

Review

# Role of Pascalization in Milk Processing and Preservation: A Potential Alternative towards Sustainable Food Processing

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**Citation:** Chughtai, M.F.J.; Farooq, M.A.; Ashfaq, S.A.; Khan, S.; Khaliq, A.; Antipov, S.; Rebezov, M.; Khayrullin, M.; Vorobeva, A.; Nelyubina, E.; et al. Role of Pascalization in Milk Processing and Preservation: A Potential Alternative towards Sustainable Food Processing. *Photonics* **2021**, *8*, 498. <https://doi.org/10.3390/photonics8110498>

Received: 12 September 2021

Accepted: 29 October 2021

Published: 8 November 2021

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**Abstract:** Renewed technology has created a demand for foods which are natural in taste, minimally processed, and safe for consumption. Although thermal processing, such as pasteurization and sterilization, effectively limits pathogenic bacteria, it alters the aroma, flavor, and structural properties of milk and milk products. Nonthermal technologies have been used as an alternative to traditional thermal processing technology and have the ability to provide safe and healthy dairy products without affecting their nutritional composition and organoleptic properties. Other than nonthermal technologies, infrared spectroscopy is a nondestructive technique and may also be used for predicting the shelf life and microbial loads in milk. This review explains the role of pascalization or nonthermal techniques such as high-pressure processing (HPP), pulsed electric field (PEF), ultrasound (US), ultraviolet (UV), cold plasma treatment, membrane filtration, micro fluidization, and infrared spectroscopy in milk processing and preservation.

**Keywords:** nonthermal processing; high-pressure processing (HPP); pulsed electric field (PEF); ultrasound (US); ultraviolet (UV); cold plasma treatment; infrared spectroscopy

## 1. Introduction

Milk is a nutrient-rich liquid food which comprises carbohydrates, fatty acids, and high-quality proteins with vitamins, minerals, and trace elements [1]. Milk is considered a perishable food due to its high-water contents which help microorganisms to proliferate. Milk and milk products help to prevent arthritis, loss of muscle mass, diabetes mellitus, cardiac disease, cognitive decline, and digestive problems due to their high nutrient content [2]. It is important to thermally process the raw milk before commercialization because it prevents the dairy products from being contaminated by pathogenic bacteria and toxic substances [3]. Thermal processing keeps the aroma, flavor, and texture of food intact by restricting the pathogenic bacteria [4]. Consumers have demanded dairy products that are minimally processed and natural in taste but also have an extended shelf life [5–9].

Thermal processing eliminates the microorganisms, but majorly destroys some nutritional components, physical, and chemical properties [4,10], resulting in some undesirable flavor changes, loss of vitamins, and volatile flavor compounds [11]. Nonthermal processing technologies can be used as a substitute for thermal processing due to their ability to provide fresh, nutritious foods that are safe and have a longer shelf life [12,13]. Thermal and non-thermal technologies such as high-pressure processing (HPP), nonthermal plasma (cold plasma), ultrasonic, pulsed electric field (PEF), ultraviolet irradiation, and membrane microfiltration techniques were employed to inhibit the growth of pathogenic microbes [14–22]. The purpose of this review is to provide a brief overview of the use of nonthermal techniques in milk and milk products, including pulsed electric field, ultrasound, ultraviolet, cold plasma treatment, membrane filtration, micro fluidization, and infrared spectroscopy, as well as their effects on the compositional and dietary value of the product.

## 2. Review of Literature

### 2.1. High-Pressure Processing (HPP)

High-pressure processing is a pragmatic substitute for heat handling. It is commercially feasible and allows food processors to pasteurize foods at or near room temperature [23]. The rise in customers' interest in better quality foods is a reason for the development of high-pressure processing as an addition to standard heat treatments [24]. It was examined that high-pressure processing inactivates bacterial spores at high temperatures. HPP can be improved using pressure applications (cyclic, pulsed, or oscillatory), antibacterial agents, and by combining with other treatments. This improvement results in the increased shelf-stability of foods while maintaining the quality and nutritional properties [25]. High-pressure processing has gained huge commercial interest, and a variety of foods treated by HPP that are nutritious and safe have become integral to the international food market [26].

High-pressure processing has other names, such as high hydrostatic pressure (HHP) and pascalization. The research on milk preservation through the application of HPP began a few decades ago [27]. High-pressure treatment of milk produces numerous changes. The most prominent changes are the inactivation of enzymes and microbes, decomposition of whey proteins, and disorganization of casein micelles. Being comparatively expensive does not hinder its commercial importance as it helps achieve certain requirements that were not possible to attain through present thermal processing technologies. Examples include the preservation of dairy products (cheese, yogurt, kefir, and ice cream) without damaging bioactive proteins or important microbes [28].

A table presenting HPP effects on milk enzymes can be found in Table 1. High-pressure processing can inactivate microorganisms; thus, it could be utilized for nonthermal pasteurization of food-producing harmless foodstuffs with sufficient nutritional value and organoleptic attributes. Water is treated as a fluid to transfer pressures to the product between 100 and 1000 megapascals. Usually, commercial systems work between 400 and 700 MPa. Pressure does not influence small molecules like pigments, vitamins, amino acids, and volatile compounds because of their simple structures. However, big molecules such as proteins, nucleic acids, enzymes, and polysaccharides may be affected.

