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Effect of non-thermal processing techniques on pathogenic and spoilage microorganisms of milk and milk products

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Abstract

Milk is a nutritious perishable product having a short shelf-life owing to the occurrence of spoilage bacteria in it. This has led to an increasing demand for ensuring safety through milk processing. Conventional techniques (such as heat treatment) increase shelf-life but damage the nutritional and sensory qualities of milk. Hence, there is a need to develop innovative, nonthermal processing techniques that increase the shelf-life while preserving its nutritional quality. This review focuses on the recent advances in assuring microbial safety of milk by using nonthermal techniques such as high-pressure processing, pulsed electric fields, ultrasound, ultraviolet irradiation and membrane microfiltration.

Keywords: microbial inactivation; emerging technologies; ultrasound; pulsed electric field; milk processing.

Practical Application: In milk industry, different types of thermal treatments are already applicable to reduce the microbial activity, but it has some nutritional losses due to heat treatment. But the same microbial reduction can be achieved (less nutritional losses) after mild heat treatments such as high-pressure processing, pulsed electric fields, ultrasound, ultraviolet irradiation and membrane microfiltration, which are the best substitute of thermal treatments. This article will be helpful for the milk processing industries to apply these nonthermal treatments for the best processing of milk at industry level.

1 Introduction

Milk is a perishable food having high moisture contents and all nutrients that helps microorganisms to proliferate. To maximize consumer safety and product quality, nonthermal processing technologies are gaining popularity in the dairy industry (Barba et al., 2017; Misra et al., 2017; Manzoor et al., 2019a, b; Monteiro et al., 2018; Ahmad et al., 2019a). The consumer demand is increasing for minimally processed and fresh-like foods with natural tastes (Roobab et al., 2018; Aadil et al., 2013, 2015a, b; Ahmad et al., 2019b). Despite the associated health hazards, some consumers still prefer to consume raw milk due to its "healthy" claims and natural, refreshing taste. Hence, the new dairy processing techniques are a need for providing both fresh and nutritious as well as safe foods with better storage stability (Alegbeleye et al., 2018; Li & Farid, 2016).

The microbial inactivation during milk processing is crucial for enhancing the shelf-life and stability of milk because milk contains nutrients that support the microbial activity and growth (Claeys et al., 2013; Monteiro et al., 2020). In the dairy industry, thermal treatments i.e., pasteurization, sterilization, ultra-high temperature have been intensively investigated to inactivate or destroy the pathogenic and spore-forming microorganisms in dairy products (Guimarães et al., 2019). Utilization of raw milk or its products (which are associated with many pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157: H7, *Bacillus cereus*, *Salmonella* spp., *Campylobacter* spp., *Clostridium botulinum*) is one of the major courses for the occurrence of foodborne illness worldwide (Claeys et al., 2013; Aadil et al., 2015c, d). Thermal treatment of milk can destroy the amount of some nutritional components along with some undesirable flavour changes. Considering these nutritional and organoleptic changes, novel nonthermal technologies (including high-pressure processing (HPP), pulsed electric field (PEF), ultrasound, ultraviolet irradiations, nonthermal plasma (cold plasma) and membrane microfiltration) have been developed with ability to inactivate both the pathogenic and spoilage microorganisms (Claeys et al., 2013; Amaral et al., 2018; Guimarães et al., 2018, 2019; Coutinho et al., 2019a, b; Zia et al., 2019; Aadil et al., 2018, 2020). The objective of this review is to give a comprehensive overview of the application of nonthermal techniques in milk and milk products together with their effects on nutritional, organoleptic and microbial quality of the product.

2 High-Pressure Processing (HPP)

In contrast to thermal processing technologies where temperature has a main influence in the inactivation of microbes and certain enzymes of interest, HPP employ high pressure generally

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100-600 MPa for up to 20 min of duration to eliminate certain pathogenic microorganisms to increase the shelf life of liquid and solid foods (Evelyn & Silva, 2015). The application of this non-thermal technique is not new and has been long employed in various non-food industries (Oliveira et al., 2012). Application of HPP on food was first reported in the late nineteenth century. Although, the commercialization of this non-thermal technique is recent, as reflected by the increase in number of HPP units installed around the world, but its application on foods has been studied for over 100 years (Sousa et al., 2016). It has been assessed that HPP can alter the characteristics of food proteins, this alteration depends upon the power employed, duration of treatment and temperature. It causes irreversible changes in secondary, tertiary and quaternary structures of protein by affecting mostly the covalent bonds (Dhakal et al., 2014).

2.1 Application in the dairy industry

HPP minimizes the nutrient loss and effects the activity of microbes. When skim milk is subjected to HPP treatment (300 MPa), it was found that the particles size substantially decreases, with the decrease of average size from about 200 to 100 nm regardless of temperature during pressurization. However, pasteurization temperature, pH of the milk, a pressure range of 200 and 300 MPa, and pressure treatment time affect the increase in the size of the particle (Anema et al., 2005). In milk, HPP at mild and room temperature, disorders only those chemical bonds that are moderately weak such as ionic bonds, hydrogen bonds and hydrophobic bonds. The small molecules such as simple-sugars, amino-acids, vitamins and flavour components remain unchanged after HPP treatment (Cheftel, 1992). Sierra et al. (2000) have reported that treatment of milk with HPP causes non-significant vitamin (B1, B6) loss at 400 MPa (at a rate of 2.5 MPa for 30 min at 25 °C). Also, the reduction in proteolytic activity was also observed due to HPP treatment (400 MPa, 40-60 °C and 15 min). In terms of maintaining the milk organoleptic properties at 25-60 °C, it was suggested that these treatments in combinations could be useful to produce the milk having an increased shelf-life with good sensory properties (García et al., 1989).

2.2 Effect on milk quality

Harte et al. (2003) showed that L-value of milk was reduced by HPP due to the disintegration of casein micelles, which cause the reduction in turbidity of milk. The slight effect on L-value was observed when milk was subjected to 200 MPa, while at 250-450MPa pressure there is a significant decrease in the L-value. At 600 MPa for 30 min, skim milk showed a decline in L-value from 78 to 42 and skim milk establishes a semi-transparent or translucent appearance (Naik et al., 2013).

HPP causes the reduction in time required for the induction of fat crystallization and this is because the value at high-pressure liquid/solid transition temperature of milk fat moves to high. At HPP treatment (100 MPa, 16.3 °C), there was an increase in the melting temperature as well as crystallization temperature of milk fat also increased at 15.5 °C with same pressure. Hence, higher the solid fat content present in HPP treated cream than untreated cream, also the time reduction in aging of ice cream substantially. Accordingly, even up to 800 MPa milk fat globule membrane (MFGM) was not deteriorate after applying HPP treated milk. After HPP treatment of milk fat globule, mean diameter of this globule remains unaffected. After HPP treatment, there is no increase in lipolysis, but some whey proteins were absorbed into the MFGM and the membrane remains undamaged (Naik et al., 2013).

