost associated with the acquisition of equipment. According to Buzrul *et al.* (2008), the existing threshold for pressure in commercial-scale applications, the tensile strength of the vessels is 680MPa.

Effect of HPP on nutritional and functional properties- Nutritional properties- The high-pressure processing (HPP) technique is well known for its protein-friendly nature. According to a study conducted by Delgado *et al.* (2013), HPP did not result in substantial denaturation or degradation of proteins in milk. Consequently, the protein content and composition of milk were effectively preserved. HPP is anticipated to result in negligible impacts on vitamins and mineral content of milk and its products. According to a study conducted by Mercan *et al.* (2018), milk treated with HPP maintained similar levels of vitamins and minerals when compared to milk that had not undergone this treatment. HPP mostly focuses on microbes and enzymes, and its impact on the lipid content or fatty acid profiles of milk is unlikely. There is a dearth of research about the effects of HPP on milk lipids, indicating a need for more inquiry in this area. HPP may also potentially contribute to the preservation of bioactive chemicals. The study conducted by Devi *et al.* (2013) provided evidence that HPP treatment has the ability to effectively preserve the bioactive peptides present in milk, suggesting possible advantages in maintaining their activity.

Functional properties- High-pressure processing, as a non-thermal treatment, is not anticipated to have a substantial impact on the emulsification characteristics of milk. According to a study conducted by Delgado *et al.* (2013) it was observed that the emulsification capability of HPP-treated milk did not undergo significant alterations, suggesting the retention of this particular functional characteristic. HPP primarily focuses on the inactivation of microbes and enzymes, and it is improbable that it will have an impact on the foaming properties of milk proteins. The study conducted by Panozzo *et al.* (2014) investigated the effects of HPP on the foaming capabilities of egg white proteins. The results indicated that there were no statistically significant variations in foam stability between the HPP-treated samples and the untreated samples, indicating that the foaming properties were little affected by the HPP treatment. The application of HPP to yoghurt did not result in any notable changes in its gelation characteristics or rheological behaviour (Tsevdou *et al.*, 2020). Although there is a scarcity of studies specifically examining the direct effects of HPP on the texture and mouthfeel of dairy products, available information from analogous food categories indicates that any potential influence is likely to be small.

Effect of HPP on organoleptic qualities- HPP method is anticipated to result in minimal effects on the flavour and scent of the product. According to a study conducted by Delgado *et al.* (2013), it was shown that milk treated with HPP had a flavour and scent comparable to milk that had not undergone any treatment. HPP has minimal effect on the visual characteristics of milk and milk products, since its main focus is on the inactivation of bacteria and enzymes. According to a study conducted by Tao *et al.* (2014), which examined a range of food products including dairy, it was found that HPP did not result in any negative impact on the visual characteristics of the products. The effect of HPP on yoghurt was found that there were no statistically significant changes in either the texture or mouthfeel of the product. In HPP-treated yoghurt, the sensory qualities seem to have been preserved, as reported by Tsevdou *et al.* (2020).

Ultrasound:

Principle of ultrasound- The use of ultrasonic processing as a novel non-thermal technology has advanced significantly in the last several years (Thi Hong Bui *et al.*, 2020). This advancement has been spurred by the identification of its potential benefits in a variety of sectors, such as medicines, biology, and the production of food. According to Deeth *et al.* (2013), the dairy sector has made substantial use of ultrasound as an emerging technology to improve the overall efficiency of the manufacturing process as well as the functional qualities of milk and milk products. The use of ultrasound as a potential alternative or complementary method to conventional treatments has resulted in a number of benefits for the processes of emulsification, milk homogenization, lactose/fat crystallization, modification of the physical and functional characteristics of dairy products, fat separation, degassing, and foaming. These benefits can be attributed to the use of ultrasound as a potential alternative or complementary method. The low frequency range of 20-25 kHz has shown to be the most useful for the application of ultrasound in the dairy processing industry (Paniwnyk, 2017). Recently, the use of high frequency ultrasound with a maximum of 2 MHz has also been investigated for application in the processing of dairy products (Johansson *et al.*, 2016; Paniwnyk, 2017).

Ultrasound (US) is defined as sound waves that have a frequency that is over the threshold of human hearing (20 kHz). The applications of ultrasound may be broken down into two categories: high intensity - low frequency

(I = 10-1000 W/cm^2 and F = 20-100 kHz) and low intensity - high frequency (I = 1 W/cm^2 and F > 1 MHz). The material being treated undergoes cycles of compression and expansion (rarefaction) as a direct result of ultrasound's ability to produce alternating high and low pressures. The basic process employed in milk systems for ultrasound is the mechanism of acoustic cavitation or implosion. This phenomenon takes place when sound waves travel through liquid food and cause the development of microbubbles, as well as their expansion and eventual collapse. The collapse causes extremely high localized temperatures that are greater than 5000 K in the area around the collapsing cavitation bubbles and creates high pressure that can reach up to several hundred MPa (Ashok *et al.*, 2010). Furthermore, due to both the extremely high localized temperatures and pressure conditions that are induced by acoustic cavitation, free radicals and other reactive species are formed inside the bubbles, and they are also transmitted from the bubbles to the liquid (Riesz and Kondo, 1992). These radicals are the ones that start a chain of oxidation processes in the food medium, and they are also participants in those reactions (Ashok *et al.*, 2010). In liquid systems, high frequency ultrasound with a low intensity creates powerful chemical reactions but has little influence on the physical state of the system. On the other hand, low frequency ultrasound with a high intensity is known as power ultrasound and generates substantial physical effects (Thi Hong Bui *et al.*, 2020).