**Table 1.** Inactivation of microorganisms by high-pressure treatment in milk and milk products.

Products	Conditions	Target Microorganism	Inactivation Effect	Ref.
Skim milk	Temperature: 84 °C; pressure: 300 MPa	<i>Bacillus stearothermophilus</i> ATCC 7953	0.67-log reduction	[29]
Commercial sterile milk	Temperature: 75–85 °C; Pressure: 300 MPa	<i>Bacillus</i> spores	5-log CFU/mL reduction	[30]
Commercial sterile milk	Temperature: 50 °C; Pressure: 400 MPa	<i>Escherichia coli</i>	1 min D value reduction	[31]
Raw milk	Temperature: 25 °C; pressure: 300 MPa	<i>Salmonella typhimurium</i>	9.21 min D value reduction	[32]
Milk	Temperature: 90 °C; pressure: 700 MPa	<i>Clostridium sporogenes</i>	13.6 min D value reduction	[33]
Raw milk (15% milk)	Temperature: 24 °C; pressure: 300 MPa	<i>Listeria innocua</i>	1.80-log reduction	[34]
Raw milk (15% milk)	Temperature: 2–4 °C; pressure: 100 MPa	<i>Listeria monocytogenes</i>	1.20-log reduction	[35]
Raw milk (15% milk)	Temperature: 20 °C; pressure: 300 MPa	<i>Staphylococcus aureus</i>	4.00-log reduction	[36]

Much research have been conducted on the implementation of appropriate pressure processing in the processing of milk and dairy products since it is regarded as an appropriate food production technology. In research to see how HPP affected Mató (fresh goat's milk cheese), 500 mega Pascal was applied at 10 °C or 25 °C for 5, 15, and 30 min. Physico-chemical characteristics such as color, texture, microstructure, whey loss, and changes in composition were assessed. The results showed that the composition remained unchanged, the color and texture of the cheese showed slight changes, and whey from pressure-treated cheese had a higher total nitrogen content. However, no noticeable changes were observed in the microscopic analysis of structure except on the surface of the fresh goat's milk cheese [37].

A study analyzed the impact of HPP on the quality of raw milk, storage, and safety. Different exposure times and pressure levels were assessed alongside artificially inoculated pathogenic *L. monocytogenes*, *Salmonella* spp., and *E. coli*. The study highlighted 5-log reductions for pathogens by HPP. Moreover, HPP increased the raw milk's storage period, preserving its quality attributes and cutting total viable counts, lactic acid bacteria, and Enterobacteriaceae. Furthermore, HPP extended the storage time of raw milk while maintaining its quality characteristics and reducing overall yield, *Lactobacilli*, and Enterobacteriaceae. As a result, HPP appears to be a viable alternative to traditional raw milk processing [38].

## 2.2. Pulsed Electric Field (PEF) Processing

The food-processing industry is developing new processing methods (such as pulsed electric field, ultrasound, ultraviolet, cold plasma treatment, membrane filtration, and micro fluidization) and products regularly. While food scientists appreciate the advancement of science, consumers are often reluctant to adopt the changes [39]. Pulsed electric field treatment is a new preservation technology with the ability to provide foods with exceptional nutrition, increased shelf life, and better sensory attributes. This includes applying huge electrical currents of 20 to 80 kV/cm for less than one second at room temperature [40]. The origin of this technique leads back to Germany [41].

Dairy products are mostly applied to examine the impact of pulsed electric field processing. This technique can be a substitute for thermal processing as it can destroy microbes and enzymes and still maintain the originality of food commodities. A few factors affect the efficiency of PEF treatment, which include the treatment time, electric-field intensity, type of enzyme or microorganism, and temperature of food [42].

PEF is a nonthermal process, and it is very likely to be successful in the future. There is research aimed at studying the application of PEF to prevent decomposition and inhibit pathogens and enzymes in dairy products. PEF has been acknowledged to successfully decrease the number of pathogens and decomposers in milk. Applying PEF at relatively low temperatures will help achieve desired results, including a decrease in microbial load without damaging the sensory and physico-chemical properties of products [43].

A treatment chamber component, a large electrical pulse generator system, and a pump for immersing liquid food to enable persistent PEF treatment are typical PEF equipment components [44]. High-intensity PEF has gained attention for its capacity to handle fluid commodities and practical usage in uninterrupted movement treating [45]. The use of this technology in industries is still limited due to the lack of reliability of electrical systems. It applies to the fact that more targeted and inexpensive electrical systems are needed to get the maximum benefits from this novel technology [46]. PEF is very likely to help obtain good quality, long-lasting milk, and dairy products with characteristics such as fresh products. It not only limits the activity of most microorganisms but also several enzymes that can be problematic for the safety of the product. There are no findings on the different safety and reliability characteristics of PEF-treated milk products; thus, more research into the safety aspects of PEF is required, which may potentially expose the technology's flaws [47].

