CHAPTER 13

Non-Thermal Processing of Milk and Milk Products for Microbial Safety

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13.1 Introduction

Due to their perishable nature and being spoiled by microorganisms, safety and microbial quality of milk and dairy products are important issues that need to be addressed. The most common way of ensuring food safety and shelf-life stability is thermal treatment of food. Despite its high efficiency in inactivating pathogenic or spoilage microorganisms, thermal treatments may well cause color modifications, and flavor changes in foods as well as significant nutritional losses. Consumers' demand towards more nutritional foods has triggered the efforts of food industry to design alternative strategies to produce safer foods with minimal thermal treatments. Such efforts have emerged novel technologies alternative to heat treatment in food processing. Different thermal and non-thermal processing technologies such as pulsed electric fields (PEF), high pressure processing (HPP), ultrasonication, cold plasma, etc. have been tested for processing of milk and dairy products (Cruz-Romero et al. 2006, del Pozo-Insfran et al. 2007, Tahiri et al. 2006). This chapter deals with potential application fields of these technologies in milk and dairy processing.

13.2 Pulsed electric fields

Application of short burst of high voltage electric pulses in the range of 20–80 kV cm^{-1} placed between two electrodes is involved in pulsed electric fields (PEF) processing. Treatment time of PEF lasts in microseconds; thus, increase in treatment temperature during PEF processing is minimized. The applied high voltage results in an electric field that causes microbial inactivation. Depending on the system design and circuit development, the electric field may be applied in the form of exponentially decaying, square wave monopolar, square wave bipolar or instant charge reversal pulses and at ambient, sub-ambient, or slightly above ambient temperatures.

The basic components of PEF system include a high voltage pulse generator, controlling and monitoring system, treatment chambers and fluid handling system. Food product to be processed is placed in either a static or a continuous design treatment chamber, where two electrodes are connected together with a non-conductive material to avoid electrical flow from one to the other. High voltage electrical pulses generated in the system are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed between the two electrodes (Zimmermann and Benz 1980).

Microbial inactivation by PEF is provided by structural damages of cell membrane, which lead to ion leakage, metabolite losses, protein releases and increased uptake of drugs, molecular probes and DNA. Structural fatigue, because of induced membrane potential and mechanical stress, causes primary effect on microbial inactivation. Material flow after the loss of integrity of cellular membrane by the electric field, local heating and membrane stress and cell swelling or shrinking and disruption due to the unbalanced osmotic pressure between the cytosol and external medium are included in the secondary and tertiary effects, respectively (Bryant and Wolfe 1987, Chang and Reese 1990). Under normal conditions, cell membrane acts as an insulator to the cytoplasm due to its six- or eight-time lower electrical conductivity than that of cytoplasm. When a cell is exposed to electrical fields, positive and negative electrical charges are accumulated in the cell membrane forming transmembrane potential. The charges attract each other and generate compression pressure, which causes the membrane to decrease in thickness. If electrical field strength increases beyond a critical transmembrane potential, it causes pore formation in cell membrane (electroporation).

When transmembrane potential is approximately 1 V, cell lysis with loss of membrane integrity occurs (Sale and Hamilton 1968). Depending on the pulse duration time, number of pulses and PEF treatment temperature, this critical electrical potential varies (Zhang et al. 1994, 1995). Studies with different microorganisms treated by PEF have demonstrated the

disruption of organelles and lack of ribosomes, leading to microbial inhibitions (Harrison et al. 1997). For example, structural damage occurred in *Staphylococcus aureus* cells after PEF treatment (64 pulses with 20, 30 and 40 kV cm^{-1}) suspended in simulated milk ultrafiltrate (SMUF) observed by electron microscopy indicated that cells exhibited rough surfaces, and the ones treated under more severe conditions showed small holes in the membrane and leakage of cellular contents (Pothakamury et al. 1997). The inactivation mechanism of thermal treatment and PEF are different, since heat treatment (i.e., 66ºC for 10 min) causes a great damage of the cell organelles, and cell-wall rupture as induced by PEF treatments is not observed (Pothakumary et al. 1997).

Applied electrical field is transmitted to food samples through the ions. Thus, PEF is mostly applied to high acid foods such as fruit juices which have higher hydrogen ions content and higher electrical conductivity to transmit applied electrical field strength. Moreover, compared to neutral pH, increased acidity provides more microbial inactivation. Composition of the food samples is also important for efficiency of PEF treatment. Air and fat are not good electric conductors; therefore, food samples which have lower fat and protein contents are more prone to be successfully processed by PEF. Fat particles of foods, especially milk, seem to protect the bacteria against applied electric pulses (Grahl and Märkl 1996). The inactivation of *Escherichia coli* using PEF is more limited in skim milk than in buffer solution when exposed to similar treatment conditions of field intensity and number of pulses due to the complex composition of skim milk (i.e., lower electrical resistance and the presence of proteins in the latter) (Martin et al. 1997).

Microbial inactivation by PEF also depends on the nature and physical state of the microorganisms. The size of the cell is an important factor for inactivation by PEF. As the size of the cell gets bigger, the chance of inactivation by PEF gets higher. Therefore, yeast cells are easier to be inactivated by PEF than bacteria. Similarly, vegetative cells are less resistant to PEF than spores. In fact, much higher electric field strengths in combination with heat should be applied to inactivate spores. Moreover, growth stage of the microorganisms also affects the level of inactivation. In general, the cells in steady state are more resistant to PEF than those in lag, exponentially growth, and exponentially decreasing phases.

Inactivation of *Listeria innocua* and *Pseudomonas fluorescens* in pasteurized skim milk by PEF at 50 kV cm^{-1} electric field strength up to 200 μs treatment time resulted in 2.6 and 2.7 log cfu ml^{-1} reductions, respectively (Fernandez-Molina 2001). When raw milk inoculated with *S. aureus* and coagulase-negative *Staphylococcus* spp. was treated with PEF, 4 and 2 log cfu ml^{-1} reductions in the counts of bacteria were obtained, respectively. On the other hand, PEF processing under the same conditions did not effectively inactivate *Corynebacterium* spp. or *Xanthomonas maltophilia* (Raso et al. 1999).

PEF processing parameters also play important role on the efficacy of microbial inactivation. There is a positive relationship between applied electric field strength and microbial inactivation. Similarly, with the increase in the treatment time, rate of microbial inactivation also increases (Peleg 1995). Treatment time is calculated by the number of pulses applied and the duration of each pulse. Thus, increase in pulse width causes increase in treatment time. Shape of the electric field affects the rate of microbial inactivation. Generally bipolar pulses are more effective than monopolar pulses (Zhang et al. 1995). The square wave pulses due to the minimization of the energy absorption in foods are more effective than both exponentially decay and oscillatory pulses for inhibiting microorganisms (Knorr et al. 1994, Zhang et al. 1994).

Microbial inactivation is also related to the treatment temperature during PEF processing. Pothakamury et al. (1996) reported that inactivation rate of *E. coli* inoculated into SMUF processed by PEF in a range of temperatures from 3 to 40ºC increased with the temperature. In addition, PEF can be combined with heat treatment to increase the inhibitory effect of both preservation methods. Combination of PEF with heat treatment at 50ºC for the inactivation of *Listeria monocytogenes* Scott A in pasteurized whole, 2% fat, and skim milk caused 4 log cfu ml^{-1} reduction in counts, while at 25ºC the reduction in the number of this bacteria was far lower (i.e., 1 to 3 log cfu ml^{-1}) (Reina et al. 1998).