2.3 Effect on microbes

Table 1 summarises the effect of HPP treatment on milk microorganisms. HPP played an important role in the inactivation of microbial activities due to applied pressure and temperature on various microorganisms such as *E. coli, L. innocua, L. monocytogenes*, *S. aureus, Bacillus* spores or different traits of these microorganisms in milk. As a result of HPP treatment (300 MPa, 84 °C on skim milk) a 0.67-log reduction of *Bacillus stearothermophilus* ATCC 7953 and *Clostridium sporogenes* PA3679 (Pinho et al., 2011). When HPP treatment (400 MPa, 21 to 31 °C and 0 to 50 min) was applied to human milk, *L. monocytogenes* ATCC 19115, and *Staphylococcus aureus* ATCC 25923 was reduced by 6-log and 8-log, respectively (Viazis et al., 2008). Similarly, when a commercial sterile milk was treated with HPP (300MPa, 75-85 °C), an approximate reduction of 5 CFU/mL of *Bacillus* spores was reported (Amador Espejo et al., 2014).

Patterson et al. (1995) demonstrated that D value of *Salmonella typhimurium* was 3 min when 350 MPa pressure and first-order rate constant (K (1/min) = 0.768) was applied on milk. Similarly, D value (3 min) of *Yersinia enterocolitica* was observed after applying a pressure of 275 MPa (K (1/min) = 0.768). The D value of *L. monocytogenes* was reduced to 3 min when 375 MPa pressure and (K (1/min) = 0.768) applied in milk. In milk, HPP has also reduced the *E. coli* and *S. aureus* when such conditions applied (400 MPa, 50 °C (K (1/min) = 0.768). The strain *E. coli* O157:H7 gave the D value (3 min). After increasing the pressure (500 MPa, 50 °C (K (1/min) = 0.92) *S. aureus* showed a D value of 2.5 min (Patterson & Kilpatrick, 1998). Erkmen (2009) observed 9.21 min D value in *Salmonella typhimurium* when 300 MPa at 25 °C was applied on raw milk. After increasing the pressure (700 MPa) and temperature (90 °C), *C. sporogenes* was reduced to 13.6 min of D value (Shao & Ramaswamy, 2011).

A 3.50-log reduction of *E. coli* (MG 1655) was observed in raw milk with 15% fat subjected to HPP treatment (300 MPa, 25 °C) (Diels et al., 2005). Picart et al. (2006) has reported that *L. innocua* reduced to 1.80-log in raw milk with 15% fat, when treated with 300 MPa at 24 °C. There was an effective pressure treatment for periodic oscillation with a condition of 200 to 500 MPa/60 min, 20 °C in destroying pathogenic microorganisms such as *Salmonella enteritidis, L. monocytogenes* and *E. coli* (Vachon et al., 2002). HPP treatment (500 to 600 MPa, 10 min at 25 °C) was needed to deactivate the Gram-positive bacteria while Gram-negatives were deactivated at relatively lower temperature, pressures and time (Smelt, 1998).

When UHT milk was treated with HPP for the inactivation of *B. cereus*, *P. fluorescens* and *L. monocytogenes*, there was less resistance of exponential phase cells to pressure as compared

Table 1. Effect of HPP on different microbes in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
B. stearothermophilus ATCC 7953	Skim milk	84 °C at 300 MPa	0.67-log	Pinho et al. (2011)
C. sporogenes PA 3679				
Bacillus spores	Commercial sterile milk	300 MPa at 75-85 °C	~5-log CFU/mL	Amador Espejo et al. (2014)
S. typhimurium	Milk	K (1/min) = 0.768, 350 MPa	3 min D value	Patterson et al. (1995)
Y. enterocolitica		K (1/min) = 0.768, 275 MPa	3 min D value	Patterson et al. (1995)
E. coli		K (1/min) = 2.303, 400 MPa, Temp. = 50 °C	1 min D value	Gervilla et al. (1997)
E. coli O157:H7		K (1/min) = 0.768, 400 MPa, Temp. = 50 °C	3 min D value	Patterson & Kilpatrick (1998)
S. aureus		K (1/min) = 0.92, 500 MPa, Temp. = 50 °C	2.5 min D value	
L. monocytogenes		K (1/min) = 0.768, 375 MPa	3 min D value	Patterson et al. (1995)
P. fluorescens		K (1/min) = 2.303, 300 MPa, Temp. = 50 °C	1 min D value	Gervilla et al. (1997)
P. fluorescens		K (1/min) = 3.838, 345 MPa Temp. =50 °C	0.6 min D value	Kalchayanand et al. (1998)
S. typhimurium	Raw milk	25 °C, 300 MPa	9.21 min D value	Erkmen, (2009)
C. sporogenes	Milk	90 °C, 700 MPa	13.6 min D value	Shao & Ramaswamy, (2011)
E. coli MG1655	Raw milk with 15% fat	300 MPa, 25 °C	3.50-log	Diels et al. (2005)
L. innocua		300 MPa, 24 °C	1.80-log	Picart et al. (2006)
L. monocytogenes		100 MPa, 2-4 °C	1.20-log	Iucci et al. (2007)
S. aureus		300 MPa, 20 °C	4.00-log	Briñez et al. (2007)

to cells having stationary phase. At 8 °C, exponential cells were more resistant in comparison to those grown at 30 °C, while the reverse was applicable for the cells in the stationary phase. In the stationary growth phase at 30 °C, *B. cereus* cells were the most pressure resistant. The most sublethal damages were observed for *L. monocytogenes* in comparison with *P. fluorescence* and *B. cereus* (McClements et al., 2001). While Chen & Hoover (2003) has investigated that UHT processed whole milk by HPP for the inactivation of *L. monocytogenes* Scott A unveiled that higher temperatures considerably enhanced the pressure caused the inactivation of *L. monocytogenes*. The endospores are highly resistant as compared to vegetative cells against HPP treatment, but a complete inactivation required a pressure >1000 MPa and >80 °C (Rastogi et al., 2007). Thus, it is proved that HPP in combination with heat, can inactivate the bacterial spores more effectively than HPP alone (Black et al., 2011), and spores were more sensitive to successive pressure treatments when germinated at lower pressures (Setlow et al., 2001). At HPP treatment of 400 MPa for 25 min at 30 °C, *B. cereus* spores were more repellent to pressure in comparison to vegetative cells, and 0.45-log CFU/mL decrease in spores of this bacterium was obtained. McClements et al. (2001) have reported that less spore germination induced at 8 °C than by pressure treatment while the inactivation of the most vegetative yeasts and molds are caused when the pressure of 300-400 MPa at 25 °C was applied for a few minutes.