Table 2 shows the inactivation of microorganisms by PEFs with moderate heat, as well as key considerations for their application in the milk business. Another study looked at using PEF in combination with light heat processing to extend the shelf life of the whole dairy. Five pulses (2.3 s pulse width and 35 kV/cm peak electric field intensity) were delivered to whole milk for less than ten seconds at 65 °C. The results showed that milk's shelf life was extended by at least 24 days. PEF and mild heat treatment had a synergistic effect. The inclusion of a thermal regeneration system increased the energy efficiency of the investigated preservation technique [48].

**Table 2.** Inactivation of microorganisms by pulsed electric field (PEF) processing in milk and milk products.

Products	Conditions	Target Microorganism	Inactivation Effect	Ref.
Milk undergone ultrafiltration	Pulses: 50 and 80; kV/cm: 60 and 70	<i>Escherichia coli</i>	6 and 9-log	[49]
Skim milk (pasteurized)	200 µs; kV/cm: 50	<i>Listeria innocua</i>	2.6–2.7-log	[50]
Ultra-High Temperature milk	8 µs; kV/cm: 35	<i>Staphylococcus aureus</i>	4.5-log	[51]
Skim milk (raw)	2 µs; kV/cm: 50	<i>Listeria innocua</i>	2.4-log	[52]
Whole milk	43.75 µs; kV/cm: 40	<i>Listeria innocua</i>	5.5-log	[53]
Skim milk	100 µs; kV/cm: 25	<i>Staphylococcus aureus</i>	3-log	[54]

### 2.3. Ultrasound (US)

Table 3 summarizes the consequences of ultrasound (US) technology on milk and milk products. Ultrasound is a technology that has been effectively proven for a number of food handling and protection applications. Most of the food processing applications usually suggest fluids. Like any other ultrasound, it is an innovative and useful technology because of its wide array of applications and increasing scientific research. It is being utilized in the food industry for several processes such as drying, homogenization, cutting, tempering, freezing, filtration, degassing, and extraction. It can work as an alternative or promoter to food processing. The usage of ultrasound for food processing has a number of advantages such as increased production rate, reduced energy and temperature, effective mixing, and increased mass transfer. This technology destroys microorganisms and enzymes without effecting nutritional quality or changing organoleptic properties [55]. Ertugay et al. in their study analyzed the effect of ultrasound processing on the homogenization of milk [56]. Milk samples were homogenized with the help of conventional and ultrasonic homogenizers. To evaluate the diameter of fat globules and to determine the homogenization efficiency of milk

samples a microscope set with a camera and ocular micrometer was used. Results exhibited that ultrasound processing with high power had a vital effect on milk homogenization.

**Table 3.** Inactivation of microorganisms by ultrasound treatment in milk and milk products.

Products	Conditions	Target Microorganisms	Inactivation Effect	Ref.
Raw milk cream	500 W, frequency of 37 kHz; Time: 2-, 5- and 10-min temperature of 30 and 40 ± 2 °C stored in the refrigerator for 10 days	Mesophyll's aerobes, total coliforms, molds, and yeasts	The treatment at 37 kHz for 10 min at a temperature of 40 °C decreased the initial microbial load by 79% For specific ultrasound parameters, the lowest Enterobacteriae count (1.06151 log CFU mL <sup>-1</sup> ) was as follows: amplitude of 120 m, treatment time of 12 min, and temperature of 60 °C	[57]
Raw milk	Temperature: 20, 40 and 60 °C Amplitude 120, 90 and 60 µm Time: 6, 9 and 12 min	Enterobacteriae	For <i>Escherichia coli</i> and <i>Pseudomonas fluorescens</i> , a maximum decrease of 1.6 log CFU/mL was obtained Following US treatment, <i>Staphylococcus aureus</i> inactivation was lower (1.05 log CFU/mL)	[58]
Homogenized milk	Temperature: 20–52 °C Intensity: 0–120 W/cm <sup>2</sup> Time; 40–240 s Constant pressure: 225 kPa	<i>Escherichia coli</i> , <i>Pseudomonas fluorescens</i> and <i>Staphylococcus aureus</i>		[59]
Cow's milk (raw, whole, 4% fat)	Temperature: 60 °C; frequency: 20 kHz; Time: 12 min Pressure: 20 kHz;	<i>Escherichia coli</i>	3.1-log reduction	[60]
Pasteurized raw milk	Time: 6 min; Intensity: 750 W	<i>Pseudomonas flurescens</i>	5.64-log CFU/g reduction	[61]
Ultra-High Temperature milk	Temperature: 60 °C; Pressure: 20 kHz	<i>Listeria monocytogenes</i>	D60&#s = 0.3 min	[62]
Skim milk	Temperature: 50 °C; Time: 30 min	<i>Salmonella typhimurium</i>	3-log reduction	[63]
Ultra-High Temperature milk (PH 6.7)	Temperature: 60 °C	<i>Escherichia coli</i> K12DH5 A	D60&#s = 23 s	[64]