13.2.1 Processing of dairy products by pulsed electric fields

Processing of milk samples by PEF is mostly carried out to determine the effect of PEF on different microorganisms that are likely present in milk. One of the earlier studies was conducted to inactivate *Salmonella* Dublin in homogenized milk by using PEF with 36.7 kV cm^{-1} and 40 pulses over a 25 min time period. Under these conditions, the target bacteria was completely inactivated in the samples stored at 7 to 9ºC for eight days; however, a 3 log reduction was obtained in *E. coli* cells under the same conditions (Dunn 1996). Population of indigenous milk microflora increased to 10^7 cfu ml^{-1} in the untreated milk; whereas, the PEF-treated milk had a bacterial load of approximately 4×10^2 cfu ml^{-1} (Dunn and Pearlman 1987). The effects of PEF on the microbial load of milk and milk products are summarized in Table 13.1.

The shelf-life of the PEF-treated (40 kV cm^{-1}, 30 pulses, and 2 µs pulse width using exponential decaying pulses) raw skim milk (0.2% milk fat) was extended to two weeks at 4ºC (Fernandez-Molina et al. 2000, 2005), and processing of raw milk with 2% milk fat by PEF with an electric field of 40 kV cm^{-1} provided a shelf-life of two weeks at refrigeration temperature (Qin et al. 1995). PEF processing (30.76 to 53.84 kV cm^{-1} electric field

Table 13.1 Effect of PEF treatment on the inactivation rate of selected pathogens in milk and milk products.

Product	Target microorganism	Processing conditions	References
Homogenized milk	*Salmonella* Dublin (full reduction) *Eschericia coli* (3 log reduction)	36.7 kV cm^{-1}, 40 pulses, 25 min	Dunn (1996)
Raw skim milk	*Listeria innocua* (4.3 log reduction)	30 and 40 kV cm^{-1}, 1–30 pulses, 20–72ºC, <10 s. Best balance of inactivation was achieved at 55ºC with 40 kV cm^{-1}	Guerro-Beltran et al. (2010)
Raw skim milk	*Listeria innocua* (2.5 log reduction)	30, 40 or 50 kV cm^{-1}	Calderon-Miranda et al. (1999)
Raw skim milk	*Listeria innocua* (2.0, 2.7 and 3.4 log reductions)	30, 40 or 50 kV cm^{-1} plus 10 IU nisin application	Calderon-Miranda et al. (1999)
UHT milk	*Geobacillus stearothermophilus* (3 log reduction)	60 kV cm^{-1}, 26–210 µs	Shin et al. (2007)
UHT milk	*Pseudomonas fluorescens*, *Bacillus cereus*, *Lactococcus lactis* (0.3–3.0 log reductions)	35 kV cm^{-1} with 64 pulses of bipolar square wave for 188 µs	Michalac et al. (2003)
UHT milk (whole)	*Pseudomonas* isolates (complete inactivation)	31 kV cm^{-1}, 20 µs, 55ºC	Craven et al. (2008)
Cheese whey	*Listeria innocua*, (3.0–5.0 log reductions) *Zygosaccharomyces bailii* (7.9–8.8 log reductions)	40 kV cm^{-1}, 4937 µs PEF plus UV (with 7.7 s, 229 mJ ml^{-1} dosage)	Dave et al. (2012)

strength and 12, 24 and 30 pulse numbers) of skim milk (SM) and whole milk (WM) in combination with mild heating (20, 30 and 40ºC) led to small variations in physicochemical properties of both milks after processing. While psychrophilic bacteria grew faster in both SM and WM, the growth of mesophilic bacteria was delayed in both milks after PEF processing, showing up to 6 and 7 log cfu ml^{-1} growths after 25 days of the storage at 4ºC, respectively (Bermudez-Aguirre et al. 2011).

The effect of PEF on physical and biochemical properties of milk samples as well as changes in some bioactive compounds have been also investigated. PEF processing with two steps of seven pulses each and one step of six pulses with an electric field of 40 kV cm^{-1} provided no apparent changes in physical and chemical properties of milk, and no significant difference in sensory attributes between heat pasteurized and PEF-treated milk was observed (Qin et al. 1995). PEF treatment of UHT skim milk inoculated with *P. fluorescens, Lactococcus lactis* and *Bacillus cereus* caused no

changes in total solids and protein levels, and color, pH, particle size and conductivity values (Michalac et al. 2003). Shin et al. (2007) demonstrated that PEF treatment did not affect the pH and titration acidity of milk samples.

Viscosity of PEF-treated milk decreased and coagulation properties were enhanced at high electric field levels (45–55 kV cm^{-1}) with 2.1 to 3.5 µs treatment time (Floury et al. 2006). Few studies exist on flavored milk processed by PEF with the main concern of product stability. After PEF processing at 40 kV cm^{-1} with 48 pulses (2.5 µs, 55ºC), only minor changes were observed in color, Allura Red concentration, and pH. Color of the samples showed significant decreases in a*, and hue angle and Chroma values of flavored milk during storage. However, PEF affected the stability of Allura Red in milk when additional ingredients were not added to the product (Bermudez-Aguirre et al. 2010).

The effect of PEF processing on milk micronutrients such as B group vitamins, cholecalciferol, tocopherol, ascorbic acid and vitamin A showed that approximately 90% of the ascorbic acid presented in milk was retained after PEF treatment, and the other vitamins remained unaltered at electric fields between 1.8 and 2.7 kV mm^{-1}. It was also observed that the macronutrients, including fat content and protein integrity, were unaffected at field strengths as high as 80 kV cm^{-1} (Deeth et al. 2007). PEF processing of milk with 36.7 kV cm^{-1} electric field strength and 40 pulses over a 25 min time period resulted in less flavor degradation and no chemical or physical changes in the quality attributes of milk used in cheese-making (Dunn 1996).

Inactivation of alkaline phosphatase by HTST pasteurization or of lactoperoxidase by high heat treatments is the principal indicator of the efficacy of these treatments to milk (Walstra et al. 2006). Therefore, inactivation of alkaline phosphatase and other enzymes (i.e., plasmin, peroxidase, microbial proteases and lipases) by PEF treatment has been tested in many studies (Elez-Martínez and Martin-Belloso 2007, Sampedro et al. 2005). Application of 2.2 kV mm^{-1} electric field strength caused the inactivation of alkaline phosphatase up to 60% in raw milk (Castro et al. 2001). Although results about the effects of electric fields on proteins especially on enzymes are controversial, generally they include the association or dissociation of functional groups, movements of charged chains, and changes in alignment of helices (Tsong and Astunian 1986). For example, alkaline phosphatase molecules treated by PEF at 22.3 kV cm^{-1} electric field strength with 0.78 ms pulse width tended to associate and aggregate. It was reported that the polarization created by electrical charges of dipoles on the enzyme could cause the aggregate formation. The polarization leading to the aggregation of the enzyme was proposed as the mechanism of the inactivation of alkaline phosphatase by PEF (Castro et al.

2001). PEF processing at 45 kV cm^{-1} with 50 pulses resulted in reduction of plasmin activity by 90% in a SMUF (Vega-Mercado et al. 1995). Inactivation of lipase, peroxidase and alkaline phosphatase has been reported as 65, 25 and <5%, respectively, after the PEF treatment of 21.5 kV cm^{-1} electric field strength with 400 kJ l^{-1} energy (Castro et al. 2001, Grahl and Märkl 1996).