3 Pulsed Electric Fields (PEF)

Pulsed electric filed (PEF), gained popularity as a potential tool for the inactivation of microorganisms especially in liquid foods (Pal, 2017). PEF has promising effect on the removal of both pathogenic and spoilage causing microbes, and enzymes related to the quality deterioration without causing any decrease in consumer demands (Alirezalu et al., 2020). This technique has major edge of providing high quality food and claimed as superior to traditional thermal processing as it decreases destructive changes in nutritional profile, quality, sensorial and physical attributes of food (Syed et al., 2017). PEF induced inactivation of certain enzymes and microbes is considered to be due to the electroporation and dielectric breakdown of cell membrane. This process is affected by some factors, such as number of pulses, electric field intensity, pulse width, flow rate and shape. Besides that, parameters like temperature, conductivity and physiological parameters of microbes (Sharma et al., 2014) also affect the process. The simple working principle of PEF is based on the application of high electric fields (cause inactivation of organisms) in the form of short pulses at an intensity of 10-80 kV/cm for the duration of micro-seconds. By multiplying the actual number of pulses with effective pulse duration, one can calculate processing time. As the electric field is applied, current flows into the liquid food sample and transferred to each point because of the presence of charged molecules. After the treatment, food needs to be packed aseptically and cold storage should be maintained in order to get longer shelf claims (Pal, 2017).

3.1 Application in the dairy industry

PEF treatment (35 kV/cm, 3 μs pulse width, 9 μs) of raw skim milk did not show any significant difference in proteins, colour, moisture and pH (Michalac et al., 2003). PEF treatment (35 kV/cm, 2.3 μs width of the pulse at 65 °C for <10 sec) immediately after high temperature short time (HTST)

pasteurization has enhanced the milk shelf-life up to 78 days at 4 °C (Sepulveda-Ahumada, 2003). PEF treatment of bovine immunoglobulin (IgG) enriched soymilk at a dose of 41 kV/cm for 54 μs did not cause any change in bovine IgG activity but resulted in a 5.3-log reduction of initial microbial-flora (Li et al., 2003). Sensory properties of PEF treated dairy products are similar to thermally treated counterparts and reported to have a good consumer acceptance rate (Sobrino-López & Martin-Belloso, 2006).

3.2 Effect on milk quality

As a novel technique, only few reports are related to nutritional properties such as vitamin or protein contents, while some of these reports include brief studies on sensorial quality such as taste and flavour of milk. Among these, studies on vitamin content in milk, water-soluble vitamins (riboflavin, vitamin C, and thiamine) and fat-soluble vitamins (tocopherol, cholecalciferol) were analysed after 400 μs at 18.3-27.1 kV/cm. Significant changes for vitamin contents were not reported in milk (Bendicho et al., 2002). The significant changes in the food quality generated by the interaction with electric current discharge into the electrode are in the chemical structure of liquids, which are mainly generated very close to the electrode surface. The important products formed due to the breakdown of water molecules (Morren et al., 2003) and other food components at the interface of electrode-food (Saulis et al., 2007). A significant difference ($p < 0.05$) was noticed between raw milk and PEF treated milk and in their physicochemical properties. The reduction in solids non-fat contents can be attributed to the electrodeposition of milk materials on the surface of electrode which may form a rubber layer. The solids non-fat are commonly called serum solids (Potter, 1986) including casein, lactose, lactalbumin, phosphorus, calcium and riboflavin (International Dairy Foods Association, 2006).

3.3 Effect on milk microbes

Table 2 shows the effect of PEF treatment on the inactivation of microorganisms in milk. Qin et al. (1998) have observed that *E. coli* were treated with PEF (26 kV/cm and 60 kV/cm at 400 μs and 40 °C with exponential decay pulses) in stimulated milk ultra filtrate (SMUF) having a 6 and 8-log reduction. Fernández-Molina et al. (1999a) reported a 2.6-2.7-log cycles reduction of *L. innocua* when pasteurized skim milk treated with PEF (200 μs at 50 kV/cm). In SMUF, a 6-log reduction of *E. coli* was reported after PEF treatment (36 kV/cm, 50 pulses) (Qin et al., 1998). (Fernández-Molina et al., 1999a) showed the effect of PEF (15 to 28 °C and 0.5 L/min 100 pulses with 50 kV/cm, 0.5 μF, 2 μsec, 3.5 Hz exponential decay) and reached to 2.6-log reduction of *L. innocua* in raw skim milk having milk fat (0.2%). Qin et al. (1995) have reported a 7-log reduction of *E. coli* in SMUF with this treatment (<30 °C, 2.5 V/μm, ± 300 pulses and 20 μs exponential decay pulse width). When raw bovine milk treated with PEF (89 μs at 40 kV/cm and 89 μs at 40 kV/cm at 32.5 °C), *E. coli* K12 was reduced to 5-log and *S. aureus* was reduced to 5.2-log (Halpin et al., 2013), while *L. innocua* in raw skim milk was reduced to 4.3-log after PEF treatment (30-pulses of 40 kV/cm, 10 s at 53 °C) (Guerrero-Beltrán et al., 2010). In milk (skim), *Lactococcus lactis* was reduced after PEF treatment (35 kV/cm, 90 μs at 22 °C) that cause 1-log reduction (Michalac et al., 2003) and 3.3-log reduction of *L. innocua* after a PEF treatment (40 kV/cm, 50 μsat 10 °C) (Noci et al., 2009). *E. coli* showed 38.4-44.8 μs D value when the rate of first-order constant K (x10-2/μs) = 5.14-6.0 and 20-45 kV/cm field is applied on skim milk (Martin et al., 1997). Additionally, in skim milk under conditions having a rate constant of K (x10-2/μs) = 0.054-0.52, pulsed of 15-40 kV/cm, and temperature of 15-40 °C gave the 4-42.4 μs D value for *Salmonella dublin* (Sensoy et al., 1997).

Lactobacillus delbrueckii and *B. subtilis* was reduced with the help of using K (x10-2/μs) = 0.096-0.115, pulses of 16 kV/cm and temperature (<30 °C) and K ($x10^{-2}$/μs) = 0.077-0.092, 16 kV/cm, temperature <30 °C showed the D value of 2000-2400 μs and 2500-3000 μs respectively in SMUF (Swanson et al., 1995). At ambient temperature with 2 μs, 100 pulses at 50 kV/cm, 2.6 and 2.7-log reductions were observed in different micro-organisms (Fernández-Molina et al., 1999b). According to Zhao et al. (2013), *E. coli* and *L. monocytogenes* in raw whole milk treated with PEF (25 kV/cm for 200 μs) showed a 2.1-log reduction of *E. coli* and 5-log cycles of *L. monocytogenes* (30 kV/cm after 200 μs). UHT milk was subjected to 150 (bipolar) pulses of 8 μs at 35 kV/cm and caused a 4.5-log reduction in *S. aureus* (Sobrino-López & Martin-Belloso, 2006). *L. innocua* in raw skim milk was reduced with the help of 2 us, 100 pulses, 50 kV/cm at ambient temperature, and almost 2.4 to 3.4-log reduction was reported (Miranda, 1998).