It was seen that exposure times and power levels were directly proportional to homogenization efficiency [56]. The focus of a study on the ultrasonic effect was on simulating milk production characteristics. Based on vitamin C, antioxidant capacity, and the buildup of exopolysaccharides in the final milk product, the effectiveness of ultrasonic processing was assessed. It was derived from the results that showed the reconstitution of dry milk by using ultrasounds increases the nutritional value of the fermented food product and promotes further accumulation of biologically active compounds [65]. Another study examined the influence of ultrasounds on the functional and physical properties of skim milk. Sterilized homogenized skim milk was treated under controlled temperature conditions with a 20 kHz ultrasound at 20 to 41 W. The study highlighted the following effects due to sonication on whey proteins and their aggregates, preservation of the integrity of the casein micelles, disruption of fat globules, and unchanged viscosity [65]. The applications of the ultrasound in the field of food processing involve enzyme and bacterial inactivation as well as the modification and analysis of foods. Currently, the usage of the ultrasound alone for bacterial damage is impractical, whereas its combination with pressure or heat is promising. The future of ultrasound lies in manosonication, thermosonication, and manothermosonication for bactericidal functions. In comparison with traditional heat treatments, sonication is more energy-efficient and reduces enzyme and microbial activity [66].

#### 2.4. Ultraviolet (UV)

Ultraviolet radiation is a non-ionizing invisible light that lies in the range of the electromagnetic spectrum with a wavelength of 100 to 400 nm, between visible light and X-rays [21]. This technique is preferred for the disinfection and preservation of food, such as liquid food and milk. Its effect is mainly dependent on microbial load, the nature of lenses used, and the infrastructure of the machines [67]. There are three regions of UV light in the electromagnetic spectrum range: ultraviolet light-A that has a wavelength range from 15 to 400 nm; ultraviolet light-B with a wavelength of 280 to 315 nm; and ultraviolet light-C having a wavelength range of 20 to 28 nm. Later UV rays are primarily germicidal against pathogenic and other microorganisms that cause food spoilage, such as viruses and protozoa, which act by disrupting DNA transcription and replication, eventually damaging the DNA and causing cell death [68,69].

A wide variety of food processing and preservation technologies exist. These include crystallization, drying, homogenization, emulsification, dispersion, changing the texture of foods and their solubility, ultrasonication for extraction, and ultraviolet radiation in milk processing [70]. The US Food and Drug Administration (US-FDA) endorsed generating innovation in processing techniques which can accomplish and satisfy the needs for microbial wellbeing in liquid food, most importantly in dairy food products [71]. Table 4 shows the outputs of using UV-light for milk preservation by irradiating the microbial load of milk. Microorganisms were found inactivated in a study in which raw milk was treated with *Serratia marcescens*, 3.2 J/cm<sup>2</sup> UV-dose at 4 °C in a collimated system [72]. A UV dose of 1.07 W/m<sup>2</sup> at 5.6 °C for 60 s was enough to disable the 7-log of *Staphylococcus aureus* [73]. Matak et al. [74] stated that UV radiations were used for lowering the *L. monocytogenes* count that showed positive outputs with the reduction rate by 5-log units in whole goat's milk through the implication of UV (UV dose 158 ± 16 J/m<sup>2</sup>). Simmons et al. [75] found a reduction in the full microbial load of 3.5-log in whey with UV (450 W/m<sup>2</sup>) implication. In another study, 1.5 J m/L ultraviolet radiations were bombarded on cow milk (whole) for the sake of reduction of the microbial load that resulted in a reduction of natural microflora by 3-log [76]. Another study with milk sources of whole cow milk, industrially processed skim and soy milk resulted in the completed deactivation of *B. cereus* and *Escherichia coli* studied under the implication of UV reactors (dean flow) [77].

**Table 4.** Inactivation of microorganisms by ultraviolet (UV) treatment in milk and milk products.

Products	Conditions	Target Microorganism	Inactivation Effect	Ref.
Raw bulk tank milk	Temperature: 4 °C Time: 120 s	<i>S. marcescens</i>	drop of 1 log cycle was achieved	[72]
Raw milk	Temperature: 5.6 °C Time: 60 s	<i>S. aureus</i>	decline of 7 log cycles was achieved	[73]
Goat skim milk	Temperature: 4 °C Time: 18 s	<i>L. monocytogenes</i>	drop of 5 log cycles was achieved	[74]
Whey	Temperature: 28 °C Time: 100 s Intensity: 450 W/m <sup>2</sup> 4 °C	Overall bacterial load	drop of 3.5 log cycles was achieved	[75]
Raw cow milk	Time: 1.5 s 0.05 J/mL	<i>E. coli</i>	drop of 4 log cycles was achieved	[77]
Skim milk	Intensity: 100 W, 20 kHz Timings: 7, 15, and 30 ice bath Voltage: 420, 900, and 1800 J mL <sup>-1</sup>	<i>B. breve</i> <i>B. infantis</i> , <i>B. longum</i> (BB-46) and <i>B. animalis</i> ssp. <i>lactis</i> (BB-12)	Reduced fermentation time for <i>B. breve</i> , <i>B. infantis</i> and BB-12	[78]
Skimmed cows' milk	Intensity: 35 kHz, 300 W Timing: 5 min 2.5 mm probe	<i>Lb. delbrueckii</i>	drop of 4 log cycles was achieved	[79]
Cow's milk	Timings 1–3 min Intensity: 30 kHz and 2–8 W; energy density 4.8–57.6 J mL <sup>-1</sup>	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>Cremoris</i>	drop of 3.5 log cycles was achieved	[80]

Other significant considerations for milk value, for example, the pH, soluble solids, color, and viscosity, have been assessed after being on the UV treatment of milk, with no statistically significant difference in all of these qualities. However, some variations may happen based on the treatment factors, like alterations in the fatty acid report, rises in the thiobarbituric acid-sensitive ingredients, as well as protein sleet [73,81].

