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Pulsed Electric Field Processing of Milk: A Review

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ABSTRACT
Pulsed electric field (PEF) is an innovative non-thermal technology which could be used as analternative to the traditional thermal process to inactivate the microorganisms and enzymes in liquid foods such as milk. Compared to thermal processing, the PEF process is considered more energy efficient as the microbial or enzymatic inactivation is achieved at ambient or mild temperatures by the application of short bursts of high intensity electric fields to liquid food flowing between two electrodes. Extensive international research has been conducted since the 1990s on the development of PEF in the food industry. This article reviews the recent findings on the application of PEF technology in milk processing, the mechanisms of microbial and enzymatic inactivation by PEF treatment and the application of PEF in combination with antimicrobials.
Keywords: Pulsed electric field (PEF), Milk Processing, Electroporation

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INTRODUCTION

Nature has bestowed humans with ultimate cherishing and relishing foods in this world. Some of these foods have an everlasting attachment with us, i.e. from birth until death in their natural or some alternate form, the most important existing in this categorybeing“milk”.Milk is one of the most important source of perfect nutrition to humans (with traces of Vitamin C and absence of Iron and dietary fiber).In fact from birth, milk fulfills our daily intake requirements in terms of energy, protein, carbohydrates, minerals etc [41, 44, 40].

Since most of the dairy foods in developed countries (and to a lesser extent in developing countries) has gone into the hands of organized sector, it is very important to keep a tandem between the quality and quantity of milk being processed in a dairy industry [36, 33].

To extract maximum benefit from the milk or its products it is very important to process the milk in such a way that, the final product either has the same or enhanced nutritional, organoleptic and functional properties with absence of adulterants and synthetic preservatives and safe for consumption. It has been the highly appreciated effort of scientists to develop such processing methods that could enable industrialists to supply milk in a more palatable and easily accessible form to the working and time-deficit class of the society that help in making the living attributes of people better and safer [31, 26].

Thermal processing has been successfully implemented in the processing of milk for ensuring its safety and enhancing its shelf-life. However the inevitable effects include browning, development of a cooked flavor,reduction of nutritional properties, development of off-flavors, alteration to functional properties of proteins, inactivation of bacterial

inhibitors, and impairment of rennetability etc. although desirability may depend on the kind of product made and on its intended use. Examples are loss of nutritional quality. This means that heat treatment should be carefully optimized.

In order to rectify these drawbacks, non-thermal processing technologies have been experimented in the field of milk processing. Out of the various non-thermal processing technologies pulsed electric field processing is one of the most promising technologies.

Pulsed electric field (PEF) is largely a non-thermal process that is able to inactivatemicroorganisms and enzymes to some degree in liquid food such as milk and fruit juice and isreported to have minimum adverse effects on the sensory attributes of these products. The PEF process is considered to be energy efficient since the microbial inactivation is achieved atambient or moderately elevated temperatures by the application of short bursts of highintensity electric fields to liquid food flowing between two electrodes. A large flux ofelectrical current in only short bursts flows through the food materials (e.g. milk), which are electrical conductors due to the presence of electrical charge carriers.

CHRONOLOGY OF USING ELECTRICITY IN MILK PROCESSING

Electrical processing of foods began in the early 1900s. In the beginning, electrical pasteurization inactivated micro-organisms by increasing the temperature of samples by means of an electrical resistance (ohmic heating), milk being the first electrically pasteurized product. Beattie [5] and Beattie and Lewis [6] designed the equipment to process milk electrically, demonstrating the lethal effects of electrical discharges on micro-organisms when voltages of 3000–4000 V were applied. Fetterman [17] processed milk using the "ElectroPure Process", which consisted of heating the milk to 70℃ and then passing it through carbon electrodes in an electric heating chamber to inactivate Mycobacterium tuberculosis and Escherichia coli. Getchell [19] pasteurized milk by heating it for 15 s at 71℃ with an alternating current of 220 V and Moses [17] reported that between 1928 and 1938, more than 200 million liters of electrical pasteurized milk were consumed. Nevertheless, this process was soon forgotten for no apparent reason [28].