HIU (high-intensity ultrasound) is a promising new technology that has been invented with the goals of being simple, cost-effective, and environmentally friendly. Due to the diverse range of uses that it offers, whether in the manufacturing or quality control of products, HIU has garnered a lot of attention in the field of food science and technology (Mason *et al.*, 1996; Dolatowski *et al.*, 2007). LIU (Low-intensity ultrasound), often at a frequency >1 MHz, is predominantly utilized in non-destructive analytical applications, including medical diagnostic imaging, food material monitoring, and the separation of multicomponent mixtures. In recent years, there has been an emphasis on using high-intensity ultrasound (HIU) on dairy products and their by-products. The primary objectives of the researches have been to decrease processing time and improve the physicochemical characteristics of various food items. The positive effects of HIU on many quality indices of fresh milk, cheese, butter, ice cream, fermented milk products, whey protein preparations, and other drinks have been demonstrated.

Effect of ultrasound on nutritional and functional properties- Effect on nutritional properties- It has been indicated that ultrasound treatment has a positive effect on the structural properties of milk proteins. Ultrasound vibrations of high frequency can induce changes in protein conformation, resulting in enhanced solubility and digestibility. According to a study by Xu *et al.* (2020), ultrasound treatment elevated the degree of casein hydrolysis, resulting in enhanced protein quality. In addition, ultrasound disrupts protein aggregates, reducing the formation of undesirable whey polymers in the milk processing process. High temperature activity can break down vitamins that are sensitive to heat, like the B-group vitamins. These vitamins can be kept fresh by pasteurizing them or sterilizing them with ultrasound. Jiang *et al.* (2017) did a study that showed that ultrasound treatment can cut down on the loss of vitamin B. Ultrasound can also help fat-soluble vitamins get out of milk fat globules more easily, which makes them more bioavailable. Ultrasound has the potential to improve the bioavailability of necessary minerals found in milk and products made from milk. Ultrasound waves with a high frequency are able to break down mineral-protein complexes, which make it easier for the digestive system to absorb minerals like calcium and magnesium. This effect may be especially helpful for people who suffer from calcium shortages. The use of ultrasound has the potential to have a beneficial effect on the lipid profiles of milk and other dairy products. It has the ability to break apart fat globules, which decreases their size and increases the surface area available for enzymatic digestion and lipolysis. In addition to this, there is a possibility that fatty acids and bioactive lipids will be absorbed more effectively, thereby increasing the positive effects that dairy fat has on human health. Another benefit of using ultrasonic is that it may efficiently inactivate pathogenic bacteria without the need for high-temperature processing, which can have a detrimental impact on dairy products. The nutritious content of milk and products made from milk may be maintained while the risk of microbial contamination is reduced using ultrasound. Numerous studies have been done on the inactivation of *E. coli* and many other bacteria (Table 1) (Herceg *et al.*, 2012).

Effect on functional properties- Milk's ability to form emulsions is an important functional quality that may be used in a variety of food-related applications. Treatment with ultrasound has the potential to change the size and stability of fat globules, which in turn can have an effect on the emulsification qualities of milk. There was a reduction in the size of the fat globules in milk as a result of the use of ultrasound, which led to improved emulsification capabilities (Zenker *et al.*, 2003). This effect can be helpful in the creation of dairy-based emulsified goods, such as salad dressings and ice creams, due to the fact that it reduces the amount of oil that must be used. In the production of aerated dairy

Table 1. Effect of non-thermal processing on microbial reduction in milk and milk products

Non-thermal Technology	Milk product	Process conditions	Target micro-organisms	Log reduction	Reference
PLT	Cheese	PL doses of 1.02 to 12.29 J/cm^2	*Listeria innocua* *P. fluorescens*, *Escherichia coli*	3.37 - 0.2 3.74 - 0.8 5.41 - 0.1	Proulx *et al*., 2015
Pulsed UV light	Goat milk	10,000 mJ/cm^2	*E. coli*	6	Kasahara *et al*., 2015
Intense pulsed light	Infant foods	5000, 600, 300, and 100 ls at 10, 15, 20, and 25 kV of voltage pulse, respectively.	*L. monocytogenes*	4-5	Choi *et al*., 2010
Pulsed light	Milk	8.4 J/cm^2 in turbulent mode,14.9 J/cm^2 (Skim milk)	*E. coli*	>2.53.4	Miller *et al*., 2012
HPP	Skim milk	84°C at 300 MPa	*B. stearothermophilus*, ATCC 7953, *C. sporogenes* PA 3679	0.67	Pinho *et al*., 2011
HPP	Commercial sterile milk	300 MPa at 75-85°C, K (1/min) = 0.768, 350 MPa, K (1/min) = 0.768, 275 MPa, K (1/min) = 2.303, 400 MPa, Temp. = 50°C, K (1/min) = 0.768, 400 MPa, Temp. = 50°C	*Bacillus spores*, *S. typhimurium*, *Y. enterocolitica*, *E. coli*, *E. coli* O157:H7	~5 3 min D value, 3 min D value, 1 min D value, 3 min D value	Amador Espejo *et al*., 2014; Patterson *et al*., 1998; Patterson *et al*., 1998; Gervilla *et al*., 1997
HPP	Milk	K (1/min) = 0.92, 500 MPa, Temp. = 50°C, K (1/min) = 0.768, 375 MPa, K (1/min) = 2.303,300 MPa, Temp. = 50°C,K (1/min) = 3.838, 345 MPa Temp. =50°C	*S. aureus*, *L. monocytogenes*, *P. fluorescens*, *P. fluorescens*	2.5 min D value, 3 min D value, 1 min D value. 0.6 min D value	Patterson *et al*., 1998; Gervilla *et al*., 1997; Kalchayanand *et al*., 1998
HPP	Raw milk	25 °C, 300 MPa	*S. typhimurium*	9.21 min D value	Erkmen, 2009
HPP	Milk	90 °C, 700 MPa,300 MPa, 25°C	*C. sporogenes*, *E. coli* MG1655	13.6 min D value,3.50	Shao and Ramaswamy, 2011; Diels *et al*., 2005
HPP	Raw milk with 15% fat	300 MPa, 24°C, 100 MPa, 2-4°C, 300 MPa, 20°C	*L. innocua*, *L. monocytogenes*, *S. aureus*	1.80 1.20 4.00	Picart *et al*., 2006; Iucci *et al*., 2007; Briñez *et al*., 2007
CP	Milk, Skim milk	35-40 kV, exit temperature < 60°C, single pass 35-40 kV, exit temperature < 60°C, double pass	*E. coli* O157:H7 ATCC 43895,*Salmonella* (5 strain mixture),*L. monocytogenes* (5 strain mixture), *B. cereus* (3 strain mixture), *E. coli*	≥3.94 2.95 2.74 0.18 4.36	Ruan, 2007