Although most of the studies have focused on PEF processing of milk, the possible applications of PEF in dairy processing have been also investigated in a few studies. For example, PEF processing of yogurt caused about 2 log cfu g^{-1} reductions in counts of *Lactobacillus brevis, Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Saccharomyces cerevisiae* (Dunn and Pearlman 1987). PEF and heat processing (at 60ºC for 30 s) of formulated yogurt-based drink caused no significant differences in L, a, b values, ºBrix, pH and selected sensory attributes between the control and the treated samples. Microbial counts of the samples treated with the combination of heat (at 60ºC) and PEF were lower than that of the control samples stored at 4 or 22ºC (Evrendilek et al. 2004).

Dairy products treated with PEF have similar sensory properties to those that are heat-treated and have good consumer acceptability (Sobrino-Lopez et al. 2009, Sampedro et al. 2005). Cheddar cheese produced from PEF-treated milk had better flavor profile than that of the cheese samples made from milk pasteurized at 63ºC for 30 min. Hardness and springiness values of the cheese made from PEF-treated milk increased while the other textural attributes (i.e., adhesiveness and cohesiveness) remained unchanged (Sepulveda-Ahumada et al. 2000).

The level of proteolysis of the cheese curd made from milk treated with PEF (2 μs pulse width, 2 Hz pulse frequency and up to 120 pulses) was lower than those made from raw milk but higher than that made from pasteurized milk. It has been recently reported that the rennet coagulation time of PEF-treated milk was increased by 10% (Garcia-Amezquita et al. 2013).

13.3 High pressure processing

High pressure processing (HPP), also called as high hydrostatic pressure (HHP) or ultra high pressure (UHP), involves application of elevated pressures with or without external heating to obtain microbial inactivation or to alter food attributes (Farkas and Hoover 2000). Although history of pressure application goes back to the late 1800s (Hite 1899), the industrial use of this technology—parallel to the developments of engineering—has relatively recently developed (Mertens and Knorr 1992). Pressure can be applied at ambient temperature, thus thermally induced cooked off-flavors can be eliminated. Finally, this technology can be used to process both liquid and solid foods in batch and semi-continuous equipment. During HHP processing, pressure is transmitted to food sample very rapidly and

uniformly thus, pressure application is not dependent on the size and shape of the product which provides homogenous processing of irregular shaped food products (Tewari 2007, Balasubramaniam et al. 2008, Smelt 1998).

Effect of HHP on food products can be explained by Le Châtelier's Principle. According to the principle, system always acts to oppose changes in chemical equilibrium; to restore equilibrium, the system will favor a chemical pathway to reduce or eliminate the disturbance so as to restabilize at thermodynamic equilibrium. It also states that "the application of pressure shifts the system equilibrium toward the state that occupies the smallest volume" (Farkas and Hoover 2000, Pfister and Dehne 2001). The effect of HHP is also explained by isostatic principle which illustrates the transmission of pressure to food samples. The details of the working principle and basic components of HHP system are discussed in Balasubramaniam et al. (2008), Farkas and Hoover (2000), Hogan et al. (2005), US-FDA (2000) and Karim (2011).

Microbial inactivation by HPP has drawn a great attention of food industry. Thus, both vegetative and spore forms of bacteria, molds, yeasts and even viruses have been studied in both model and food systems. The processing conditions such as initial sample temperature, water temperature circulating in pressure level, pressurizing medium and holding time not only influence the level of inactivation but also affects the nutritional and sensory characteristics of foods (Zhang et al. 2011).

High-pressure treatments are generally effective in inactivating most vegetative pathogenic and spoilage microorganisms at pressures ranging between 200 and 600 MPa with temperatures at or below ambient temperature (Patterson 2005). HHP causes damage in cell membrane integrity (Pagán and Mackey 2000) as well as ribosomal destruction (Niven et al. 1999), enzyme inactivation (Degraeve et al. 1996, Simpson and Gilmour 1997), inactivation of membrane-bound transport systems (Ulmer et al. 2002), and damage to the proton efflux system (Wouters et al. 1998). Dramatic changes of the cell structure exposed to HHP such as the collapse of intracellular vacuoles at relatively mild pressures (i.e., 500 MPa), separation between the cell-wall and cytoplasmic membrane, and ribosomal destruction occur (Ritz et al. 2001, Kaletunc et al. 2004). The damages in the cell morphology cause the impairment of cell functions, slowing growth rate or causing cell death. Damages in the cell membrane caused by HHP result in the leakage of intracellular components and loss of homeostasis (Farkas and Hoover 2000). HHP treatment causes an increase in extracellular levels of adenosine triphosphate (ATP) (Smelt et al. 1994) and an increase in the uptake of propidium iodide (Ulmer et al. 2000) and ethidium bromide (Benito et al. 1999), indicating loss of membrane permeability and function.

Microbial cell death occurs depending on the level of the injuries caused by HHP (Fig. 13.1) (Masschalck et al. 2000, Smelt 1998, Tay et al. 2003). Generally, Gram-positive bacteria are more resistant to environmental stresses such as heat and pressure than Gram-negative bacteria, and cocci are more resistant than rod or spirochete-shaped bacteria (Alpas et al. 1999, Patterson 2005, Pilavtepe-Celik et al. 2008, Ritz et al. 2002, Smelt 1998). Moreover, different level of inactivation of strains of the same bacteria can be obtained under the same processing conditions. Processing of Cheddar cheese inoculated with *E. coli* O157:H7 and *L. monocytogenes* Scott A by HHP indicated that *E. coli* was more baroresistant, with decimal reduction values (D-values) of 14.5 and 3.6 min at 300 MPa and 25ºC, respectively (Shao et al. 2007).

HHP application of 500–600 MPa at 25ºC for 10 min required to inactivate Gram-positive microorganisms, while Gram-negative microorganisms were inactivated at relatively lower pressures under the same time and temperature conditions (Smelt 1998). It is thought that temperature including adiabatic heating during HPP can have a significant effect on microbial survival (Farkas and Hoover 2000). Moderate pressure treatments and shorter pressure holding times when combining with high temperatures can end up with greater anti-microbial impact; however, pressure application at high temperatures can lead to undesirable effects in certain cheese quality parameters. For example, pressure application at 50ºC resulted in higher volume of whey losses and unacceptable textural characteristics in the cheese (Shao et al. 2007).

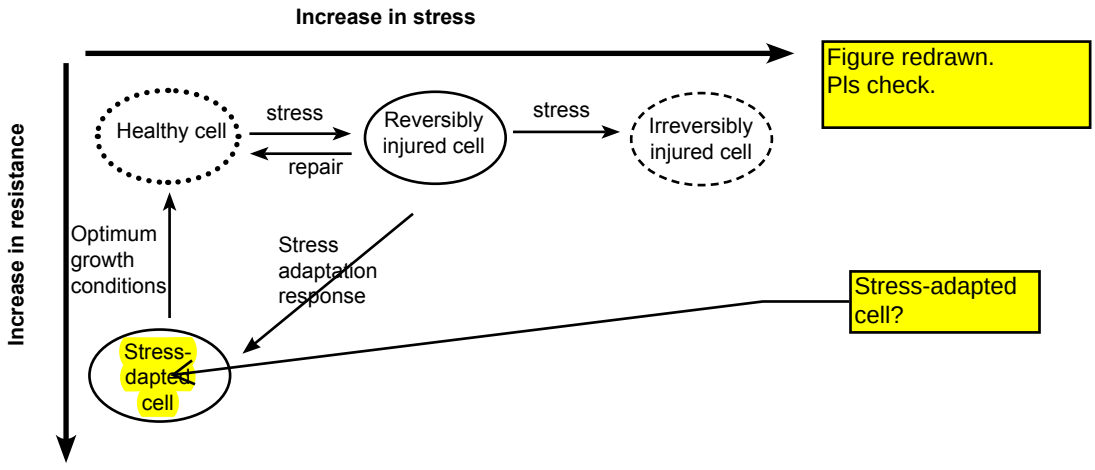

Figure 13.1 Microbial inactivation mechanism by HPP. After: Lado and Yousef (2002).