In the last decade, several studies have been performed in order to develop non-thermal electrical pasteurization processes. Different types of equipment for the application of high intensity pulsed electric fields (PEF) have been patented and several studies have demonstrated the effectiveness of this non-thermal technique in food processing.

A great part of the PEF research is focused on studying its effect on milk and dairy products due to the importance of the dairy industry. Most of the studies carried out with these products have been performed to evaluate PEF's effect on microbial inactivation. The level of microbial inactivation achieved with PEF treatment mainly depends on the field strength and the number of pulses applied during the process [31].As regards to the effect of PEF on enzymes, some controversial results have been obtained. In several cases, high levels of inactivation have been achieved, whereas in other cases no effect or increase in initial activity has been detected [7].

PEF, as a non-thermal process, is believed to maintain the original composition of milk. If this technique keeps the initial content of minority food components, such as vitamins [8], it could be introduced to the dairy industry for obtaining products with excellent nutritional and sensory qualities.

PEF BASICS AND OVERVIEW

The main components of typical PEF equipment are a high voltage pulse generation system, a treatment chamber assembly, and a pump for subjecting liquid food, such as milk, to enable continuous PEF treatment. Heat exchangers can be installed for pre-heating and cooling of the food before and after PEF treatment. Exponentially decaying pulses and square wave pulses (monopolar, bipolar) are the two most common wave shapes generated during PEF processing (Fig. 1A), which are normally monitored during PEF treatment using an oscilloscope. Bipolar square wave pulses are reported to be more lethal as these lead to a charge reversal across the cell membrane, hence inducing more cellular damage [37]. Barbosa-Canovas, *et al.*,[2] reported several developments in the design of PEF treatment chambers; however, parallel plates, coaxial and co-linear configurations are the most commonly used (Fig. 1B).

The effectiveness of PEF treatment at inactivating microorganisms depends on various factors such as process parameters (electric field intensity, number of pulses, pulse shape, frequency, and duration of pulse), product parameters (composition, conductivity, pH, and water activity), and microbial characteristics (type of microorganism, growth conditions, growth phase, and recovery conditions) [42]. The potential of PEF treatment to inactivate microorganisms and enzymes in milk for improving quality has been extensively studied in the last decade [8, 9]. PEF induced microbial inactivation is hypothesized to be due to dielectric breakdown and electroporation of the cell membrane (Fig. 1C), based on microbial and other physicochemical studies using phospholipid vesicles and planar bilayers model systems [20]. Despite this, the mechanism of PEF-induced inactivation of enzymes is not clearly understood although some authors have suggested that it is due to the structural and configurational changes induced under the influence of high electric fields [21].