Mañas et al. (2001) reported 2-log reduction of *E. coli* in cream through application of PEF (33 kV/cm, below 100 μs), and (Evrendilek & Zhang, 2005) has indicated the equivalent reduction of *E. coli* O157:H7 in PEF treated skim milk (24 kV/cm for 141 μs). In PEF treated skim milk (25 kV/cm for 45 μs), a decrease in *E. coli* bacteria was more than 2-log cycles (Martin et al., 1997). In UHT skim milk, >4-log cycles of *E. coli* cells were inactivated after PEF treatment (22.4 kV/cm for 46 μs) (Grahl & Märkl, 1996). In pasteurised fat-free milk inoculated with *E. coli* prior to PEF treatment (41 kV/cm for 158 μs), more than 5.5-log cycles did not survive in these processing conditions (Dutreux et al., 2000). In simulated milk ultra-filtrate (SMUF), a reduction of up to 9-log cycles of *E. coli* was observed (Zhang et al., 1995) after PEF treatment (70 kV/cm for 160 μs).

4 Ultrasound

Ultrasound (US) technology is one of the most widely used non-thermal processing technique around the world because of its environment friendly, non-toxic and benign nature, additionally it has a wide range of applications in food industry (Shanmugam et al., 2012). It has been a century that we know the destructive effect of US on both pathogenic and spoilage causing microorganisms, but induction of this technology in food industry for controlling and promoting their activities is much more recent. Firstly, Harvey & Loomis (1929) reported significant effect of US to kill luminous bacteria in aqueous medium (Ojha et al., 2017). US technology was applied in food industry because of its ability to improve

Table 2. Effect of PEF on different microbes in milk.

Microorganisms	Dairy food	Treatment conditions	Reduction	References
E. coli	Milk submitted to ultrafiltration	50 pulses of 60 kV/cm or 80 pulses of 70 kV/cm	6 and 9-log	Qin et al. (1998)
L. innocua	Pasteurized skim milk	200 µs at 50 kV/cm	2.6-2.7-log	Fernández-Molina (2001)
E. coli K12	Raw bovine milk	89 µs at 40 kV/cm at 32.5 °C	5-log	Cregenzán-Alberti et al. (2015)
S. aureus			5.2-log	
E. Coli	Raw whole milk	25 kV/cm for 200 µs	2.1-log	Zhao et al. (2013)
L. monocytogenes	Raw whole milk	30 kV/cm after 200 µs	5-log	Zhao et al. (2013)
S. aureus	UHT milk	150 bipolar pulses of 8 µs at 35 kV/cm	4.5-log	Sobrino-López & Martin-Belloso (2006)
L. innocua	Raw skim milk	2 µ sec, 100 pulses, 50 kV/cm at ambient temperature	2.4-log	Calderon-Miranda (1998)
E. coli	UHT milk	15 pulses, 22 kV/cm	3-log	Grahl et al. (1992)
E. coli	Stimulated milk ultrafiltrate	36 kV/cm, 50 pulses	6-log	Qin et al. (1998)
L. innocua	Raw skim milk	15 to 28 °C, 0.5 L/min 100 pulses, 50 kV/cm 0.5 µF, 2 µsec, 3.5 Hz Exponential decay	2.6-log	Fernández-Molina et al. (1999a)
E. coli	Skim milk	15 ± 1 °C, 4.0 V/µm, 3 µsec, 64 Pulses	3-log	Zhang et al. (1994)
E. coli	Stimulated milk ultrafiltrate	< 30 °C, 1.6 (1.2, 1.4, 1.6 tested) V/µm, 200-300 µsec, 60 (20, 30, 40, 50, 60) pulses	4-log	Pothakamury et al. (1995)
E. coli	SMUF	< 30 °C, 2.5 V/µm, ± 300 pulses, exponential decay pulse width 20 µsec	7-log	Qin et al. (1995)
L. lactis	Skim milk	35 kV/cm, 90 µs, 22 °C	1-log	Michalac et al. (2003)
L. innocua	Milk	40 kV/cm, 50 µs, 10 °C	3.3-log	Noci et al. (2009)
E. coli	Skim milk	K (x10-2/µs) = 5.14-6.0, 20-45 kV/cm	38.4-44.8 µs D value	Martin et al. (1997)
Salmonella Dublin	Skim milk	K ($x10^{-2}$/µs) = 0.054-0.52, 15-40 kV/cm, Temp = 15-40 °C	4-42.4 µs D value	Sensoy et al. (1997)
L. monocytogenes (Scott A)	Milk	K ($x10^{-2}$/µs) = 0.012-0.015, 30 kV/cm, Temp = 10-50 °C	150-200 µs D value	Reina et al. (1998)
L. innocua	Skim milk	K (x10-2/µs) = 2.995, 50 kV/cm, Temp= 15-28 °C	76.9 µs D value	Fernández-Molina et al. (1999a)
L. delbrueckii	SMUF Raw skim milk (0.2% milkfat)	K (x10-2/µs) = 0.096-0.115, 16 kV/cm Temp, < 30 °C	2000-2400 µs D value	Pothakamury, (1995)
B. subtilis		K (x10-2/µs) = 0.077-0.092, 16 kV/cm, Temp < 30 °C	2500-3000 µs D value	Pothakamury, (1995) Qin et al. (1994)
B. subtilis	SMUF	K (x10-2/µs) = 0.44-0.54, 16 kV/cm	425-520 µs D value	Pothakamury, (1995) Qin et al. (1994)
P. fluorescens	Raw skim milk (0.2% milkfat) Milk	15-28 °C, 0.51/min 30 pulses, 50 kV/cm, 0.5 µF, 2 µsec, 4.0 Hz Exponential decay	2.7-log	Fernández-Molina et al. (1999b)
B. subtilis *P. fluorescens* *S. Dublin*		63 °C, 3.67 V/µm, 36 µsec, 40 pulses	4.0-log	Dunn & Pearlman (1987)
Lactobacillus brevis	Yogurt	(50 °C, 1.8 V/µm	2.0-log	Dunn & Pearlman (1987)
B. stearothermophilus	3.4% fat milk	60 kV/cm, 200µs, 50 °C (T_{in})	3-log	Shin et al. (2007)
L. lactis	Skim milk	35 kV/cm, 90 µs, 22 °C	1-log	Michalac et al. (2003)
P. fluorescens	3.4% fat milk	60 kV/cm, 200 µs, 40 °C (T_{in})	5-log	Shin et al. (2007)
Cronobacter sakazakii	Infant formula milk	35 kV/cm, 500µs, 5 °C (T_{in})	1.2-log	Pina-Perez et al. (2009)
L. innocua	3.6% Fat whole milk	29 kV/cm, 250 µs, < 45 °C	1.5-2-log	Picart et al. (2002)
Mycobacterium paratuberculosis	Milk	30 kV/cm, 2500 pulses, ~50 °C	5.6-log, 5.9-log	Rowan et al. (2001)
L. innocua	Whole milk	40 kV/cm, 43.75 µs, 68 °C	5.5-log	Guerrero-Beltrán et al. (2010)
S. aureus	Skim milk	25 kV/cm, 100 µs, ~50 °C	3-log	Sobrino-López & Martín-Belloso (2008)

functional, physical and chemical properties of various food items (Higuera-Barraza et al., 2016). The basic principle of US technology is based on the mechanical waves which are at a frequency, above the threshold level (>16 kHz) of human hearing (Soria & Villamiel, 2010). In liquid food samples, US causes periodic cycles of high and low pressure, as it conducts high intensity and frequency sound waves. During the high and low pressure cycle vacuum bubbles form and collapse violently (Zhao et al., 2014). Based on frequency range US technology can be divided into power ultrasound, high frequency ultrasound and diagnostic ultrasounds having ranges 20-100 kHz, 20 kHz-2 MHz and (>1 MHz), respectively. Based on application it can be divided broadly into high (10-1000 W/cm^2) and low (<1 W/cm^2) intensity sonication (Ojha et al., 2017).