Cont. Table 1.

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Non-thermal Technology	Milk product	Process conditions	Target micro-organisms	Log reduction	Reference
			O157:H7 (5 strain mixture), *Salmonella* (5 strain mixture), *L. monocytogenes* (5 strain mixture)	5.55 4.73	
CP	Whole milk, Semi-skimmed milk, Skimmed milk	9 kV of AC power supply, 20 min, temp below 35°C	*E. coli* ATCC 25922	3.63 3.40 3.34	Korachi *et al*., 2010; Korachi and Aslan, 2011
US	Raw whole 4% fat cow's milk	20 kHz, 120 μm, 12 min,60°C	*E. coli*	3.1	Herceg *et al*., 2012
US	Raw milk (pasteurization)	20 kHz, 10 min, 750 W	*E. coli*	5.34	Cameron *et al*., 2009
US	UHT milk (pH 6.7)	110 μm, 60°C	*E. coli* K12 DH5 α	D60 and S = 23s	Zenker *et al*., 2003
US	Skim milk	30 min at 50°C, 30 min at 40°C	*S. typhimurium*	3 2.5	Wrigley and Llorca, 1992
MF	Skim milk, Reconstituted skim milk	MF-1.4 μm, ceramic membrane, Tp = 50°C	Total bacterial load	5.18 4.5	Awad *et al*., 2010; Pafylias *et al*., 1996
MF	Skim milk	MF- 0.8μm, tubular ceramic ISOFLUX membrane	Total bacterial load	>2.3	Gosch *et al*., 2014
MF	Simulated milk ultrafiltrate	MF- 1.4 μm, ceramic membrane, MF- 0.5μm (micro sieve)	*Bacillus cereus* spores, *Bacillus subtilis*	3.5 6.6	Olesen and Jensen, 1989; Brans *et al*., 2004
PEF	Milk	submitted to ultrafiltration 50 pulses of 60 kV/cm or 80 pulses of 70 kV/cm	*E. coli*	6 9	Qin *et al*., 1998
PEF	Pasteurized skim milk	200 μs at 50 kV/cm	*L. innocua*	2.6 - 2.7	Fernández-Molina, 2001
PEF	Raw bovine milk	89 μs at 40 kV/cm at 32.5°C	*E. coli* K12	5	Cregenzán-Alberti *et al*., 2015
PEF	Raw whole milk	25 kV/cm for 200 μs	*S. aureus*, *E. Coli*	5.2 2.1	Zhao *et al*., 2013
PEF	Raw whole milk, UHT milk	30 kV/cm after 200 μs	*L. monocytogenes*	5	Zhao *et al*., 2013

products such as whipped cream and mousse, the presence of foaming qualities is very necessary. It has been demonstrated that subjecting milk proteins to ultrasound treatment can increase their foaming properties. Ultrasonic treatment was shown to enhance the foam stability as well as the volume of milk protein-based foams (Wierenga and Gruppen, 2010). The production of dairy products with increased texture and mouthfeel may result from this modification if it is implemented. Milk's natural ability to form a gel is an important component in the manufacture of dairy products like yoghurt and cheese. Modification of protein structures brought forth by ultrasound can have an effect on the gelation process. According to the findings of Soria and Villamiel (2010), ultrasonic treatment of milk proteins led to changes in the structural properties of the proteins, which in turn led to improvements in the gelation and textural features of the milk. Based on these findings, it can be inferred that ultrasonography may be utilized to modify the consistency of dairy products to better suit individual preferences. When it comes to dairy products, texture is an important sensory quality to consider since it might influence customer acceptability. The texturization and rheological qualities of milk and products made from milk can be altered by the use of ultrasound. Ultrasonic treatment of yoghurt increased its texture as well as its rheological qualities, resulting in a more silky and creamy consistency (Aslam *et al.*, 2022).