### 2.5. Cold Plasma Technique

The electrically energized fourth state of matter in a vaporous form containing ions, free radicals, and some radiation, is called plasma that is produced through electron discharge [82,83]. The plasma state contains almost the same number of chargers, so it is globally assumed to be electrically neutral [84]. When kinetic energy possessed by electrons is increased through the use of high-frequency radio waves, microwaves, thermal or magnetic power, the matter can reach this plasma state, while the kind of plasma depends upon the procedure characteristics and gas secondhand for electron discharge purposes [85]. Plasma was classified into denominated thermal plasma and nonthermal plasma or cold plasma, which can be characterized by the thermodynamics between charged species. Cold plasma requires less power and is produced in vacuum conditions at 30–60 °C. Cold plasma expresses much higher electron temperatures in contrast to the equivalent gas and is unable to show a specific thermal equilibrium [86].

In this treatment, only small energy is gained by charged and uncharged particles while temperature rise is also low, and that is why heat sensitive liquid food products are treated preferably with the cold plasma technique [84,85]. Despite this, very high pressure and high power are required for the working of the thermal plasma technique; however, a local thermodynamic equilibrium is present between electrons and high-mass species [87]. The literature described that for all plasma components, the gas temperature is almost the same, which is graded as extremely high [88].

This technique is innovative for food processing specifically to get rid of the microbial load of food that efficiently decreases the shelf life of food, including dairy and non-dairy products [89]. Many agents, such as free radicals and other chemically active species, likely reactive oxygen species, unstable nitrogenous species, and ultraviolet rays with high potential can act during plasma application. These products are the main reasons that cold plasma is preferred for the inactivation of a microbial load because, mostly, they are unable to resist these unstable and reactive species that attack and damage their DNA structures [90].

Table 5 shows the studies in the literature that were conducted to examine the potential of using cold plasma for the purpose of bacterial microfilm inactivation in different states of milk, including skim, semi-skimmed, and whole milk [11,91]. All studies significantly approved the substantial ability of cold plasma to avoid or reduce the number of damaging microorganisms in milk.

Ruan et al. [92] conducted a study to examine the potential of UV for the reduction as well as inactivation of microbes in milk and skim milk that are harmful. Skim milk containing three different microbes, including *Salmonella* spp., *L. monocytogenes*, and *B. cereus* containing a mixture of five, and three strains, respectively, was subjected to cold plasma at 35–40 kV at more than sixty-celsius temperatures. Outcomes revealed a 2.95, 2.74, and 0.18-log decline, respectively. However, in another study, the same strains showed a 5.55, 4.36, and 4.73-log reduction, under the same plasma condition [93]. Further, skim milk, semi-skim milk, and whole milk approved the potential of the cold plasma technique when it showed a reduction of 3.34-log, 3.40-log, and 3.63-log, respectively [94]. Furthermore, another study that used whole, semi-skim, and skim milk as a source containing *E. coli*, *S. aureus*, and *Salmonella typhimurium* subjected to characteristic parameters of plasma techniques (AC power supply 20 kV) after being stored for 40 days at 4 °C expressed reduction to 3.63, 2.00, and 2.62 colony-forming units per milliliter, respectively.

**Table 5.** Inactivation of microorganisms by cold plasma treatment in milk and milk products.

Products	Conditions	Target Microorganism	Inactivation Effect	Ref.
Raw milk	Temperature: 35 °C Time: 20 min Intensity: 9 kV	<i>Escherichia coli</i> (ATCC 25922)	drop of 3.63 log cycles was achieved	[11]
Milk	Intensity: 250 W Time 10 min Temperature: 25 °C	<i>E. coli</i> (KCTC 1682) <i>L. monocytogenes</i> (KCTC 3569) <i>S. typhimurium</i> (KCTC 1925)	drop of 2.40 log cycles was achieved	[91]
Milk	Intensity: 35–40 kV Temperature <60 °C, Single pass CHIEF	<i>E. coli</i> O157:H7 ATCC43895 <i>Salmonella</i> (5 strain mixture) <i>L. monocytogenes</i> (5 strain mixture)	drop of 2.95 log cycles was achieved for <i>E. coli</i> O157:H7 ATCC43895 reduction of 2.74 log cycles was achieved for <i>Salmonella</i> (5 strain mixture) reduction of 0.18 log cycles was achieved for <i>L. monocytogenes</i> drop of 4.73 log cycles was achieved for <i>E. coli</i> O157:H7	[92]
Skim milk	Intensity 35–40 kV, exit temperature <60 °C, double pass CHIEF	<i>E. coli</i> O157:H7 (5 strain mixture) <i>Salmonella</i> spp. (5 strain mixture) <i>L. monocytogenes</i> (5 strain mixture)	drop of 4.36 log cycles was achieved for <i>Salmonella</i> drop of 5.55 log cycles was achieved for <i>monocytogenes</i>	[93,94]
Raw skim milk	Intensity: 30–40 kV One to thirty pulses Temperature: 20–72 °C	<i>Listeria innocua</i>	drop of 4.3 log	[53]
Skim milk	Intensity: 40 kV cm 1, 4937 microsecond PEF plus UV	<i>Listeria innocua</i> , <i>Zygosaccharomyces bailii</i>	(3.0–5.0 log reductions) (7.9–8.8 log reductions)	[95]