4.1 Applications in the dairy industry

It has been used for homogenization of milk (Al-Hilphy et al., 2012), novel dairy products with unique physico-chemical and functional properties can be prepared alone with ultrasonication or in combination with different traditional homogenization techniques. (Jin et al., 2014) showed that there was increased crossflow ultrafiltration of skim milk by applying in situ ultrasonication. Inactivation of microbes through sonication is one of its applications in dairy industry. The effectiveness of microbial inactivation in retaliation to ultrasound depends on the type of targeted microorganisms. Gram-positive bacteria contain a thick and tightly adherent peptidoglycan cell wall layer which is resistant to sonication (Chemat et al., 2011). Gram-positive bacteria are generally more sensitive than Gram-negative microbes, while spores are more resistant than vegetative cells (Halpin et al., 2013).

4.2 Effect on milk quality

Use of ultrasound in food processing as compared to other novel technologies is limited. However, the utilization of ultrasound can have various advantages on milk processing such as removal of gases, homogenization of fat globules and increase in the activity of antioxidants (Evrendilek, 2014). Hence, continuous ultrasound flow treatment can be a favourable technology for the processing of milk. A total elimination of *E. coli* was observed due to ultrasound (20 kHz, 10 min) application. After 6 min, viable counts of *P.* fluorescens were reduced by 100% and *L. monocytogenes* were decreased by 99% after 10 min (Cameron et al., 2009). For both raw and pasteurized milk, protein or lactose contents were not changed with ultrasound, although it may induce rise in the fat concentration. Woefully, ultrasound does not cause the inactivation of lacto-peroxidase and alkaline-phosphate activities (Cameron et al., 2009). There is no change in the viscosity, but turbidity was reduced by the processing of homogenized pasteurized milk (skim) by sonication treatment (20 kHz at 20 and 41 W) at different time intervals (up to 60 min) under controlled conditions. The fat globules size, particles that are soluble and casein micelles change after 60 min of sonication with a change in energy generation. In milk whey proteins denaturation was observed, which forms aggregates of soluble whey proteins. During the first 30 minutes of sonication treatment, the interaction of these aggregates with casein micelles form micellar aggregates. When there was an increase in the times for sonication, some of the whey proteins were partially disrupted by these aggregates (Shanmugam et al., 2012). After sonication treatment, liquid egg and skim milk inoculation with *Salmonella Typhimurium* at 20 and 40 °C for 30 mins cause reduction of 1 and 3-log CFU/mL in counts of this bacteria, respectively (Wrigley & Llorca, 1992). In the milk samples treated with frequency of 800 kHz for 1 min with 8.4 W/cm^2 power intensity, coliform bacteria counts were reduced by 93%.

4.3 Effect on milk microbes

Table 3 shows the influence of ultrasound treatment on milk microorganisms. Cameron et al. (2009) has reported the effect of sonication treatment (20 kHz for 10 min at 750 W) in raw pasteurized milk that showed reduction to 5.34-log CFU/g in *E. coli* and 2.07-log CFU/g in *L. monocytogenes* but at 6 min with same ultrasonic conditions, it was reduced to 5.64-log CFU/g in *P. fluorescens*, which means microbes are more sensitive against ultrasound treatment. When raw whole cow's milk (4% fat) was sonicated (20 kHz, 120 µm for 12 min at 60 °C), a 3.1-log reduction in *E. coli* was observed (Herceg et al., 2012). After sonication treatment (15.8 ± 1.6 mJ/cm^2, 18 sec), *L. monocytogenes* was reduced to 10^7 CFU/mL in goat milk (Matak et al., 2005). In another study, after ultrasound treatment (20 kHz with 60 °C) in UHT milk resulted in 0.3 min D value for *L. monocytogenes* (Earnshaw et al., 1995). García et al. (1989) has investigated that the inactivation of *B. subtilis* with a rate of 70 and 49% after sonicated milk treatment (20 kHz with 150 W at 100 °C temperature), 2.5 to 3-log reduction of *Salmonella Typhimurium*

Table 3. Effect of ultrasound on different microorganism in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
E. coli	Raw whole 4% fat cow's milk	20 kHz, 120 µm,12 min, 60 °C	3.1-log	Herceg et al. (2012)
E. coli	Raw milk (pasteurization)	20 kHz, 10 min, 750 W	5.34-log CFU/g	Cameron et al. (2009)
P. fluorescens		20 kHz, 6 min, 750 W	5.64-log CFU/g	
L. monocytogenes		20 kHz, 10 min, 750 W	2.07-log CFU/g	
L. monocytogenes	UHT milk	Heat at 60 °C with Sonication at 20 kHz	$D_{60\&S}$ = 0.3 min	Earnshaw et al. (1995)
S. typhimurium	Skim milk	30 min at 50 °C 30 min at 40 °C	3-log 2.5-log	Wrigley and Llorca (1992)
E. coli K12DH5 A	UHT milk (pH 6.7)	110 µm, 60 °C	$D_{60\&S}$ = 23s	Zenker et al. (2003)

in skim milk was attained after ultrasound treatment for 30 min at 40 °C and 50 °C (Wrigley & Llorca, 1992).

5 Ultraviolet irradiation

UV radiation (spectrum ranges from 100-400 nm) technology has been used for decades (60 years) primarily for the disinfection of water, surfaces and air (Guneser & Karagul Yuceer, 2012). In food industry, it is being effectively used for the microbial decontamination of packaging materials and surfaces. The major drawback of using this technology is that, it has low penetration power and suspended solids reduce its effectiveness in liquid samples (as 254 nm UV radiation suffers 30% loss; below 5 cm surface, in intensity in 10% sucrose solution (Falguera et al., 2011). In the electromagnetic spectrum, UV light has 3 regions UV-A, UV-B and UV-C spectrum ranges from 315-400 nm, 280-315 nm, 200-280 nm respectively, provided that the UV-C region have the germicidal properties. UV-C radiation act on both pathogenic and spoilage causing microorganisms (viruses and protozoa) by damaging the DNA which eventually prevents the transcription and replication process, resultantly causing cell death (Choudhary & Bandla, 2012). The effect of UV-C radiation depends on the microbial load, flow and optical properties of the product, wavelength, power, geometric configuration of the device, radiation path length and physical arrangement of UV source (Guneser & Karagul Yuceer, 2012).