Effect of ultrasound on organoleptic qualities- Although ultrasonic technology has several uses in dairy processing that hold significant potential, a notable concern linked with this technology is the generation of off-flavours, which have been described as "rubbery," "burnt," or "foreign" in nature. There has been a possibility that the use of ultrasound might result in the formation of radicals, which in turn may facilitate lipid oxidation processes within milk systems. This process can give rise to the production of volatile chemicals that are considered unpleasant, hence contributing to the development of off-flavours. Also, the process of protein breakdown can result in the breakage of amino acid side chains, thus producing aromatic hydrocarbons. The study of volatile compounds in sonicated milk systems has been mainly centred on addressing concerns related to off-flavour development. This is because the application of ultrasound treatment, has been found to contribute to the production of volatile compounds. Researchers have been particularly interested in understanding the mechanism and origin of these compounds under various ultrasonic processing conditions. In addition, the variability in composition within milk systems has a significant role in the generation of volatile compounds (Popova *et al.*, 2019). Hence, it is vital to comprehend the structural modifications of key milk constituents in order to anticipate the generation of volatile molecules.

Cold plasma:

Principle of cold plasma- The use of cold plasma treatment, a non-thermal technology, has recently gained prominence as an innovative approach to maintain the nutritional integrity of milk and its derivatives, enhancing their safety and extending their shelf life. Prior to the turn of the 21st century, technologies such as high hydrostatic pressure, pulse electric field, and ultrasound were of utmost importance, whereas cold plasma has only appeared relatively recently in food-based research. Cold plasma has been shown to impact the quality of a variety of foods, including whole and juiced fruit (Misra *et al.*, 2014; Almeida *et al.*, 2017; Rodríguez *et al.*, 2017). In the context of animal-derived products, diverse dairy products have been exposed to cold plasma treatment under varied experimental settings. Numerous investigations have been conducted to examine the impact on pH, fatty acid content, volatile chemicals, as well as rheological, microstructural, and thermal characteristics of milk, chocolate milk beverages, and whey-based beverages, pH, color, oxidation and sensory evaluation of the sliced cheddar cheese. The impact of cold plasma on various constituents of food can provide both favourable outcomes, such as the alteration of carbohydrate and protein, as well as unfavourable consequences, such as the oxidation of lipids. The use of cold plasma treatment has been found to have a positive impact on the inactivation of food enzymes (Misra *et al.*, 2016). This effect is attributed to the structural alterations of enzyme proteins, resulting in a subsequent loss of enzymatic activity.

Plasma is the state of ionized gas that is quasi-neutral, consisting of ions, free electrons, and atoms or molecules in their fundamental or excited states, collectively maintaining a net neutral charge. Atmospheric cold plasma (ACP) refers to a condition of gas that is slightly ionized, with the ionization level being roughly 5% or less of the gas. The gas temperature in this state is carefully regulated to remain at approximately room temperature. The presence of non-equilibrium conditions leads to the removal of free electrons from a limited number of gas molecules, determined by their ionization potential. The occurrence of free elections afterwards intersecting with molecules that are unionized gives rise to radical elements. The phenomenon referred to as a cascade reaction leads to the production of several reactive species, including radicals and reactive gas species. Various plasma sources are used for food treatment, such as plasma jets, dielectric barrier discharges (DBDs), corona discharges, and microwave discharges (Misra *et al.*, 2016).

Effect of cold plasma on nutritional and functional properties- Effect on nutritional quality- A treatment with cold plasma has been found to have a beneficial effect on the structural characteristics of milk proteins. Changes in the structure of proteins can be induced by reactive species created by plasma, which can lead to an increase in the protein solubility and digestibility. According to the study conducted by Dabade *et al.* (2023), the addition of cold plasma to milk improved the *in vitro* digestibility of soy proteins, indicating that the protein quality was improved. Treatments supported by cold plasma, which functions at lower temperatures, have the potential to better maintain the heat-sensitive vitamins. In their study, Nikmaram and Keener (2022) stated that treating milk with cold plasma significantly reduced the loss in the amount of vitamin C that was lost while the milk was being processed. Treatment with cold plasma can increase the amount of vital minerals that are bioavailable in milk and milk products. Reactive species that are created by plasma have the ability to break down mineral-protein complexes, which makes minerals such as calcium and magnesium more available for absorption in the digestive tract. This effect may be especially helpful for people who are deficient in certain minerals. Cold plasma treatment also has the potential to change the lipid profile of milk and other dairy products. Plasma-generated species can cause lipid oxidation, which can have repercussions for dairy products in terms of both their sensory qualities and their shelf life. However, these impacts can be minimized with adequate management of the parameters of the cold plasma, which will also protect the milk's lipid quality.

Effect on functional properties- Emulsification is an important functional feature that is required in a variety of applications involving processed dairy, such as creamers and dressings. Studies suggest that treating milk proteins with cold plasma can improve their emulsification capabilities by altering their surface features. Increased emulsion stability can be achieved as a result of plasma-generated reactive species' capacity to stimulate protein unfolding, which in turn increases those proteins' adsorption at the oil-water interface. In dairy products like whipped cream and mousse, foaming is an absolutely necessary step. The interfacial characteristics of proteins can be changed by treatment with cold plasma, which can then have an effect on foaming properties. The findings of a study conducted by Dabade *et al.* (2023) indicated that treatment with cold plasma increased the foaming capability and stability of milk proteins. These improvements can be linked to changes in the structure of the protein. The influence that cold plasma treatment has on the structure of proteins has the potential to change the gelation and consistency of dairy products. Cold plasma treatment of milk proteins led to alterations in gelation kinetics, which ultimately resulted in gels that were both more stable and more robust (Terpiłowski *et al.*, 2017). Texture is an important aspect to consider when judging dairy products. Treatment with cold plasma can change the surface characteristics of milk proteins, which in turn can affect the texture and rheological qualities of the milk. According to the findings of Sharma and Singh (2023), the treatment of yoghurt with cold plasma enhanced both its texture and its viscosity, resulting in a mouthfeel that was less gritty and a texture that was creamier.