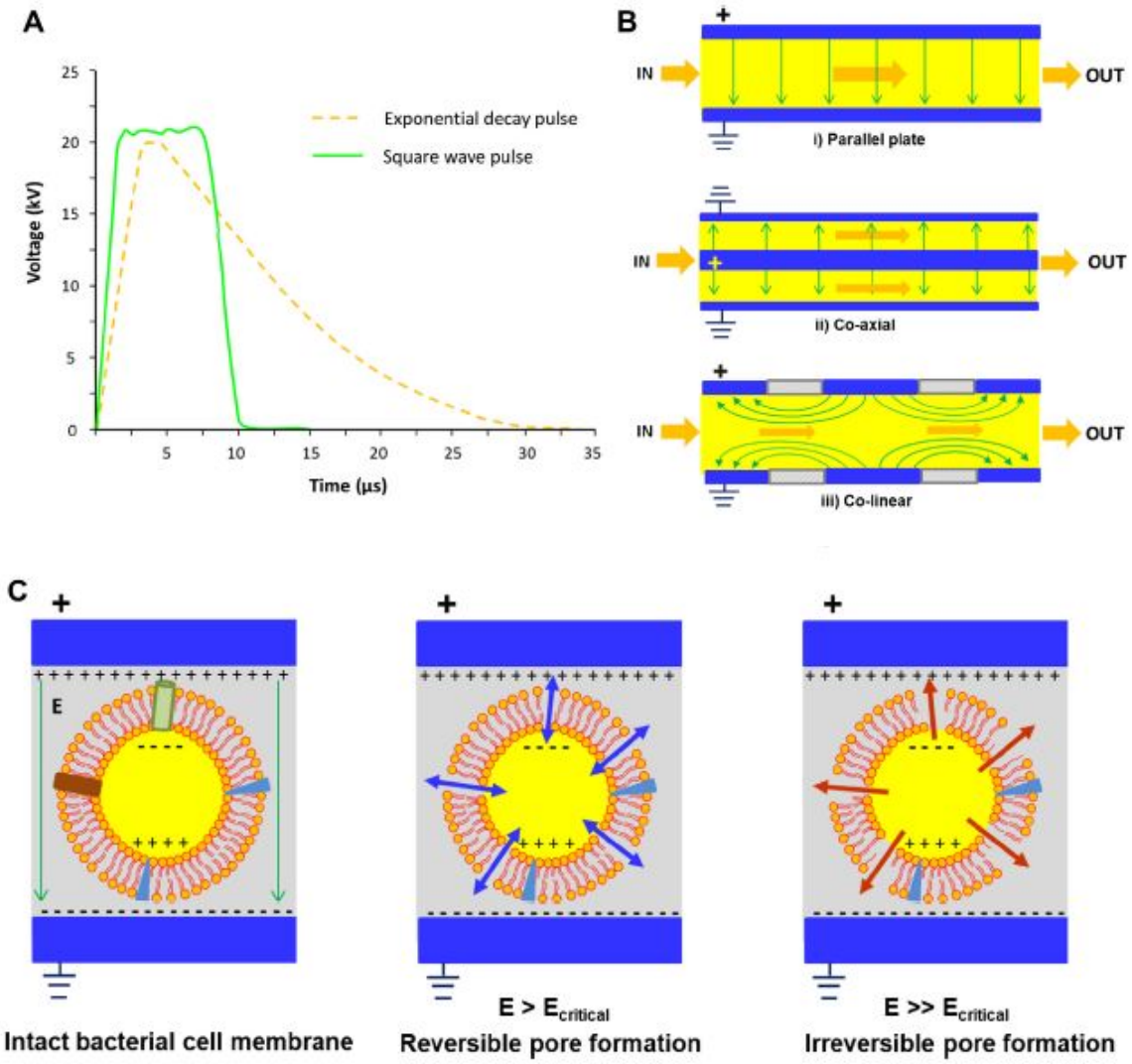

Fig. 1. Schematic depiction of, (A) the voltage patterns of exponential decay and square wave pulses, (B) configuration of treatment chambers for continuous PEF treatment, (C) mechanism of membrane permeabilisation by electro-compressive forces induced by an external electrical field. E: electric field intensity; Reversible: electrical breakdown when pores are smaller compared to membrane area; Irreversible: electrical breakdown when pores are large, causing mechanical destruction of the membrane leading to cell death. Adapted from [37].

Mechanism of Microbial Inactivation by PEF: Electroporation and Electrical Breakdown

The mechanism underlying the inactivation of microorganisms by PEF is yet to be fully understood and knowledge of the microbial inactivation mechanism is essential in order to design and develop more efficient PEF equipment and define conditions for effective inactivation of microorganisms in food products**.** According to Sale and Hamilton, 1967 membrane damage is the direct cause of cell inactivation. The inactivation of microorganisms is related mainly to the changes in the cell membrane and its electromechanical instability [22].

Two mechanisms have been proposed for the mode of PEF action on microbial membrane: electroporation and electrical breakdown. However, both mechanisms are in fact referring to a phenomenon starting by electroporation resulting in electrical breakdown by which the cell wall is perforated and cytoplasm contents leak out resulting in cell death. The electroporation theory suggests that the main effect of an electric field on microbial cells is to increase the membrane permeability due to membrane compression and poration and cell inactivation results from osmotic imbalance across the cell membrane [38]. Figure 2 shows the mechanism of electrical breakdown and cell poration which was initially proposed by Zimmermann [45]**.** He suggested that the membrane can be considered as a capacitor filled with a dielectric medium.