Anti-microbial effect of HPP is largely influenced by the pH, water activity or chemical composition of foods. The barotolerance of the microorganisms is getting higher as the growth medium gets richer, but microorganisms injured by pressure are generally more sensitive to low water activity. For example, carbohydrates are generally more protective than salts, and recovery of pressure treated cells is much lower when 2% salt is added to the medium (Patterson et al. 1995, Oxen and Knorr 1993). The net effect of lower water activity is not always easy to predict; however, the protective effect of different carbohydrates against high pressure is same as the protective effect of different carbohydrates on the membrane during freezing and it is in the order of fructose < glucose < sucrose < trehalose (Smelt et al. 1997).

Pressure resistance (at 483 MPa for 30 min) of *Bacillus subtilis* inoculated into milk changed as pH of milk raised to 8 (Timson and Short 1965). Pressure tolerance or resistance of bacteria can change from species to species and even may also be different in a single species grown under different conditions or in different growth media (Hoover et al. 1989). The growth phase of microorganisms also plays a role in determining their pressure sensitivity and inactivation. Cells in the lag or stationary phase of growth are generally more pressure resistant than those in the log or exponential phase; this is probably due to the fact that the cellular membrane is more robust, and stress induced genes that synthesize proteins that protect against stress conditions, can be turned on more readily in the stationary phase cells (Alpas et al. 1999, Bowman et al. 2008, McClements et al. 2001, Manas and Mackey 2004). The effect of HPP on the stationary and exponential phase cell membrane of *E. coli* reveals that the increased sensitivity to pressure of exponential phase cell membranes is linked to physical perturbations of the cell envelope, loss of osmotic responsiveness, and exclusion of intracellular proteins and RNA (Manas and Mackey 2004). Moreover, loss of viability which is related to a permanent loss of membrane integrity in exponentially phase cells and leaky cell membrane has also been reported. However cells in the stationary phase are able to reseal partially or wholly after decompression (Pagán and Mackey 2000). Reduced activity of the integral membrane protein F0F1 ATPase in *Lactobacillus plantarum* in addition to impairment of acid reflux and maintenance of intracellular pH was also reported after pressure application at 250 MPa (Wouters et al. 1998).

Inactivation of *L. monocytogenes*, *B. cereus* and *P. fluorescens* in UHT milk treated by HHP presented that exponential phase cells were significantly less resistant to pressure than stationary phase cells for all of the three species studied. Growth temperature was found to have a significant effect at two growth stages studied. Exponential cells grown at 8ºC were more resistant than those grown at 30ºC, but the reverse was true for the

stationary phase cells. *B. cereus* stationary phase cells grown at 30ºC were the most pressure resistant, and *L. monocytogenes* showed the most sub-lethal damage compared to *B. cereus* and *P. fluorescence* (McClements et al. 2001). Inactivation of *L. monocytogene*s Scott A processed by HHP in UHT whole milk revealed that elevated temperatures substantially promoted the pressure-induced inactivation of *L. monocytogenes* (Chen and Hoover 2003). Pressure inactivation (400 MPa at 20–25ºC) of *L. monocytogenes* in the mid-stationary phase of growth at 4, 15, 25, 35 or 43ºC, inoculated into whole UHT milk showed that both growth temperature and plating medium had a significant effect on the inactivation of stationary phase *L. monocytogenes* by HPP. It was also reported that cells of *S. aureus* ATCC 6538 and *E. coli* K-12 in the exponential phase of growth were more sensitive to HPP in Cheddar cheese slurry than cells in the stationary phase (O'Reilly et al. 2000a).

Compared to vegetative cells, endospores are far more resistant against high pressure, requiring treatment at pressures exceeding 1000 MPa and temperature of more than 80ºC for full inactivation (Abee and Wouters 1999, Rastogi et al. 2007, Smelt 1998). Thus, high pressure processing is proved to inactivate bacterial spores more effectively when used in combination with heat (Ahn et al. 2007, Black et al. 2007, 2011, Heinz and Knorr 2002, Smelt 1998, Wuytack et al. 1998), and spores germinated at lower pressures are in turn more sensitive to subsequent pressure treatments (Setlow et al. 2001). *B. cereus* spores are more resistant to pressure than vegetative cells, and pressure treatment at 400 MPa for 25 min at 30ºC provided about 0.45 log cfu m^{-1} reduction in spores of this bacterium. Pressure treatment at 8ºC induced significantly less spore germination than 30ºC (McClements et al. 2001).

Most vegetative yeast and molds are inactivated within a few minutes by 300–400 MPa at 25ºC. However, heat-resistant molds such as *Byssochlamys*, *Neosartorya* and *Talaromyces* are generally considered to be extremely resistant to high pressure (Voldrich et al. 2004). Studies have shown that virus inactivation by pressure is due to the denaturation of capsid proteins essential for host cell attachment to initiate infection (Buckow et al. 2008, Khadre and Yousef 2002, Kingsley et al. 2002).

13.3.1 High pressure processing of dairy products

Most of the studies with milk processed by HHP have performed for the inactivation of pathogenic and spoilage microorganisms. Depending on the microbiological quality of milk, processing at 400–600 MPa was comparable to that of heat pasteurization (72.8ºC, 15 s) (Buffa et al. 2000, 2001, Kolakowski et al. 1997); however, it was not comparable to sterilized milk due to the resistance of spores to high pressure. Effects of HPP on milk can be summarized as disruptive to the casein micelles and the structure of

whey proteins, shifting the mineral balance in milk, inducing crystallization of milk fat, increasing the pH of milk, reducing the turbidity of milk, reducing the rennet or acid coagulation time of milk, and increasing cheese yield (Considine et al. 2007, Huppertz et al. 2002, Lopez-Fandino 2006, Trujillo et al. 2002). The disintegration of casein micelles is accompanied by increases in caseins and calcium phosphate levels in the diffusible or serum phase of milk and by decreases in both non-casein nitrogen and serum nitrogen fractions, suggesting that the whey proteins become sedimentable by centrifugation and precipitable at pH 4.6 (Law et al. 1998). High-pressure treatment markedly increases the transfer of individual caseins from colloidal to soluble phase of milk (Lopez et al. 1998), and dissociation of individual caseins in milk by high pressure is dependent on pH (Arias et al. 2000, Huppertz et al. 2002).

β-lactoglobulin is one of the pressure sensitive proteins, being easily denatured under the pressure treatment up to 500 MPa at 25ºC. On the other hand, denaturation of immunoglobulins and α-lactalbumin occurs only at much higher pressures and particularly at 50ºC (Schrader et al. 1997). HPP not only causes solubilization of colloidal calcium phosphate, but also resolubilizes insoluble heat-induced crystalline calcium phosphate (i.e., in UHT milk) (Felipe et al. 1997).