Effect of cold plasma on organoleptic qualities- As a non-thermal process, the treatment of milk and milk products with cold plasma does not appreciably alter the flavour or aromatic qualities of the milk or the products made from milk. According to the findings of a study conducted by Chutia *et al.* (2020), the application of cold plasma to orange juice had a minimal impact on its sensory qualities, such as its flavour and aroma. Consumer perception of milk and dairy products is significantly influenced by its visual attributes, such as colour and visual texture. The application of cold plasma treatment often does not result in any negative impacts on the visual characteristics of dairy products. Pankaj *et al.* (2018) reported that the application of cold plasma treatment did not result in any noticeable changes in the colour and visual characteristics of apple juice. This finding implies that the impact of cold plasma treatment on the look of milk and milk-based products is expected to be minimal. The study conducted by Ji *et al.* (2018) provided evidence indicating that the use of cold plasma treatment had a beneficial impact on the texture and mouthfeel of soy protein gel. This finding suggests a promising opportunity for enhancing the texture of dairy products. However, further research especially dedicated to examining the impact of dairy products is necessary in order to confirm this effect. In order to validate its potential influence, conducting studies that evaluate consumer preferences and acceptance of dairy products treated with cold plasma is crucial. However, the objective of cold plasma treatment is to maintain and improve the overall acceptability of these items by guaranteeing food safety while preserving sensory quality.

Membrane filtration:

Principle of membrane filtration- Membrane filtration is a nonthermal method of selectively separating components in foods. A semi-permeable membrane serves as a physical barrier that has the ability to either fully reject or decrease the flow of a certain substance, therefore facilitating its separation from the other components of the stream. Membrane filtration processes are extensively used in the dairy sector for the purpose of standardizing or concentrating milk proteins. There are various forms of membrane filtration viz. microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO). Out of these, ultrafiltration has been widely used in dairy industries. Ultrafiltration (UF) is a process in which lactose and tiny molecular weight components pass through a membrane, while proteins are obtained in the retentate (Waungana *et al.*, 1998; Li and Corredig, 2020). Despite extensive use of membrane processes, there is a lack of comprehensive understanding about the changes in the physico-chemical characteristics of milk's casein micelles during membrane filtration, as well as the resulting implications for the functional features of the retained substances. The renneting functionality of casein micelles is altered with the use of ultrafiltration (UF), particularly when applied at concentrations above three times the normal concentration (Alexander *et al.*, 2011). The impact of the soluble fraction's composition on reconstituted milk concentrates has also been studied. Understanding the influence of UF combined with diafiltration (DF) on the gelation behaviour of milk proteins under acidic conditions is crucial, particularly due to the potential use of milk protein concentrates as constituents in fermented milk gels.

Effect of MF on nutritional and functional properties-

Effect on nutritional qualities- The process of membrane filtration, more specifically ultrafiltration (UF), has been commonly used for the purpose of protein concentration in milk and dairy products. Ultrafiltration (UF) is capable of preferentially concentrating proteins, such as casein and whey proteins, while simultaneously permitting the passage of smaller molecules, such as lactose and minerals, across the membrane. Research findings have indicated that the use of UF (ultrafiltration) can yield notable enhancements in both the protein content and composition of many dairy products, particularly milk. The lipid content of milk and dairy products can be influenced by membrane filtration, particularly when fats are selectively separated. Membrane filtration is used in cream separation operations to effectively get the necessary fat content in the products. The selection of membrane type and optimization of process parameters have the potential to significantly impact the efficacy of separation and, thus, the lipid composition of the end product.

Retention of vitamins and minerals: Membrane filtration, particularly microfiltration (MF) and nanofiltration (NF), facilitates the segregation of different constituents by virtue of their molecular dimensions, hence facilitating the preservation of vital vitamins and minerals in dairy products. Numerous studies have demonstrated that these procedures have the capability to retain essential vitamins, such as vitamin C and B-group vitamins, as well as minerals like calcium, while effectively segregating unwanted constituents. Membrane filtration has the potential to effectively preserve the beneficial components that are naturally found in milk and dairy products. Bioactive peptides, which possess promising health-enhancing properties, may be effectively concentrated using UF techniques, while other bioactive substances, such as immunoglobulins, are selectively maintained by MF processes. These mechanisms contribute to the preservation of the functioning and health-promoting attributes of dairy products.