5.1 Application in the dairy industry

Milk turbidity is a major challenge presented by UV light treatment when used for pasteurization. Turbidity in milk decreases microbial inactivation due to lower UV light penetration in turbid milk. Suspended and colloidal solids present at high level in milk make it turbid that causes the opaqueness of milk. In modern UV reactors, there are two strategies that have been used to increase the UV light penetration into milk based on the fluid flow, which opened the ways in food and dairy industries for pasteurization using application of this technology. The first approach employs laminar flow of milk or fluid by very thin film formation on a UV irradiated surface which results in complete penetration of light through the milk. Second approach employs the use of turbulent flow of milk by bringing all liquid parts into close proximity of UV light exposed surfaces which decreases the required path length and results in good UV light penetration in milk (Datta et al., 2015). Few studies regarding effects of UV processing on whole milk quality demonstrated that there was no notable change in the viscosity, colour, pH, soluble solid contents and viscosity of milk. The pH range of milk treated under UV was 6.66 to 6.70, viscosity was on average of 2.00 ± 0.01 (m Pa s), the colour change ΔE^* was in range of 0-0.5 and contents of soluble solid was 12.78 ± 0.10 (% g/g) when pasteurized whole milk was treated with UV having dose of 10 mJ/cm2 (12 to 235 min) (Orlowska et al., 2013).

5.2 Effect on microbes

Table 4 represents the UV irradiation effect on microorganisms present in milk. The raw cow milk treated with UV dose (1.5 J m/L using 1 and 2 pure version of reactors) resulted in a 3-log reduction of natural microflora (Reinemann et al., 2006). UV reactors (dean flow) efficiency and their effects on inactivation of *B. cereus* endospores and *E. coli* W1485 in raw cow milk, commercially processed skim milk and in soymilk has been studied (Bandla et al., 2012).

By using a reactor (dean flow) with a diameter of 1.6 mm having UV dose (0.05 J m/L), reduction of *E. coli* W 1485 (>7-log) in skimmed milk and soymilk (>5-log reduction) was observed. The 4-log reduction of *E. coli* W 1485 was resulted by using a same UV dose and reactor in raw cow milk. For raw cow milk because of less transmission of UV, a higher dose of UV was recommended than soymilk and skimmed milk. For treatment of UV, milk and dairy products pose a challenge due to containment of high amount of spoilage and pathogenic microorganisms as compared to fruit juices. In sweet, acid and brine whey, the total bacterial count reduced to 7-log, showing the possible use of treatment with UV in brine and whey for processing of dairy products (Gupta, 2011).

According to Matak et al. (2005), UV radiation was employed for reducing the *L. monocytogenes* population. Population of *Listeria* was reduced by 5-log units in raw goat milk when UV was applied (UV dose 158±16 Jm^2). In whey, there was a reduction of the total bacterial viable count of 3.5-log when turbulent reactor was used while UV intensity was at 450 W/m^2 (Simmons et al., 2012).

6 Plasma and low plasma treatment

Plasma (quasi-neutral gas) technology (PT), is one of the newly developed technology, having various applications in food industry. PT improves the quality and ensures the safety of the food product from both pathogenic and spoilage causing microorganisms, without affecting the functional, sensory and nutritional profile (Mir et al., 2016). PT is based on a simple physical principle, the gas is fed by additional energy by means of electrical discharge, which resultantly turns it into energy rich

Table 4. Effect of UV irradiation on different microbes in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
L. monocytogenes	Goat milk	15.8 ± 1.6 mJ/cm^2, 18 s	5-log	Matak et al. (2005)
	Skim milk (0%fat)		2.29-log	
E. coli ATCC 25922	Reduced fat milk (2%fat)	5.8 mJ/cm^2 for 1.5 sec, 4 °C	1.82-log	Bandla (2010)
	Whole milk (3.25%fat)		0.73-log	
	Soya milk		> 5-log	
E. coli	Raw cow milk	Dean flow reactor (1.6 mm), μv dose (0.05 J/mL)	4-log	Bandla et al. (2012)
	Skimmed milk		> 7-log	

plasma (fourth state of matter) state. Plasma is completely or partially ionized state consisting of free electrons and radicals, intermediate highly reactive species, negatively and positively charged ions, UV photons, molecules and atoms with a neutral charge (Sarangapani et al., 2015). Low temperature property and higher efficiency in microbial inactivation are the most attractive features of PT (Guo et al., 2015). Moreover, it is important to note that, PT induce modifications only on the surface of the food as plasma reactive species do not have penetrating power (Fernández & Thompson, 2012). In food industry, PT is mostly used for enzyme inactivation, waste water treatment, food packaging modification, toxic removal and food decontamination (Pankaj et al., 2018).

6.1 Application in the dairy industry

Regarding microorganism inactivation, there are few studies executed on cold plasma treatment in milk and dairy industries, but it is mostly used in chemistry, polymer and medical industries.

6.2 Effect on microbes

Table 5 and Table 6 show the summary of microbial inactivation by Plasma technology in milk. Ruan (2007) showed a 2.95, 2.74, 0.18-log reduction of *Salmonella* (5 strain-mixture), *L. monocytogenes* (5 strain-mixture) and *B. cereus* (3 strain-mixture) respectively when skim milk was subjected to 35-40 kV with an exit temperature <60 °C, and single pass concentrated high-intensity electric field (CHIEF). In skim milk, when strains of *Salmonella, E. coli* and *L. monocytogenes* was subjected to double pass CHIEF with 35-40kV and an exit temperature of <60 °C caused a 5.55, 4.36 and 4.73-log reduction, respectively. *E. coli* ATCC 25922 was reduced to 3.40-log in semi-skimmed milk, 3.63-log in whole milk and 3.34-log in skimmed milk when it was subjected to low-temperature plasma treatment (AC power supply 9 kV, 20 min, <35 °C) (Korachi & Aslan, 2011; Korachi et al., 2010). The inactivation rate of plasma on *E. coli*, *Salmonella typhimurium* and *S. aureus* in whole, semi-skimmed and skimmed milk that stored at 4 °C for 42 days were conducted, and after plasma treatment (20 kV), the counts of *E. coli, S. typhimurium,* and *S. aureus* was reduced to 3.63, 2.00 and 2.62-log CFU/mL, respectively. There was no remarkable change in colour and pH of samples of milk. After 1-week examination, there were no viable cells detected in whole milk and the samples remained stable after six weeks of storage (Evrendilek, 2014).

7 Membrane filtration

Membrane filtration (MF) technology is basically a separation process which particularly employs semi-permeable membranes to concentrate or fractionate liquids into two diverse compositions, generally by allowing some selective compounds to pass and preventing others. The retained liquid is referred as retentate and passed out liquid is known as permeate. The effectivity of membranes is mainly directed by the hydrostatic pressure (or transmembrane pressure) through the membrane and concentration gradient of liquids. Dairy industry has been applying MF technology since 1960 (Kumar et al., 2013). In food industry, primarily in dairy industry, MF processes (for example nanofiltration or ultrafiltration) are used for higher outputs of concentration and separation of proteins (Leeb et al., 2014). As a non-thermal technology it reduces the total viable count of bacteria along with their spores thus prolonging shelf life without damaging the nutritional and sensory profile of dairy products (Kumar et al., 2013).