Various factors are involved in the working potential of cold plasma for the removal of microbes from food, especially milk, including strains of bacteria that are required to be inactivated, voltage, duration, the composition of the gas used, and food chemistry. Cold plasma technology's antimicrobial efficacy in dairy products is influenced by a number of parameters, including the target microorganism's species, input power, treatment time, gas composition, and food composition. The literature results showed cold plasma as a forthcoming innovative technique in contrast to the previously used techniques for milk processing because there are very few changes in color and flavor that may cause a reduction in commercial value [11,96]. However, the various results give directions to the researchers for further innovations in technique parameters for commercial productions [91].

## 2.6. Membrane Filtration

Membrane filtration is essentially an extraction method that significantly deals with membranes that are specialized for filtration of selective components like dust particles as well as certain sized microbes, to focus on fractionating fluids into various compositions. The retentate is referred to as a retained fluid and permeate is passed out of the liquid. The efficacy of membranes is particularly assessed by the pressure and amount of liquid across the membrane. Since 1960, the dairy sector has used membrane filtration technology [97]. In the foodstuff trade, mainly in dairy commercialization, membrane filtration processes, either nanofiltration or ultrafiltration, are preferred for efficient protein separation or



purification [98]. It acts on the side of their fertility to lower the complete viable count of microorganisms, thereby extending the shelf life without compromising the nutritional and sensory characteristics of dairy products [97].

Table 6 summarizes the inactivation of microorganisms and bacteria in milk in order to preserve it for a long period and make it easier to handle or process for different dairy products. It is formed by the membrane microfiltration of milk. Pafylas et al. [99] observed the potential to reduce the microbial count from treated skim milk via membrane filtration, particularly on an ionic membrane with a hole size of 1.4 microns. It was also summarized that lessening of the microbe number, particularly in skim milk, is possible to obtain the exclusive use of any discriminative alterations in milk biochemistry.

It was investigated that the membrane filtration method has the potency to get rid of microbiota, vegetative spores, and other actively fertile cells from skim milk at decreased temperatures. They detected no bacteria in skim milk permeate that was initially having a count of 5.25 and 2.15-log colony-forming units per milliliter, of vegetative microbes and spores, respectively, under membrane filtration treatment conditions such as 1.4  $\mu\text{m}$  pore size at 6 °C, while the somatic cell's viable count was also observed to be reduced by up to 3.0-log. The researcher observed a 2.1-log decrease in mesophile microbes in low-fat milk when treated through the crossflow membrane filtration [100]. Another author reported a significant reduction in vegetative cells (>3.5-log), spore formation (>4.5-log), and free somatic cells of microbes under specific treatment conditions [101].

Gosch et al. [102] examined more efficient microbial removal and reduction up to >2.5 by using a membrane having a pore size of 0.8  $\mu\text{m}$  than the MF with a membrane pore size of 1.4  $\mu\text{m}$  that showed more than a 3.5-log lessened viable number. In combination, both types of the membrane caused a reduction in the complete sustainable number up to 2.3-log CFU/mL in milk. Results also revealed that both membranes caused the complete removal of microbes from food sources either with an aperture size of 0.14 or 0.2. The research study conducted by Daufin et al. [103] proved that reducing bacterial numbers to 4.13-log cycles in the skim milk processed it under the following MF conditions: pore size of 1.4  $\mu\text{m}$ , temp. 51 °C. Another study applied a 1.4  $\mu\text{m}$  porcelain membrane with a hole size of 1.4 micrometers. This was an MF treatment for the sake of checking the potency of membrane filtration in the removal of the microbes and spores from milk [104]. A microbial viable count reduction of 2.1 to 3.1-log CFU/mL has also been reported by passing the milk through 1.4  $\mu\text{m}$  of the microbial filter as compared to the initial value of the microbiome. On the other side, the ceramic membrane of the same pore size reduced the value up to 2–3-log [105]. A microbial reduction of 5 to 6-log and 3 to 4-log CFU/mL occurred through Sterilox<sup>®</sup> membranes using 0.8 and 1.4  $\mu\text{m}$  MF, which is much more efficient than the former. Elwell and Barbano [106] investigated the potency of a method by using membranes made of ceramic with pore sizes of 1.4 micrometers for preservation purposes and found that bacterial counts were 3.79-log. A study investigated the shelf life of skim milk preserved through the membrane filtration method and found more than 4.5-log reductions. In addition, as compared to the number of microbes in the milk before treatment, the microbial value was reduced to a negligible level [107]. Another study investigated skim milk preservation by using membrane filtration and observed a lessened bacterial load up to >3.5-log, in contrast to the outcomes when the 0.5-micrometer membrane was used, and the bacterial number of the viable count was increased to 2–3-log [108]. In another scientific study by Brans et al. [109], they observed that *B. subtilis* was minimized in number up to 6.6-log by using a membrane that has a narrow pore size such as 0.5  $\mu\text{m}$  micro-sieve and could act at a low pressure of the transmembrane.