Effect on functional properties- The emulsifying capabilities of milk are significantly influenced by membrane filtration, particularly UF, through the alteration of protein composition and fat content. The protein fraction generated using UF has enhanced emulsification ability, hence facilitating the creation of stable emulsions in dairy-based formulations. Membrane filtration has the potential to impact foaming properties through the alteration of protein composition and structure. Numerous studies have provided evidence indicating that the utilization of membrane filtering in the production of dairy products leads to notable enhancements in foam stability and volume. These improvements can be attributed to the increased concentration of proteins with favourable foaming characteristics. The gelation characteristics and rheological behaviour of dairy products play a vital role in determining their texture and sensory perception. Membrane filtration has the capacity to adjust these characteristics through the process of protein concentration and structural modification. Studies have demonstrated that the concentration of milk proteins by the utilization of UF techniques yields notable enhancements in gelation capabilities and rheological behaviour. Consequently, this advancement has facilitated the creation of dairy products that possess superior texture and mouthfeel attributes. Membrane filtration plays a crucial part in the modification of the texture and mouthfeel of milk and milk products through the process of protein concentration and composition adjustment. For example, the process of UF is employed to concentrate milk proteins, resulting in enhanced attributes such as creaminess, viscosity, and smoothness in dairy products like yoghurt. Consequently, this enhancement contributes to a superior sensory experience for consumers.

Effect of MF on organoleptic qualities- Membrane filtration is a widely used method within the dairy industry for a multitude of applications involving the concentration, fractionation, and purification of milk and milk products. It is vital to comprehend the impact of this substance on the organoleptic characteristics, encompassing flavour, aroma, appearance, and texture, of these commodities in order to guarantee customer acceptance. Studies indicate that the implementation of membrane filtering techniques has the ability to concentrate the flavour constituents present in dairy products, hence potentially enhancing their taste characteristics. As an instance, Kim and Morr (1996) documented that the use of UF on skim milk resulted in an enhanced sweetness, mostly attributed to the heightened concentration of lactose and other sugars. However, it is important to take caution in the choice of membrane type, pore size, and process parameters in order to prevent the undesired accumulation of unfavourable flavours or off-notes. The utilization of membrane filtering techniques has the potential to induce significant changes in the sensory attributes of dairy products, particularly in relation to their textural and consistency characteristics. For instance, the utilization of ultrafiltration (UF) has been shown to result in enhanced creaminess and viscosity in yoghurt, as demonstrated by Gursoy *et al.* (2016). In a study conducted by Muse and Hartel (2004), it was found that the use of MF has been implemented as a means to enhance the texture and sensory experience of low-fat dairy products. Membrane filtration, specifically UF and MF, has the potential to modify the texture and sensory perception of milk and dairy products. It was observed that the addition of MF resulted in an improvement in the creaminess of low-fat yoghurt, while also reducing its grittiness. Furthermore, it was suggested by Vieira *et al.* (2020) that UF has the potential to enhance the texture attributes of dairy desserts, particularly in terms of smoothness and creaminess.

UV:

Principle of UV- Thermal pasteurization is widely used and recognized as the predominant approach for the inactivation of pathogens present in skim milk (Jayarao *et al.*, 2006). However, this process is not without its limitations. Thermal pasteurization is associated with significant energy expenditures, the potential denaturation of proteins, and often results in alterations to the sensory characteristics of goods (Cappozzo *et al.*, 2015; Islam *et al.*, 2016). Certain liquid meals may not be able to withstand the heat treatments included in the pasteurization process due to the presence of biomolecules that are sensitive to high temperatures. Consequently, there exists a keen interest in exploring alternate techniques that do not rely on heat processes to effectively disinfect skim milk and other liquid food products. The use of UV technology has recently emerged as a potential method for non-thermal preservation in the beverage industry. The potential of ultraviolet light within the germicidal spectrum, notably UV-C light with wavelengths ranging from 200 to 280 nm, has also been studied for its ability to deactivate pathogens in skim milk (Cappozzo *et al.*, 2015; Gunter-Ward *et al.*, 2018). The primary mechanism by which UV-C light eliminates microorganisms is by the formation of cross-linked pyrimidine dimers in nucleic acids as a consequence of their absorption of UV-C radiation. The presence of pyrimidine dimers in critical loci has been found to have a substantial impact on DNA repair mechanisms, leading to the inhibition of organism replication and subsequent reduction in pathogenicity (Hamkalo and Swenson, 1969; Bintsis *et al.*, 2000; Guerrero-Beltr·n *et al.*, 2004).

Effect of UV on nutritional and functional properties- Effect on nutritional properties- Proteins provide an important nutritional constituent of milk. The non-thermal nature of UV treatment indicates that it is unlikely to induce protein denaturation or substantial breakdown. The study conducted by Heinz *et al.* (2003) revealed that milk treated with ultraviolet (UV) radiation had a protein content and composition that were similar to those of untreated milk. Vitamins and minerals are important micronutrients found in milk that significantly contribute to its nutritious composition. The non-thermal nature of UV treatment is expected to result in negligible impacts on the nutritional content of these substances. A study showed that the vitamin content in UV-treated milk was similar to that of untreated milk (Delormea *et al.*, 2020). Lipids, comprising fats and fatty acids, are essential constituents of milk. The primary objective of UV treatment is to achieve microbial inactivation, with no anticipated impact on the lipid content or fatty acid profiles of milk. The impact of UV on milk lipids has not been studied well, thus more research is required. Bioactive substances with potential health advantages are present in milk and milk products. The non-thermal characteristic of UV therapy has the potential to effectively maintain bioactive substances. It was suggested that the utilization of UV treatment might effectively maintain the presence of bioactive peptides in milk, hence demonstrating potential advantages in terms of retaining their functioning (Siriamornpun and Kaewseejan, 2017).