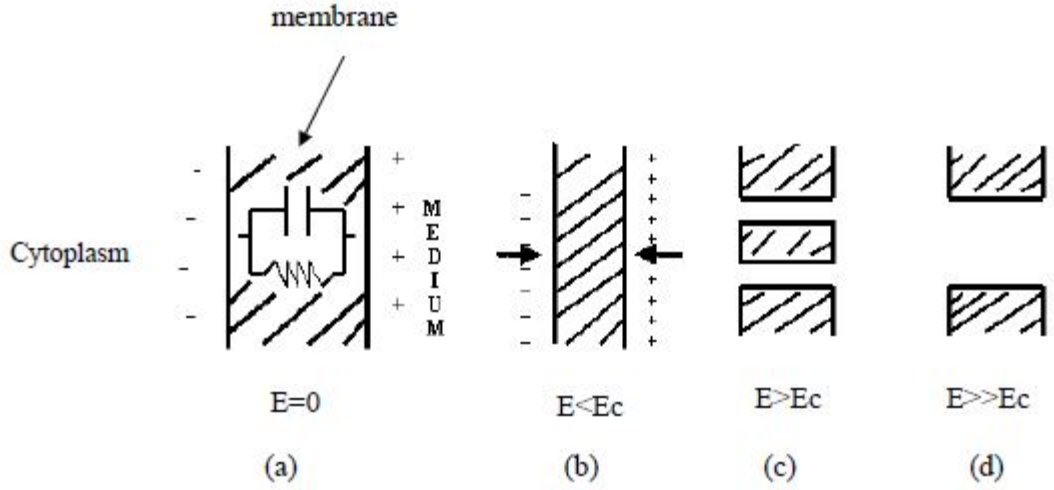

Figure 2: Schematic diagram of reversible and irreversible breakdown of a microbial cell indicating compression by electroporation when exposed to electric field. The membrane acts as a capacitor and is represented by hatched area. E_c is the critical electric field (a) Intact cell membrane; (b) membrane compression; (c) pore formation with reversible breakdown; (d) irreversible breakdown with large pores formation**.**

Based on this theory, when the transmembrane potential is exposed to a higher external fieldintensity this results in membrane damage. According to Chen and Lee, [13] the membrane of a biological cell insulates the shell from cytoplasm while the electrical conductivity of the cytoplasm is 6 to 8 times greater than conductivity of the membrane. When the cell is exposed to an electric field, the free charges generated on the membrane surface are attracted to each other due to the difference in the signs (- and +) which causes a compression pressure resulting in a decrease in membrane thickness (Fig. 2b). Increasing the field intensity leads to more accumulation of surface charges, resulting in a higher electromechanical stress and reversible breakdown of membrane (Fig. 2c).The membrane thickness decreases by increasing the field intensity which eventually results in an irreversible breakdown through creating larger pores in the membrane (Fig. 2d). If the area of the pores in relation to the membrane surface becomes larger, an irreversible breakdown occurs in the membrane leading to the total destruction of the cell [45]. In large cells the induced potential is greater which makes them more vulnerable to damage compared to smaller cells [13].

Osmotic imbalance is a theory through which the electroporation and electrical breakdown

has been described (Figure 3). The cell exposed to an external electric field is "electroporated" through the leakage of ions and small molecules and thus the membrane becomes permeable to water that causes swelling and eventual rupture (electrical breakdown) and lysis of the cell. Therefore, based on the above observations it could be concluded that the inactivation of cells follows a sequence of a primary electroporation with small pores on the cell membrane followed by a secondary electroporation with larger pores which finally causes electrical breakdown and cell lysis. Large pores are obtained by increasing the intensity of the electric field and pulse duration or reducing the ionic strength of the medium [34].