**Table 6.** Inactivation of microorganisms by membrane filtration treatment in milk and milk products.

Product	Conditions	Target Microorganism	Inactivation Effect	Ref.
Skim milk	MF-1.4 $\mu\text{m}$ , ceramic membrane, $T_p = 50\text{ }^\circ\text{C}$	Full microbial load	drop of 4.5 log cycles was achieved	[99]
Simulated milk	1.4 $\mu\text{m}$ at $6\text{ }^\circ\text{C}$	Full microbiome	drop of 3 log cycles was achieved	[100]
Skim milk	$55\text{ }^\circ\text{C}$ , 1.4 $\mu\text{m}$ pore size	Bacterial vegetative, spore-forming, and somatic cells	drop of >3.5, >4.5, and no log cycles were achieved	[101]
Skim milk	0.8 $\mu\text{m}$ , tubular ceramic ISOFLUX membrane	Total bacterial load	drop of >2.3 log cycles was achieved	[102]
Skim milk	1.14 $\mu\text{m}$ ceramic membrane	Total bacteria	drop of 3.1 log cycles was achieved	[104]

### 2.7. Micro Fluidization

Micro fluidization is a revolutionary technique of the nonthermal procedure. Proficiently in one pass, the milk homogenizes into an emulsion inhabited through the components with changed protein–protein as well as protein–fat interactions and submicron-sized fat droplets. Micro fluidization is a procedure that is proposed for milk with two streams smashing together at an angle of  $180^\circ$  [110]. To the milk, the main alterations are persuaded when milk’s two streams are enforced (up to 200 MPa) under elevated pressure within a reaction chamber to smash together in opposing directions, as well as the resultant hurly-burly, cavitation, and shear disrupt of the droplet of lipid and its adjacent membrane. When compared to the homogenization of lower pressure, the micro fluidized milk has minor-sized lipid droplets at the lipid interface through a smaller amount of intact or semi-intact casein micelles. A few of the smaller lipid droplets in fact are entrenched in a micelle portion [111]. Micro fluidization has been utilized to improve the yogurt texture and manufacturing [112]; however, micro fluidized milk forms denser, fewer supple cheese matrices, which are harmful to the mozzarella and cheddar cheeses’ textures [113,114]. However, immediately after the micro fluidization on the milk explosive profiles, no information is obtainable. In milk, the fat droplets’ severe disruption is of concern as to the flavor of milk. The components of fatty acids are major contributors as well as extremely vulnerable to rancidity issues, oxidative (aldehydes) and (free fatty acids) hydrolytic. The ultrahigh-pressure homogenization (UHPH), which utilizes a homogenization nozzle at >200 MPa pressure, was reported to alter the milk aroma [115] as well as yogurts [116,117], and in aging cheeses to change the lipolysis and proteolysis [118]. Hardly any studies have assessed the volatile profile in UHPH milk [119,120]. Prepared within micro fluidized milk in cheese it was noticed that there is no alter in milk composition except for a minor lessening in protein. The fat droplet size was abridged, a good emulsion was noticed by confocal microscopy by scattered agglomerations. Milk coagulation possessions treated at  $54\text{ }^\circ\text{C}$  as well as 125 or 170 MPa have exposed extended times of coagulation as well as weaker gels. As for micro fluidized milk in the gels form, an opaque matrix with well-dispersed droplets of fat–protein was observed [121]. The micro fluidization decreased globule sized milk fat as well as a number of globules’ multiplication, and the cheeses prepared as of micro fluidized cream were superior in moisture and softer in texture. The enlarged cheese yield had been allocated additional fat and moisture retention [113]. The cheese milk micro fluidization demonstrated a reduction in the mozzarella cheese’s aptitude to melt and flow. The cheese milk micro fluidization reduced the droplet size of lipid, enhanced the small lipid droplet allocation, as well as entrenched the smaller-sized drops into the matrix of protein, and transformed the interactions of fat with protein and the mozzarella cheese’s rheological possessions [114]. In additional work, it has been established that there