Effect on functional properties- While UV treatment is largely applied for microbial inactivation and preservation, its impact on the functional characteristics of milk and milk products is not well documented. UV treatment, being a non-thermal method, is anticipated to exert little influence on functional attributes such as emulsification, foaming, gelation, texture, and mouthfeel. Nevertheless, it is vital to possess a comprehensive comprehension of the effects of this technology in order to effectively exploit its potential inside the dairy sector.

Effect of UV on organoleptic qualities- Ultraviolet (UV) treatment is considered a beneficial non-thermal process that plays a crucial role in maintaining safety and increasing the shelf life of milk and milk products. This method is particularly advantageous since it minimizes any notable negative impacts on the sensory qualities of these goods. In general, it preserves the sensory attributes such as flavour, aroma, appearance, texture, and overall palatability. Nevertheless, the sensory impact can be influenced by modifications in product kind, treatment time, and UV intensity. The use of UV radiation to milk is generally known to have minimal impact on its flavour and aroma due to its non-thermal nature. According to a study conducted by Delormea *et al.* (2020), it was shown that UV-treated milk exhibited comparable taste and aroma characteristics to untreated milk. In general, it preserves the sensory attributes of flavour, aroma, visual presentation, texture, and overall desirability. Nonetheless, the sensory impact can be influenced by factors such as differences in product kind, treatment time, and UV intensity.

Pulse electric field processing (PEF):

Principle of PEF- Pulsed electric fields (PEF) is extensively used in the fields of genetic engineering and biotechnology for the purpose of cell hybridization and electrofusion. Microbial cells are subjected to the application of a pulsed electric field (PEF) with the purpose of inducing electroporation of the cellular membrane. Exogenous substances, such as deoxyribonucleic acid (DNA), are then introduced to incorporate into the cellular structure. Upon removal of the pulsed electric field (PEF), microbial cells undergo membrane repair, thereby closing the electropores. The fusion of two microbial cells is achieved by the creation of electropores and subsequent cell alignment. In the field of genetic engineering and biotechnology, it is essential to exercise precise control over the process to ensure the continued survival of microorganisms while applying pulsed electric fields (PEFs).

PEF has garnered significant attention in the food sector as a promising method for enhancing mass transfer operations. This technology has acquired increased interest in recent years (Jeyamkondan *et al.*, 1999). The procedure relies on the use of external electric fields to induce electroporation in eukaryotic cell membranes, hence facilitating the enhanced diffusion of solutes. The permeabilization of cell membranes may be accomplished by applying moderate electric fields, often below 10 kV/cm, and using low specific energies, generally less than 10 kJ/kg.

Effect of PEF on nutritional and functional properties-

Effect on nutritional properties- The use of pulsed electric field (PEF) treatment has the potential to induce alterations in protein structure and therefore impact protein functioning. The study conducted by Toepfl *et al.* (2006) provided evidence supporting the notion that the use of pulsed electric field (PEF) treatment has the potential to induce protein denaturation and aggregation, hence influencing their digestibility and bioavailability. The application of PEF treatment has been found to effectively mitigate thermal deterioration, hence preserving the essential vitamins and minerals naturally occurring in milk. An investigation by Heinz *et al.* (2003) revealed that milk treated with PEF exhibited greater retention of vitamins A and B2 in comparison to milk that underwent standard pasteurization methods. The application of PEF treatment has the potential to impact the lipid profile and fatty acid content of milk and dairy products. Sale and Hamilton (1967) found that the use of PEF treatment resulted in an increase in the release of free fatty acids. This increase in free fatty acids has the potential to impact both the sensory characteristics and nutritional composition of milk and its products. The use of PEF treatment has been proven to be effective in preserving the bioactive components. The study conducted by Roobab *et al.* (2022) provided evidence that the use of pulsed electric field (PEF) treatment effectively conserved the enzymatic activity in milk, hence suggesting its potential for preserving the functional and nutritional attributes of dairy products.

Effect on functional properties- The emulsification characteristics of milk can be affected by PEF treatment through the modification of fat globule size and stability. The study conducted by Marco-Molés *et al.* (2011) provided evidence that the use of PEF treatments resulted in a reduction in the size of fat globules and an improvement in emulsification capabilities. These findings highlight the potential of PEF treatment in the formulation of dairy products. The use of PEF treatment has the potential to enhance the foaming properties shown by milk proteins. The research conducted by Taha *et al.* (2022) demonstrated that the use of PEF treatment resulted in an improvement in both the stability and volume of foams made from milk protein. These findings indicate that PEF treatment has the potential to enhance the foaming characteristics of dairy products. Protein structure can be changed by PEF treatment, which can change these traits. The use of PEF treatment on milk proteins induced alterations in their structural characteristics, leading to enhanced gelation and rheological capabilities (Sharma *et al.*, 2014).

Effect of PEF on organoleptic qualities- The use of pulsed electric field (PEF) therapy is often seen to have minimal effect on the visual characteristics of milk and milk products, mainly because of its non-thermal nature. The research conducted by Salehi (2020) on fruit juices revealed that the use of PEF treatment preserved the visual characteristics of the materials, presenting a possibility that there will be limited influence on the visual attributes of dairy items as well. The use of PEF treatment possesses the capability to impact the texture and mouthfeel attributes of food products through the alteration of protein and fat structures. Turk *et al.* (2012) confirmed that the application of PEF treatment resulted in enhanced texture and mouthfeel of apple juice. These findings imply that PEF treatment may have the ability to influence the textures of dairy products. While there are limited studies of particular research on dairy products, previous studies conducted on other food categories, such as fruit juices, offer valuable insights into the possible beneficial effects of PEF treatment on the general acceptance of these products (Salehi, 2020).