Some modifications in size and distribution of milk fat globules were observed on ewe's milk treated by HHP up to 500 MPa (Gervilla et al. 2001). No damage occurred on the milk fat globule membrane, being proved by the lack of increased lipolysis. Lactose in milk and milk products may isomerize in lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds were observed after pressurization (100–400 MPa for 10–60 min at 25ºC), suggesting that no Maillard reaction or lactose isomerization occur in milk after pressure treatment (Lopez-Fandino et al. 1996). HHP induces the crystallization of milk fat during pressure treatment of cream (Buchheim et al. 1996). No increase in the products of lipolysis was observed following treatment of milk with pressures ranging from 400 to 800 MPa (Buchheim et al. 1996).

Enzyme inactivation by high pressure is more difficult than microorganisms, since enzymes in milk are more resistant to high pressure treatment (Huppertz et al. 2004a). Studies with enzyme inactivation reveal that alkaline phosphatase, lactoperoxidase, phosphohexose-isomerase and γ–glutamyltransferase are resistant to pressures lower than 400 MPa at 25ºC (Lopez-Fandino 1996, Rademacher et al. 1998, Seyderhelm et al. 1996). On the contrary, lactate dehydrogenase activity was reduced substantially at 206 MPa (Kouassi et al. 2007). Rennet coagulation time of milk treated with high pressure at >300 MPa was increased, while at lower pressures the opposite was observed (Buffa et al. 2001, Lopez-Fandino et al. 1996).

The small molecules such as vitamins, amino acids, simple sugars and flavor compounds are not affected by HPP, because contrary to thermal treatments where covalent as well as non-covalent bonds are affected, HHP at room and mild temperatures only disrupts relatively weak chemical bonds (i.e., hydrogen and ionic bonds, and hydrophobic effect) (Cheftel 1992). Therefore, HPP of milk at 400 MPa (at a rate of 2.5 MPa s^{-1} for 30 min at 25°C) resulted in no significant loss of vitamins B_1 and B_6 (Sierra et al. 2000).

Effect of HPP on cheese indicated that high pressure ranging from 200 to 600 MPa results in cheese with acceptable quality and increased yield (Drake et al. 1997, Huppertz et al. 2002, Lopez-Fandino 1996, Trujillo et al. 2002). No significant difference was noted in the flavor profile of Cheddar cheese made from pasteurized or high-pressure treated milk; however, HPP-treated milk produced a pasty, weak texture attributed to the presence of whey proteins and greater moisture content (Drake et al. 1997). With the increase in pressure, the volume of separated whey is reduced.

Application of high pressure can reduce the variability of the moisture content existing within or between cheese blocks and can create new texture in cheese (Torres-Mora et al. 1996). Effect of pressure application on cheese ripening in Gouda-type cheese revealed that salt uptake increased as the pressure and processing time increased from 100 to 400 MPa and from 0.5 to 4 hr, respectively. Disruption of *para*-casein network by HHP at 300 MPa for 5 min led to increase in the concentrations of peptides and proteins in whey (Messens et al. 1998).

Treatment of Turkish white cheese with high pressure from 50 to 600 MPa for 5 or 10 min at 25°C resulted in decreases in the counts of *L. monocytogenes*, total aerobic mesophilic bacteria, molds and yeasts, *Lactococcus* spp. and *Lactobacillus* spp. (Evrendilek et al. 2008). No changes were reported in the pH and water activity of the cheeses treated with high pressure. Gallot-Lavalle (1998) demonstrated that the counts of *L. monocytogenes* in goat's milk cheese made from raw milk processed at 450 MPa for 10 min or at 500 MPa for 5 min decreased more than 5.6 log cfu g^{-1} without significantly affecting the organoleptic characteristics of the end product. Processing of vacuum packaged and salted curds with pressure at 400 MPa for 10 min at 2°C yielded cheese with very low levels of contaminant flora and little modifications of rennet or plasmin activities (Trujillo et al. 2000). Initial number of *E. coli* in cheese samples treated at 400 MPa at 25°C for 5 min was reduced by 7 log cfu g^{-1} and it was not detected after one week at 4°C (Capellas et al. 1996). Combined treatment of nisin and high pressure was found to be the most effective way in extending the shelf-life of the cheese samples, because nisin mainly acted over the sporulated population (Ray 1992), while the combined treatment inactivated a fraction of the microbial

population that is resistant to both treatments when applied separately (Hauben et al. 1996, Kalchayanand et al. 1994).

No viable cells of *E. coli* O59:H21 and O157:H7 were detected in washed-curd cheese treated at 500 MPa (De Lamo-Castellvi et al. 2005, 2006). Pressure applications at both 300 and 400 MPa to cheese (pH about 4.82) caused complete inactivation of S. Enteritidis CECT 4300 and S. Typhimurium CECT 443 with no recovery following 15 d of storage at 12ºC (De Lamo-Castellvi et al. 2007). Inactivation of seven hemolytic strains belonging to serotype 1/2a of *L. monocytogenes* in Gorgonzola cheese rind by pressure treatment at 400 to 700 MPa for 1 to 15 min at 30ºC ended up with strong resistance of the microorganism to pressures up to 500 MPa; however, pressure treatment at 700 MPa for 15 min led to a 5 log cfu g^{-1} reduction in *L. monocytogenes* counts (Carminati et al. 2004). HPP at 450 MPa for 10 min or 500 MPa for 5 min at 11ºC caused 5.6 log cfu g^{-1} reductions of *L. monocytogenes* F13 in 14 day-old Sainte Maure de Touraine cheese (Gallot-Lavalle 1998). Pressure application of 400 MPa for 5 min yielded 3 log cfu g^{-1} reductions in starter flora (Saldo et al. 2000a). It was found that acidification capacity of lactic acid bacteria was reduced after pressure treatment at 400 MPa, even without changes in cell viability (Casal and Gomez 1999). Table 13.2 shows the effect of HHP on pathogenic microorganisms in cheese.

Previous studies have revealed that HPP is able to accelerate cheese ripening by causing alterations in enzyme structure, conformational changes in the casein matrix making it more susceptible to the action of proteases, and/or bacterial lysis enhancing the release of microbial enzymes that promote biochemical reactions (Garde et al. 2007, Martinez-Rodriguez et al. 2012, Messens et al. 1998, O'Reilly et al. 2000b, 2003, Saldo et al. 2000b, 2002, Voigt et al. 2010).

Pressure induced bacterial lysis is strain dependent and affects aminopeptidase activity (Juan et al. 2007, Malone et al. 2002). When pressure was applied at 300 MPa for 10 min at 12ºC, the autolysis of cheese starter bacteria (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) was higher in ewes' milk cheese at the beginning of ripening than that at day 15 (Juan et al. 2008).

Treatments below 150 MPa resulted in the greatest increase in the pH 4.6 SN/TN ratio in cheese; however, total free amino acids (FAA) production decreased as pressure increased from 100 to 400 MPa, and increased processing time up to 60 hr raised total FAA levels (O'Reilly et al. 2000b). Processing of 1 and 4 month-old commercial cheeses with pressures ranging from 200 to 800 MPa for 5 min at 25ºC showed that pressure treatments higher than 400 MPa could be useful for controlling cheese ripening without any textural changes (Wick et al. 2004). Rynne et al. (2008) reported that processing of one day-old full-fat Cheddar cheese pressure-treated at 400 MPa for 10 min at room temperature slowed down ripening effectively.

Table 13.2 Effect of high hydrostatic pressure on the inactivation of some pathogenic bacteria in cheese.