7.1 Application in the dairy industry

Khanal (2014) has described the uses of MF in the dairy industry for casein concentration (milk fractionation), fat separation, bacterial removal and spore removal. It can be used to clarify the food material and separate the suspended particles of 0.10 to 5 μm range in the food industry. MF is used to enhance the milk shelf-life by reducing the microbial load and remove spores from the milk while keeping the organoleptic quality as before (Khanal, 2014). The membranes made of cellulose acetate are among the most popular in dairy industry because of their low cost and low fouling characters. The membrane processing was first used in the dairy industry for separating milk components in the late 1960s, and now is widely used for whey and cheese processing (Gésan-Guiziou, 2010), but Fernández García et al. (2013) have confirmed that

Table 5. Effect of plasma technology on different microbes in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
E. coli O157:H7 ATCC43895	Milk	35-40 kV, exit temperature < 60 °C, single pass CHIEF	≥ 3.94-log	Ruan (2007)
Salmonella (5 strain mixture)	Skim milk		2.95-log	
L. monocytogenes (5 strain mixture)			2.74-log	
B. cereus (3 strain mixture)			0.18-log	
E. coli O157:H7 (5 strain mixture)		35-40 kV, exit temperature < 60 °C, double pass CHIEF	4.36-log	
Salmonella (5 strain mixture)			5.55-log	
L. monocytogenes (5 strain mixture)			4.73-log	

Table 6. Effect of low temperature plasma treatment on different microbes in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
E. coli ATCC 25922	Whole milk	9 kV of AC power supply, 20 min, temp below 35 °C	3.63-log	Korachi et al. (2010); Korachi & Aslan, (2011)
	Semi-skimmed milk		3.40-log	
	Skimmed milk		3.34-log	

that membrane processing was first used to separate cream and skim milk using polymeric filters with 0.2-10 μm pore sizes while 2 μm ceramic membranes were successfully used to obtain the skim milk virtually free from fat.

7.2 Effect on microbes

Table 7 shows the summary of microbial inactivation by membrane microfiltration in milk. Rodríguez-González et al. (2011) counted a 2.1-log reduction in mesophilic micro-organisms in skim milk, using cross-flow MF of 1.4 μm pore size. Maubois (2002) has reported a reduction of >3.5-log in the vegetative cells of skim milk after MF processing (55 °C, 1.4 μm pore size). The MF treated milk was free from somatic cells, and the spore reduction was >4.5-log. Pafylias et al. (1996) have investigated the efficiency to remove the bacteria from inoculated reconstituted skim milk through MF ceramic membrane (1.4 μm pore sized) while average of 4.5-log reduction in bacterial count was reported. They also concluded that a reduction in bacterial count substantially in skim milk can be attained without any significant changes in the milk composition.

Fritsch & Moraru (2008) investigated the efficiency of MF to remove the microorganisms, spores and somatic cells from skim milk at cold temperatures. They were unable to detect any bacteria in permeate from skim milk having an initial count of 5.25 and 2.15-log CFU/mL of vegetative bacteria and spores, respectively following the application of MF treatment (pore size of 1.4 μm at 6 °C) and somatic cell count was reduced to 3.0-log. (Gosch et al., 2014) used 0.8 and 1.4 μm MF (tubular ceramic ISOFLUX® membrane) to process colostrum and skim milk. The microbial removal with a 0.8 μm MF membrane was more efficient than >5.4-log reduction in total viable count, while >3.5 log reduction in the count using a membrane having pore size of 1.4 μm. On the other hand, both types of MF reduced the total viable counts to >2.3 log CFU/mL in skim milk. They also used 0.14 and 0.2 μm MF and reported that permeate from both of these membranes were almost free (<1.0-log CFU/mL) from microorganisms. To check the efficiency of membrane filtration in removing the bacteria and spores from milk, 1.4 μm ceramic membrane MF treatment was applied (Caplan & Barbano, 2013). The skim milk processed through 1.4 μm MF at 51 °C, reduced the bacterial count to 4.13-log cycles while the spore count was found <1.0-log. (Daufin et al., 2001) reported a microbial reduction of 2.1 to 3.1-log CFU/mL when milk was passed through 1.4 μm MF, depending on initial count and morphology of the bacteria, while (Gésan-Guiziou, 2010) counted a 2-3-log reduction in using a ceramic membrane with 1.4 μm (pore size). However, the efficiency of Sterilox® membranes (Pall-Exekia Company) is much better due to narrow pore distribution size and can reduce the microbial load by 5 to 6-log and 3 to 4-log CFU/mL using 0.8 and 1.4 μm MF, respectively. Elwell & Barbano (2006) investigated the quality and storage stability of skim milk following MF using ceramic membranes having pore size of 1.4 μm and they found 3.79-log reduction in the bacterial count and reported that spore count was reduced to an undetectable level from initial counts of 2-log CFU/mL in raw milk. Another study reported >3.5-log reductions of bacterial count, and retention of all somatic cells in skim milk, when filtered through a pore size of 1.4 μm membrane at 50 °C. On comparing the results with 0.5 μm membrane processing, the bacterial reduction was increased to 2-3-log when the smaller pore size membrane was used (Saboyainsta & Maubois, 2000). Trouvé et al. (1991) observed >4.5-log reductions of spore-forming bacteria when skim milk was treated with a 1.4 μm membrane. In another study, Brans et al. (2004) investigated the use of a 0.5 μm micro-sieve, an advanced type of membrane filter. This type of membrane has a narrow pore size distribution and can work at a low trans-membrane pressure and attained a 6.6-log reduction in *B. subtilis* that was inoculated in SMUF.

8 Combined treatments

Table 8 illustrate the effect of combined nonthermal treatments on inactivation of milk microbes. It has been proved that combination of two different nonthermal techniques has shown the best results than single treatment (alone) for the reduction of microorganisms. When a combination of HPP and heat was applied on UHT milk (400 MPA, 50 °C for 15 min) it resulted in 5.0-log reduction of *E. coli* O157:H7 (Patterson & Kilpatrick, 1998). At 20 to 30 °C, the bacterial vegetative cells showed the greatest resistance to HPP, while at lower and higher temperature microbes were much sensitive to HPP. The resistance showed by bacterial vegetative cells to HPP decreases even at non-lethal temperatures when pressure is used in combination with heat. This combination allows the inactivation (>6-log cycles) of pathogenic and spoilage microorganisms substantially at lower pressures or shorter times than that required at room temperature. In UHT milk the *L. monocytogenes* did not inactivate at 200 MPa up to 45 °C, while 6-log reduction was obtained in cell count after 200 MPa, 55 °C and 15 min (Simpson & Gilmour, 1997). The resistance showed by different strains (*E. coli* O157:H7, *Salmonella* species, *L. monocytogenes* and *S. aureus*) at 25 °C with

Table 7. Effect of membrane microfiltration (MF) treatment on different microbes in milk.