Environmental impact of non-thermal processing treatments

Energy consumption and energy savings in the food industry have been the focus of the last 30 years. This is attributable, in part, to the extensive automation of manufacturing processes, as well as the growing need for food safety. Non-thermal processing techniques have gained popularity in the food processing industry due to their ability to preserve food while using as minimal heat as possible. These techniques, which include pulsed electric fields, ultrasound, high-pressure processing, etc. may improve the quality and shelf-life of food. However, in order to develop more environmentally friendly food production systems, it is crucial to evaluate their impact. Examining the energy use, resource use, and general sustainability of non-thermal processing methods can help us understand how they may affect the environment (Pereira and Vicente, 2010).

The energy consumption of non-thermal processing processes is one of the key environmental problems. They are not energy-free, but they consume less energy than typical thermal processes. High-pressure processing, for example, necessitates a significant amount of hydraulic energy, and pulsed electric fields rely on electricity. The environmental impact differs depending on the energy source. To reduce the carbon footprint, it is critical to employ renewable and clean energy sources (Sampedro *et al.*, 2014).

Non-thermal processing techniques often involve the use of specialized equipment and consumables, such as high-pressure chambers and electrodes. The production and disposal of these components might have an impact on the environment. Furthermore, non-thermal treatments, such as ultrasound-assisted cleaning, can result in an increased requirement for water due to washing and rinsing procedures. These effects can be mitigated through resource-efficient design and recycling procedures (Knorr, 1999).

Non-thermal processing often requires less water than standard thermal processes since it does not require significant heating or cooling phases. Non-thermal treatments, such as ultrasound and high-pressure processing, have the potential to save water and contribute to more sustainable water management in the food and beverage sector (Chemat *et al.*, 2011).

Non-thermal processing methods offer the potential to help to more sustainable food processing by lowering energy usage and enhancing product quality. Their environmental impact, however, must be carefully examined. It is critical to use renewable energy sources, optimize resource utilization, and implement recycling methods to reduce this impact. Furthermore, non-thermal processing technologies should be incorporated into a larger sustainability strategy that takes into account the full product life cycle.

Bactofugation

Bactofugation is another type of non-thermal method used for removing bacteria and somatic cells from raw milk in the dairy business. (Stack and Sillen, 1998). It uses centrifugal force as its operating principle to separate materials according to their densities. In order to create a concentrated fraction, heavy particles—such as bacteria and somatic cells—are driven to the centrifuge's outer edge. Milk that has been clarified and has less bacteria in it is taken out of the centre (Júnior *et al.*, 2019).

Bactofugation has a number of benefits, such as less heat damage, higher microbiological quality, longer shelf life, and non-thermal treatment. It is frequently employed in the manufacturing of cheese, yoghurt, fermented goods, and fluid milk, among other dairy products. It does, however, need ongoing maintenance, varying efficiency, an upfront cost, and ongoing operating expenses. Applications for bacteria-fermentation include lowering the bacterial load in fluid milk, enhancing the quality of cheese, and creating premium yoghurt and other fermented dairy products. (Ribeiro-Júnior *et al.*, 2020)

Hybrid technologies

A single nonthermal method may not provide the desired lethality or other quality characteristic as a thermal process. As a result, combination of different non-thermal procedures would be an alternative. Listed below are some examples of non-thermal technologies and their potential combinations:

The potential of combination of PEF and HPP has been investigated for improvement in inactivation of microorganisms in milk while maintaining the milk's nutritious quality. PEF has the ability to produce pores in the membranes of microbial cells, which makes them more vulnerable to subsequent HPP treatment. (Da Cruz *et al.*, 2010).

On the basis of its potential to promote microbial inactivation and enhance mass transfer in milk, the combination of ultrasound and PEF can be subjected to research. Cavitation can be produced by ultrasound, and it is possible for PEF to rupture cell membranes, which can result in enhanced microbial inactivation (Sebastià *et al.*, 2023).

The use of UV light treatment, followed by HPP, has been investigated in some studies for its ability to lower the microbial burden in fluids in a synergistic manner. UV radiation has the ability to specifically target bacteria, and high-performance photons (HPP) can add an additional layer of microbial inactivation. (Koutchma *et al.*, 2019).

The use of ozone treatment in conjunction with ultraviolet light can be examined for its potential to lessen the number of microorganisms that are present in dairy industry wastewater. UV radiation has the potential to boost the antibacterial impact of ozone, which can operate as a powerful oxidizing agent (Suresh *et al.*, 2021).

Conclusion

The changing consumer perceptions and expectations have forced the scientific communities to adopt new and novel technologies. Milk and milk products segment have greater importance in industrial production in India. Keeping in view the nutritional loss and unhygienic practices adopted in conventional heat treatment processes, non-thermal technologies have successfully been adopted for milk and milk products. Wide adoption of these technologies for mass production would reduce the cost of production. Fundamental understanding of their effect on microbes and other milk constituents is needed so that milk and milk products of high quality can be made. Furthermore, the search for successful non-thermal ways in milk processing has led to the development of hybrid technologies that take use of the synergistic benefits of mixing multiple non-thermal treatments. There is enormous potential in combining two or more non-thermal approaches to handle milk and milk products effectively. The underlying problem is a lack of appropriate technology and cost effectiveness in managing process parameters.

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