Product	Target microorganisms	Treatment conditions	References
Washed curd cheese	*Yersinia enterocolitica* CECT 4055 (serotype O3) CECT 559 CECT 4054	Strain CECT more barotolerant at 300 MPa	De Lamo-Castello et al. (2005)
Washed curd cheese	*Listeria monocytogenes* NCTC 11994 Scott A	Strain NCTC 11994 was more sensitive to HPP at 400–500 MPa for 10 min	Lopez-Pedemonte et al. (2007a)
Swiss cheese slurry	Coliforms, yeasts, molds, presumptive coagulase-positive *Staphylococcus*, starter lactic acid bacteria	The counts of all microbial groups reduced greatly at 345–550 MPa for 10–30 min	Ding et al. (2001)
Gouda cheese	*Aeromonas hydrophila*	D-values of 32.05, 12.97 and 2.43 min at 100, 200 and 300 MPa at 50ºC, respectively.	Fonberg-Broczek et al. (2005)
Washed curd cheese	*Staphylococcus aureus* CECT 4013 ATCC 13565 Staphylococcal enterotoxin A (artificially inoculated)	Rate of inactivation increased with increasing pressure from 300 to 500 MPa	Lopez-Pedemonte et al. (2007b)
Mato cheese	*Staphylococcus carnosus* 4491	No remarkable decrease in counts of bacteria at 500 MPa for 30 min at 10 or 25ºC. 7 log reduction at the same pressure at 55ºC for 5 min	Capellas et al. (2000)

Moderate HPP conditions (50 to 150 MPa) applied to young Cheddar cheese may be effective in accelerating proteolysis; whereas, higher HPP conditions (≥400 MPa) may help cheese manufacturers arresting the ripening process at a desired stage, thus maintaining optimum "commercial attributes" for a longer period. Pressure treatments to commercial Irish Cheddar cheese at 50 MPa for 72 hr at 25ºC caused significant decrease in the level of α_{s1}-casein and increase in the accumulation of α_{s1}-I-casein in cheese during ripening (O'Reilly et al. 2003). On the other hand, pressure treatments performed between 350 and 400 MPa reduced the rate of accumulation of α_{s1}-casein degradation products significantly (O'Reilly et al. 2003). Accelerated proteolysis as a result of HPP has also been achieved in other cheese varieties such as smear and mold-ripened cheese, Garrotxa, and ewes' milk cheese, but not in Gouda, Edam, or Mozzarella cheese (Martinez-Rodriguez et al. 2012). The effects of HPP on cheese proteolysis vary depending on the cheese type and ripening conditions (Voigt et al. 2010), and the effect of HHP applied directly to cheese on the activity of proteolytic enzymes of secondary cultures is not well known (Bansal et al. 2007, 2009).

Studies related to whey proteins—especially with β-lactoglobulin—show that HPP at 200 MPa causes enhancement of proteolysis (Hayashi et al. 1987). Compared to native form of the β-lactoglobulin, pressure application at 300–900 MPa reduces the foamability of β-lactoglobulin (Pittia et al. 1996). Pressures between 200 and 900 MPa caused irreversible molecular modifications in tertiary structure, surface hydrophobicity and association state of β-lactoglobulin (Belloque et al. 2000, Yang et al. 2003). α-lactalbumin is more baroresistant than β-lactoglobulin in both milk and whey, and both proteins—when treated by pressure in whey—are more resistant than that of in milk (Huppertz et al. 2004b).

Yogurts made from high-pressure-treated milk are coagulated at a higher pH, and have similar physical properties (i.e., firmness and water-holding capacity) to yogurts made from heat-treated milk (Ferragut et al. 2000, Harte et al. 2002). Increasing pressure treatment causes an increase in the firmness of the yogurt made from high pressure treated milk and the firmness of the yogurt has the maximum value when milk is treated with HPP at 55ºC compared to the milk treated at 10 or 25ºC (Needs et al. 2000, Ferragut et al. 2000).

Although studies related to HPP of different dairy products such as yogurt drink (ayran) is very limited, influences of HPP on some physical properties of ayran as well as inactivation of *L. monocytogenes* and *L. innocua* in this product with or without addition of mint essential oil revealed that pressure treatment alone or in combination with mint essential oil did not cause significant changes in pH, water activity, color and whey separation. Addition of mint essential oil enhanced inactivation of both pathogenic bacteria by more than 1 log cfu ml^{-1} (Evrendilek and Balasubramaniam 2011).

13.4 Ultrasonication

Ultrasound (US) defined as the use of pressure waves with a frequency of above 20 kHz, which is undetectable by human ear, also referred to as "power ultrasound", has a potential to be used as food processing method for microbial inactivation (Butz and Tauscher 2002). The use of ultrasound to inactivate microorganisms was reported in the late 1920s (Harvey and Loomis 1929), but due to its limited lethal effect on both spoilage and pathogenic microorganisms, the use of US as a sterilization method is not commonly employed by the food industry (Cameron et al. 2009).

Low or high energy US has a potential to be used in food industry for different purposes (Piyasena et al. 2003). Lower than 1 W cm^{-2} intensities and higher than 2–3 MHz frequencies are used in low energy US, while higher than 1 W cm^{-2} intensities with frequencies between 18 and 100 kHz are used in high energy US. Generally, low energy US is used for non-

invasive purposes such as determination of unwanted foreign matters in raw materials and final products, and characterization of food stuffs (Režek Jambrak et al. 2010). High energy ultrasound is employed in milk homogenization, bacteria and enzyme inactivation (i.e., chymosin) and β-galactosidase extraction (Ashokkumar and Mason 2007, Bosiljkov et al. 2011, Jeličić et al. 2010, Patel et al. 2008, Režek-Jambrak et al. 2009).

Destructive effect of US on bacterial cells stems from the formation and implosion of bubbles in a liquid, what is known as cavitation (growth and collapse of microbubbles) which can produce high localized temperatures, pressures and turbulence (Ashokkumar and Grieser 2007, Brnčić et al. 2010). Since US does not affect a large area on a food surface, it may not efficiently destroy resistant bacteria and spores (Piyasena et al. 2003, Raso et al. 1998). Combination of US with heat treatment known as thermosonication or thermoultrasonication shows better lethal effect on microorganisms. It was demonstrated that combination of ultrasonication (20 kHz and 160 W) with heat (5 to 62°C) resulted in better efficiency regarding time and energy consumption compared to either treatment individually (Ordonez et al. 1984). Similar results were obtained when US was combined with extreme pH and chlorination. Sonication, manosonication, thermosonication and manothermosonication processes have been studied for their efficiencies on microbial inactivation by Manas et al. (2000), McClements (1995), Miles et al. (1995), Ordonez et al. (1984) and Raso et al. (1998).

Several factors such as temperature, pressure, amplitude of the ultrasonic waves, exposure/contact time, volume of food being processed, and the composition of the food are important for efficacy of US processing. Temperature control is provided by dissipating excess heat evolved during US, using cold water circulated through the cooling coil that is placed inside the treatment chamber. Pressure control is monitored by a manometer that is also placed in the treatment chamber.

Microbial inactivation efficacy of US ranges depending on several factors such as cell morphology, pH and viscosity. In general, Gram-negative, rod shape bacteria are more sensitive to US treatment than Gram-positive, coccus-shaped bacteria (Hulsen 1999). However, it was also reported that there was no difference between Gram-negative (i.e., *Pseudomonas aeruginosa* and *E. coli*) and Gram-positive (i.e., *S. aureus* and *B. subtilis*) bacteria regarding US-induced inactivation kinetics (Scherba et al. 1991). According to these results, it is argued that this morphological feature does not seem to be a sole factor for grouping of the organisms based on their resistance against ultrasonic treatment, and instead it is proposed that the target of ultrasonic damage might be the inner (cytoplasmic) membrane, which consists of a lipoprotein bilayer (Villamiel and de Jong 2000). The effect of pH on microbial inactivation reveals that the best inactivation is observed at pHs between 6.8 and 7.1 for milk samples. Effectiveness of

the US for microbial inactivation decreases with increasing viscosity, and increases with raising pressure up to 3000 kPa (Hulsen 1999).