Microorganisms	Dairy Food	Treatment conditions	Reduction	References
Total bacterial load	Skim milk	MF-1.4 μm, ceramic membrane, Tp = 50 °C	5.18-log	Awad et al. (2010)
	Reconstituted skim milk		4.5-log	Pafylias et al. (1996)
Spore forming bacteria	Skim milk	MF-1.4 μm, ceramic membrane, Tp = 55 °C	>3.5-log	Maubois (2002)
		MF-1.4 μm	>4.5-log	Trouvé et al. (1991)
Spores		MF-1.4 μm, ceramic membrane, Tp = 55 °C	>4.5-log	Maubois (2002)
Bacillus cereus spores		MF-1.4 μm, ceramic membrane	3.5-log	Olesen & Jensen (1989)
Bacillus subtilis	Simulated milk ultrafiltrate	MF-0.5 μm (micro sieve)	6.6-log	Brans et al. (2004)
Total bacterial load	Skim milk	MF-0.8 μm, tubular ceramic ISOFLUX® membrane	>2.3-log	Gosch et al. (2014)

Table 8. Effect of combined treatments on different microbes in milk.

Treatment	Microorganisms	Dairy food	Treatment conditions	Reduction	References
Thermosonication	*S. aureus*	Milk 4% fat	20 kHz, 4,8 min, 120 µm, 60 °C	1-log CFU/g	Herceg et al. (2012)
	L. innocua	Raw and whole cow's milk	63 ± 0.5 °C, 24 kHz, 129 mW/mL, 400 W, 120 mm, 10 min	5-log CFU/mL	Bermúdez-Aguirre et al. (2011)
PEF and electrically induced heat	*E. coli*	Milk 4% fat	20 kHz, 2.78 min	1-log CFU/g	Herceg et al. (2012)
	L. innocua	Pasteurized whole milk	40 kV/cm, 15 pulses, 23 °C (T_i), 10 s	5.5-log	Guerrero-Beltrán et al. (2010)
		Raw whole milk	120 µm, 2.85 W/cm^2/63 ± 0.5 °C/30 min	3.58-log	Earnshaw, Appleyard and Hurst, (1995)
Thermosonication	*B. subtilis spores*	Whole milk	US: 20 kHz,150 W, V=30 mL, 100 °C	$D_{TUS=}$ 1.6	García et al. (1989)
	L. monocytogenes	UHT milk	US: 20 kHz, 150 W, V = 30 mL, 60 °C	$D_{TUS=}$ 0.3	
	E. coli	UHT milk (pH 6.7)	US: 20 kHz, 110 µm, V = 10 mL, 60 °C	$D_{TUS=}$ 0.4	Zenker et al. (2003)
HPP and Heat	*L. monocytogenes*	UHT milk	375 MPA/15 min/35 °C	3.5-log	Simpson & Gilmour (1997)
HPP and Heat	*E. coli* O157:H7	UHT milk	400 MPA/15 min/50 °C	5.0-log	Patterson & Kilpatrick (1998)

345 MPa, but at 50 °C these differences were greatly decreased (Alpas et al., 1999). In general, the kinetics of inactivation of most vegetative cells by HPP at low temperatures shows an initial exponential rate, followed by pronounced tailing (Smelt, 1998). This trail disappears when HPP is combined with heat (Kalchayanand et al., 1998).

Herceg et al. (2012) used combination of heat with ultrasound and showed 1-log reduction of *S. aureus* when milk having 4% fat was subjected to 20 kHz frequency for 4-8 min and 120 µm with a temperature of 60 °C and *E. coli* also reduced by 1-log reduction when milk was subjected to thermosonication (20 kHz, 2.78 min and 60 °C). Ultrasound treatment with lethal or sub-lethal temperatures (ultrasound-assisted thermal processing) has numerous benefits and proved to be an effective technique in prolonging the shelf life of foods. It can take a product to the better-quality with enhancements in the appearance, taste and texture than conventionally treated by heat, which reduces the cost and energy requirements. During sonication, sensitivity of microbes towards temperature could be the factor in addition to the effect on the phenomenon of cavitation. The changes in pressure happen during cavitation, which are responsible for the inactivation effect, then temperature rise, and disruption enhances the membrane fluidity i.e. weakening the intermolecular forces (Russell, 2002). García et al. (1989) first introduced that bacterial cells become highly sensitive to heat treatment, if they have undergone sonication treatment. In UHT milk, *S. aureus* was reduced to 6.0-log after 500 MPa, 5 min at 50 °C, while <1.0-log in numbers was achieved either with a single treatment. It has been reported that to get better spore inactivation, pressure can also be used with temperature. Destruction of spores (*B. subtilis* and *C. sporogenes*) was increased by temperature elevation (Stewart et al., 2000). HPP can cause the effective inactivation of spores (*B. stearothermophilus*) at elevated temperatures (Ananta et al., 2001). A single sonication treatment had no effect, but applying thermosonication in glycerol, 63 to 73% (<1-log cycle CFU/mL) population of spores was reduced from 40 to 79% in milk. The reduction in water (distilled) ranges from 70 to 99.9% (3-log cycle CFU/mL). As the treatment temperature reached to 100 °C, the thermosonication effect was dramatically diminished. For the maximum spore's inactivation, the optimum temperature was 70 °C under the experimental conditions.

9 Cost effectiveness of non-thermal technologies

The capital and operating cost of HPP equipment will continue to decrease according to the demand of equipment (Campus, 2010). So, the average processing cost (depending upon the processing conditions) of HPP is US$0.05-0.5 per litre or kilogram of different food items, which is lower than the thermal processing cost. HPP technology is suitable and can be cost effectively used for premium products (Bermúdez-Aguirre & Barbosa-Cánovas, 2010). Töpfl (2006) reported that operation cost of PEF was in the span of US$ 0.011-0.022 per litre for the preservation of liquid media and this was 10-fold greater than needed cost for the conventional thermal processing. PEF can effectively accelerate the drying process in food industry, as compared to conventional drying that employ elevated heat by precisely controlling the process temperature, leading to decrease in energy cost and gas consumption (Pereira & Vicente, 2010). US technology can be cost effectively used for extraction processes and rapid crystallization of food material, and provide benefits such as less processing time, increased final yield, greater penetration power and reduced cost (Chandrapala et al., 2013). Current limitations, for the application of non-thermal technologies, including high investment cost, lack of regulatory support and full control of variables have been delaying the broader application of these technologies in the industrial sector (Pereira & Vicente, 2010).

10 Conclusions

Novel nonthermal technologies have the capability to inactivate the microbes present in milk and milk products. These techniques facilitate less destruction in nutritional contents of milk as compared to thermal techniques and enhance the shelf life as well. Major nonthermal approaches for decontamination of milk and milk products include HPP, PEF, sonication, thermosonication and

various other methods. Combined effects of these technologies appear to be the most successful in processing of milk. This could inactivate the pathogenic and spoilage microbes and minimise the nutritional quality deterioration in milk and milk products. Hence, these techniques may operate in the dairy and food industries in large scale in future processing operations.

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