was no major difference in opus or microstructure among the mozzarella prepared as no homogenized milk as well as milk was homogenized at 10–30 °C and 34 megapascal as this temperature does not adequately turn to liquid the fat on behalf of whole micro fluidization, as well as this pressure does not decrease the size of the fat globules. In the cheese, the fat globules turned out to be smaller at elevated temperatures and pressures [122]. When micro fluidization was applied to the milk to make frozen products, diverse pressures had an effect on a few frozen dairy desserts' possessions. Nonfat as well as low-fat ice creams prepared with micro fluidized milk showed slower rates of meltdown [123]. It has been established that the thermally denatured whey proteins can be incompletely or completely solubilized by micro fluidization as well as being able to reduce the heat-treated whey protein sedimentation [124]. It has been exposed that dynamic high-pressure micro fluidization is able to persuade the  $\beta$ -Lacto globulin antigenicity in bovine milk that was carried about through changes in  $\beta$  strand content, particle size, and SH groups [125]. For micro fluidized milk's changed functionalities as innovative dairy food applications are explored, the micro fluidization's instant effect on the explosive compound profile of the milk, a crucial factor in user receipt of the product, requires additional clarification.

### 2.8. Infrared Spectroscopy

Infrared spectroscopy is widely being used for verifying adulteration and authenticity in foods [126]. It is one of the most pertinent technologies in process control, analysis of raw materials, and characteristics of final products in the dairy industry [127]. Techniques such as NIR (near-infrared) and MIR (mid-infrared) have been productively applied in the evaluation of milk and dairy products' quality, including whey, cheese, WPCs, and milk powder. During routine liquid milk testing, FT-MIR is the global method of choice for composition and quality control. It helps with speedy, non-destructive quantification of milk chemical properties to elude reference methods, which are usually costly, tiresome, and time-consuming [128]. Mid-infrared displays distinct bands for organic functional groups, protein, fat, and lactose which are constituents of milk and are good for quantitative and qualitative identification [129]. As compared to the NIR apparatus, medium infrared equipment usually uses the smallest possible sample volume for the milk analysis. However, its major drawback is the occurrence of an enormous band of absorption of water, since milk has about 87% water [130]. Another disadvantage of FT-MIR is the experimental complexity for the milk analysis and the requirement for sample preparation (in the absence of ATR) [129]. Mid-infrared devices are commonly much more expensive. The NIR has several advantages over the MIR such as a cheaper light source and simple and economical transmission instruments with glass optics. Botelhoet et al. [131] were able to perform the simultaneous detection of five adulterants in raw cow milk by using a multivariate classification and mid-infrared spectroscopy. The adulterants were water, sucrose, starch, formaldehyde, and sodium citrate. For every adulterant, a particular area of the spectrum differentiates from the original milk spectrum according to the observations made. A distinct peak of about  $1000\text{ cm}^{-1}$  could be noted for formaldehyde adulteration. Moreover, in sucrose adulteration, several peaks appeared near  $1200\text{--}1000\text{ cm}^{-1}$  in the fingerprint region. Gondim et al. [132] investigated a sequential strategy in the same line of research for the detection of usual adulterants in milk. These included thickeners, neutralizing agents, preservatives, and water. The classification technique SIMCA (Soft Independent Modelling of Class Analogy) was applied to mid-infrared (MIR) data and the cross-validation method was used for building models.

Residues of some veterinary drugs such as enrofloxacin, ceftiofur hydrochloride, tetracycline, penicillin, and diclofenac sodium were analyzed in milk samples by using PCA-associated FT-NIR for fast and accurate detection [130]. The results helped to distinguish different types of antimicrobics dissolved in milk. These were compared with the maximum residue limits approved by the European Medicines Agency and the Ministry of Agriculture, Livestock and Supply of Brazil. Spectroscopy procedures have been used in research with whey and its products [133–139]. O'Loughlin et al. [136] character-

ized the alterations going on in whey protein dispersions due to heat treatment. They examined the thermal denaturation of whey protein solutions and performed inter- and intramolecular level analyses using infrared spectroscopy. According to the researchers, infrared (IR) spectroscopy for distinguishing between 2° and 3° protein structures is fast and reasonable when compared to other methods. The results revealed that MIR is a useful technique in differentiating the structural changes in homogeneous protein systems relating to physical properties.

### 3. Conclusions

Pascalization or nonthermal techniques including high-pressure processing, ultrasound, micro fluidization, ultraviolet radiation, cold plasma technique, microfiltration, and infrared spectroscopy have the potential to be used in milk and milk products. These techniques not only inactivate the vegetative microorganisms in foods but also have a slight effect on the sensory and nutrient value of foodstuffs. These techniques are being used as a substitute for thermal techniques in the milk industry because of their promising effects on the processing of dairy food sources. Despite the various beneficial effects of these technologies on dairy food items, including milk and products made from milk, dairy industries still feel reluctant to adopt nonthermal technologies due to their high cost and large production value.

**Author Contributions:** Conceptualization, M.F.J.C. and A.K.; methodology, S.K., M.A.F. and S.A.A.; collection of literature, reviewing, and editing, S.A., M.R., M.K., A.V., E.N., M.T. and M.A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research work was supported by a grant from the Ministry of Science and Higher Education of the Russian Federation (under Agreement No 075-11-2019-044 of 27 November 2019).

**Acknowledgments:** The authors are highly grateful to the Department of Food Science and Technology for their contribution.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

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