13.4.1 Processing of dairy products by ultrasound

Compared to other novel technologies, studies related to US processing of foods are rather limited. However, the use of US on milk processing can have several advantages such as homogenization of fat globules, removal of gases, and enhancement of anti-oxidant activity (Mason 1998, Villamiel and de Jong 2000). Therefore, continuous-flow ultrasonic treatment can be a promising technique for milk processing.

Application of US with 20 kHz frequency for 10 min caused reduction in *E. coli* counts by 100%. It was reported that viable counts of *P. fluorescens* were reduced by 100% after 6 min and *L. monocytogenes* was reduced by 99% after 10 min. Protein or lactose contents of both raw and pasteurized milk do not change with US, but it may cause an increase in the fat concentration. Unfortunately, US does not cause inactivation of alkaline phosphatase and lactoperoxidase activities (Cameron et al. 2009). Processing of pasteurized homogenized skim milk by ultrasound with 20 kHz at 20 and 41 W under controlled temperature conditions for different time intervals up to 60 min causes no changes in viscosity, but reduces the turbidity. The sizes of the casein micelles, fat globules and soluble particles after 60 min of sonication change with changes in energy generated. Denaturation is observed in milk whey proteins, forming soluble whey protein aggregates. These aggregates further interact with casein micelles to form micellar aggregates during the first 30 min of sonication. Increase in sonication time causes the partial disruption of some whey proteins from these aggregates (Shanmugam et al. 2012).

US treatment of brain heart infusion broth, skimmilk and liquid egg inoculated with S. Typhimurium at 20 and 40ºC for 30 min led to 1 and 3 log cfu ml^{-1} reductions in the counts of this bacterium, respectively (Wrigley and Llorca 1992). The counts of coliform bacteria in milk samples treated with 800 kHz for 1 min with a power intensity of 8.4 W cm^{-2} was reduced by 93%. Combination of UV irradiation with sonication increased the inactivation rate of coliforms to 99% with a possible explanation of that fat globules are broken up by the ultrasound, allowing deeper penetration of the UV, thus resulting in a more efficient process for microbial inactivation (Munkacsi and Elhami 1976).

The combined effect of ultrasonic (20 KHz, 150 W) and heat treatment on the survival of two strains of *Bacillus subtilis* in three suspending media (distilled water, glycerol and milk) revealed that when spores are suspended in water or milk were subjected to ultrasonic waves before heat treatments a little or no decrease of the heat resistance was observed.

However, when both sporicidal agents were applied simultaneously (thermo-ultrasonication), initial number of *B. subtilis*, var. *niger*-40 and *B. subtilis* ATCC 6051 decreased by 63 and 74%, respectively.

13.5 Pulsed ultraviolet light

Although it has been mainly used for surface treatment, there is a growing interest to use ultraviolet (UV) light for food preservation. Studies focused on food preservation by UV light have been mostly performed in recent years even though the earlier studies using UV light as a bactericidal agent goes back to 1828 (Demirci and Krishnamurthy 2011, Sizer and Balasubramaniam 1999). UV processing wavelength ranges from 100 to 400 nm having higher energy than infrared and lower energy than X-rays (Bintsis et al. 2000, Sastry et al. 2000).

The UV light in the range of 220 and 300 nm is considered germicidal against microorganisms such as bacteria, viruses, protozoa, molds and yeasts, and algae (Bintsis et al. 2000, Perchonok 2003, Sizer and Balasubramaniam 1999). The highest germicidal effect is obtained between 250 and 270 nm, but it may decrease as the wavelength is increased (e.g., above 300 nm, the germicidal effect is negated) (Bachmann 1975). Therefore, 254 nm wavelength (UV-C, generated by LPM lamps) is used for disinfection of surfaces, water and some food products. Bacteria suspended in air are more sensitive to UV-C light than those suspended in liquids due to the different penetration capacity of UV light through different physical media (Bintsis et al. 2000).

Energy transfer from UV light to an exposed material is realized by photons. Energy of photons in UV light range is very high and this energy can even cause ionization of molecules, whereas visible light and infrared region cause vibration and rotation of molecules, respectively. Molecules that absorb energy are elevated from the ground state to an excited state. The excited molecule can either (i) relax back to the ground state by releasing the energy as heat, (ii) relax back to the ground state by releasing energy as photons, or (iii) can induce some chemical changes (Demirci and Krishnamurthy 2011).

The effect of UV radiation on microbial inactivation may vary from species to species and, in the same species, may depend on the strain, growth media, stage of culture, number of microorganisms and other characteristics, such as type and composition of the food (Wright et al. 2000). Generally, fungi and yeasts which have bigger cell size are more resistant than bacteria during disinfection; however, high microbial levels should be taken into account when using UV-C for disinfection (Bachmann 1975).

Inactivation of microorganisms by radiation may occur due to the radiation absorbed by DNA which may stop cell growth and lead to cell

death (Liltved and Landfald 2000). Physical shifting of electrons to render splitting of the DNA bonds, delay of reproduction or cell death is caused by the absorbed UV-C light (Anonymous 2002) indicating that the bactericidal effect of UV-C occurs mainly at the nucleic acid level (Wright et al. 2000). UV-C radiation causes a cross-linking between neighboring thymine and cytosine (pyrimidine nucleoside bases) in the same DNA strand. One of the most commonly occurring DNA photoproducts is the cyclobutyl pyrimidine dimers. DNA mutations might be produced due to the cross-linking effects of UV-C irradiation in the injured organism (Sastry et al. 2000). UV-A (315–400 nm) has better penetration capacity than UV-C, and it affects bacterial cells by causing membrane damages and/or generate active oxygen species or H_2O_2 on cell structure (Kramer and Ames 1987). UV-C light only penetrates a very short depth into the surface of liquids other than clear water (Sharma 1999). UV light penetration into juices is about 1 mm for absorption of 90% of the light (Sizer and Balasubramaniam 1999). Intensity of penetration decreases as the level of solids in liquids increases (Bintsis et al. 2000, Sharma 1999). It is reported that in order to ensure an adequate reduction in the counts of microorganisms in milk, it is necessary for all parts of the fluid to be exposed to at least 400 J m^{-2} of UV light at 254 nm (Anonymous 1999).

Because of the structural differences, spore inactivation mechanism by UV light is different from that of vegetative cells (Riesenman and Nicholson 2000). Resistance of spores is induced by the thick protein coat present in spores. The DNA of bacterial spore has a different conformation than those of the vegetative cell DNA, and thus, no detectable amount of thymine-containing dimers are reported in *Bacillus* spores. The main photoproduct is reported as 5-thyminyl-5,6 dihydrothymine adduct; later, it is termed as "spore photoproduct" (Setlow and Setlow 1987).

13.5.1 Processing of dairy products by pulsed UV light

Processing of milk and other dairy products by UV light aims mostly inactivation of pathogenic and spoilage bacteria. The efficacy of pulsed UV light at 5, 8, or 11 cm distance from a UV light strobe with 20, 30, or 40 ml min^{-1} flow rate up to three times by re-circulation for continuous-flow milk treatment caused 0.55 to 7.26 log cfu ml^{-1} reduction in *S. aureus* counts (Krishnamurthy et al. 2007). Processing of raw cow's milk by continuous flow coiled tube ultraviolet reactor for a residence time of 17 s with cumulative UV-C dose of 16.822 mJ cm^{-2} resulted in 2.3 log cfu ml^{-1} reduction in total microbial count with no significant difference on the odor compounds of UV light-treated, untreated and control samples. Although UV-processing caused detectible changes in the odor of milk samples, no significant difference between the malondialdehyde and other reactive substances

were detected between the untreated and UV-treated milk samples right after the treatment and during the storage. It is also reported that fresh or untreated raw milk samples have lower lipid oxidation products than that of UV-treated milk (Bandla et al. 2012).

UV light was less effective for the inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in milk (0.5 to 1.0 log reduction per 1000 mJ ml^{-1}) than that of suspended in Middlebrook 7H9 broth (2.5 to 3.3 log reduction per 1000 mJ ml^{-1}) (Altic et al. 2007). Processing of milk by UV light and heat treatment causes loss of vitamin C. UV light treatment causes a decrease in the concentration of vitamin B_2 after several passes of milk through the UV system, and it also causes decreases in the levels of vitamins A and E. UV light sensitivity of vitamins is in the order of C > E > A > B_2 (Guneser and Karagul Yuceer 2012).

Goat's milk samples processed for 12 consecutive times using a UV fluid processor for a cumulative exposure time of 18 s and targeted UV dose of 15.8 +/− 1.6 mJ cm^{-2} reveal a significant difference between the odor of raw goat's milk and UV irradiated milk. As the UV dose is increased, the oxidation and hydrolytic rancidity of raw milk are increased. UV-C treatment caused more than 5 log cfu ml^{-1} reduction in numbers of *L. monocytogenes* in goat's milk (Matak et al. 2005). Processing of milk by UV dose of 21.3 mJ cm^{-2}, with a flow rate of 28.8 l hr^{-1} and a tube's inner diameter of 1.5 mm caused more than 6 log cfu ml^{-1} reduction in the initial counts of bacteria. A recent study showed that application of the UV dose with 21.3 mJ cm^{-2} caused 3–4 log cfu ml^{-1} reduction in *Salmonella* spp., *Shigella* spp., *L. monocytogenes*, *Staphylococcus* spp., Enterobacteriaceae, LAB, pseudomonads and the total aerobic bacteria (Lu et al. 2011).

Processing of whey protein isolate (WPI) solutions at concentrations of 1% and 5% (w/v) circulated at flow rates ranging from 30 to 800 ml min^{-1} suggests that UV treatment increased the concentration of total and accessible thiol groups in 1% WPI solutions, while no change in 5% WPI solutions was observed. As a result of UV treatment, oxidation products of aromatic amino acids (e.g., N-formylkynurenine and dityrosine) occurred, and protein conformation was changed leading to increased susceptibility of whey protein to hydrolysis by pepsin (Kristo et al. 2012).

13.6 Cold plasma

Non-thermal plasma (NTP), also referred to as cold atmospheric plasma or cold plasma, is one of the most novel non-thermal technologies being investigated as an alternative food processing tool. It was first described by Sir William Crookes in 1879 (Crookes 1879), and electron beams were first identified by Sir J.J. Thomson in 1897 (Sarghini et al. 2008, 2011). This state of matter is described as: "Except near the electrodes, where there are *sheaths*

containing very few electrons, the ionized gas contains ions and electrons in about equal numbers so that the resultant space charge is very small. We shall use the name *plasma* to describe this region containing balanced charges of ions and electrons" (Langmuir 1928). Plasma is considered to be the fourth state of matter, and defined as a quasi-neutral gas, following the more familiar states of solid, liquid and gas, and constitutes more than 99% matter of the universe. The concept of fourth state of matter occurs with the idea that phase transactions happens by progressively providing energy to the matter from the solid to the liquid up to the gas state. As more and more energy is supplied to the system, further phase transition from the gas state to the plasma occurs. It has net zero electrical charge formed by a gaseous mixture of both negatively and positively charged particles. It is more or less an electrified gas with a chemically reactive media that consists of a large number of different species such as electrons, positive and negative ions, free radicals, free electrons, gas atoms and activated neutral species (exited and radical) (Sasai et al. 2011).

It is possible to obtain plasma with extremely broad range of temperature and pressure. By coupling energy to a gaseous medium by several means such as mechanical, thermal, chemical, radiant, nuclear, or by applying a voltage, or by injecting electromagnetic waves and also by a combination of these to dissociate the gaseous component molecules into a collection of ions, electrons, charge-neutral gas molecules, and other species, plasma can be produced at low-pressure or atmospheric pressure (Sarghini et al. 2008). Plasma, based on the differences in their characteristics, is classified into two types as thermal and cold plasma (Sasai et al. 2011).

The thermal plasma can be generated under atmospheric pressure. It is described as the state of fully ionized gas characterized by a high gas temperature and an approximate equality between the gas and electron temperature ($Tg \approx Te$). Because the energy level of this plasma is very high, it is able to break any chemical bond. On the other hand, cold plasma has a low gas temperature and a high electron temperature ($Tg << Te$), and thus, it is easier to obtain cold plasma by electrical discharges under reduced pressure. Cold plasma irradiation of surface treatment has limited penetration (ca. 500–1000 Å); therefore, only the surface properties of a food can be changed without affecting the bulk properties (Georghiou et al. 2005, Gibalov and Pietsch 2004).

13.6.1 Processing of dairy products by cold plasma

Although it is used in medical, chemistry and polymer industries, limited studies have been carried out on the application of cold plasma to milk and dairy products. In one study, the effects of plasma application on the inactivation rate of *E. coli, S. aureus* and S. Typhimurium in whole, semi-

skimmed and skimmed milk stored at 4ºC for 42 days were investigated, and the reductions in the counts of *E. coli*, S. Typhimurium and *S. aureus* after plasma application at 20 kV were reported as 3.63, 2.00 and 2.62 log cfu ml^{-1}, respectively. No significant changes were noted in pH and color of the milk samples. No viable cells were detected after one week examination in whole milk samples, and the samples were remained stable for over six-week storage.

In order to determine the reduction in the allergenicity of isolated major milk proteins, milk was processed by ultrasound, non-thermal atmospheric plasma and UV-C light treatments. Ultrasound and plasma treatments did not show any differences in SDS-PAGE patterns of casein, β-lactoglobulin and α-lactalbumin indicating no significant change in protein concentration and/or composition. No significant difference was detected in IgE binding values of the control and the samples treated with ultrasound and cold plasma. It is reported that while the UV-C light treatment can reduce the allergenicity of major milk proteins to some extent, high intensity ultrasound and non-thermal atmospheric plasma treatments fail to generate effective results for reducing allergenicity (Tammineedi 2012).

Inactivation of *L. monocytogenes* inoculated into sliced cheese by atmospheric pressure plasma (APP), which is capable of operating at atmospheric pressure in air with 75, 100, 125 and 150 W input powers and 60, 90 and 120 s plasma exposure times revealed that the rate of microbial reduction increased with increased input power and plasma exposure time. The viable cells of *L. monocytogenes* was reduced by 1.70, 2.78 and 5.82 log cfu g^{-1} in sliced cheese after 120 s APP treatments at 75, 100 and 125 W, respectively. The exposure time required to inactivate 90% of microbial population of sliced cheese using APP (with 75, 100, 125 and 150 W) were 71.43, 62.50, 19.65 and 17.27 s, respectively. No viable cells were detected at 125 and 150 W of APP treatments in sliced cheese (Song et al. 2009).

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