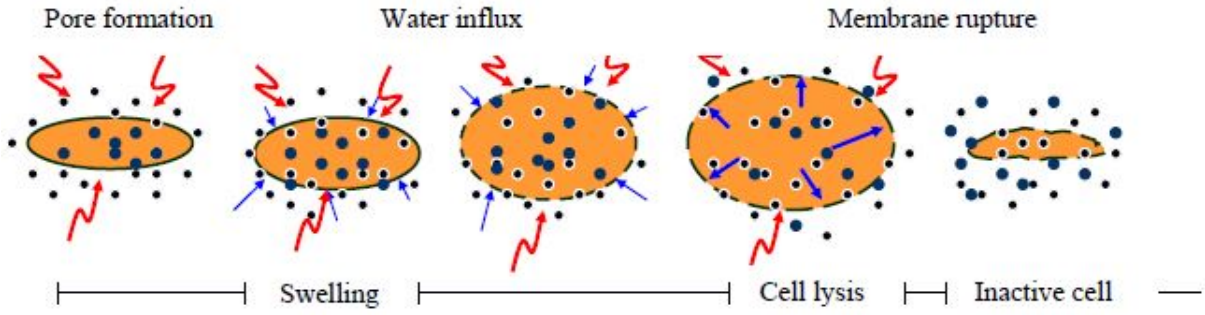

Figure 3: Stages of electroporation in a cell membrane through osmosis

Castro *et al.*, [13] further explained electroporation as a phenomenon in which the high voltageelectric field temporarily destabilizes the lipid bilayer and proteins of cell membranes. The plasma membranes thus become permeable to small molecules that cause swelling and eventual rupture of the membrane.. Tsong and Kinosita, [24] demonstrated that an electric field of 2.2 kV/cm induced pores of *ca.* 1 nm in diameter in human erythrocytes. They suggested a two-step mechanism for pore formation in which the initial perforation is a response to an electrical potential greater than the E_c (critical field intensity) followed by a time-dependent expansion of the pore size.

Pothakamury *et al.*, [30] treated a suspension of *Staphylococcus aureus* in simulated milkultrafiltrate (SMUF) by 64 pulses of 20, 30 and 40 kV/cm and scanning electron microscopic (SEM) examination showed rough surfaces and small pores in the membrane which led to the leakage of cellular contents. This finding was confirmed by Aronsson *et al.*, [1] who studied *Escherichia coli, Listeria innocua, Leuconostocmesenteroides* and *Saccharomyces cerevisiae*by means of SEM examination and found a clear difference between untreated and PEF treated cells (25-35 kV/cm, 20-40 pulses of 2-4 µs).

Microbial Studies on PEF-treated Milk and SMUF

The bulk of research activities reported in the literature have focused on the impact of PEF treatment on microbial and enzymatic inactivation in milk or SMUF. The SMUF is a salt solution with composition similar to milk ultrafiltrate. It was proposed by Jeness and Koops [23] and is now widely used in dairy-related research [9].The level of microbial inactivation has been found to be mainly dependent on the electric field strength, number of pulses applied during the process and treatment time Bendicho *et al.*, [8].

Various studies published on treatment of milk by PEF have proven this technology as aneffective method for the inactivation of moulds, yeasts and vegetative bacterial cells. The microorganisms inactivated by PEF belong to the major G+ and G- bacteria. Various researchers have reported 1 to 6 logs inactivation of different strains of *E. coli* (pathogenic and non-pathogenic) in milk (UHT, skim, whole, partially skim), egg pulp, pea soup, apple juice, SMUF, 0.1% NaCl saline, phosphate buffer (pH 7.0) and sodium alginate.

Dutreux *et al.*, [14] PEF treated *E. coli* and *L. innocua* suspended in pasteurised skim milk and in phosphate buffer (with similar pH and conductivities) with inlet and outlet temperatures of 17°C and 37°C, a flow rate of 0.5 L/min, frequency of 3 Hz and field intensity of 41 kV/cm. The number of surviving organisms was determined after the application of 0, 3, 10, 20, 35 and 60 pulses (pulse width unknown). Transmission and scanning electron microscopy wasused to examine *E. coli* cells subjected to 60 pulses.

Changes in the cytoplasm were observed and the cell surface appeared rough. The cells' outer membranes were partially destroyed allowing leakage of the cytoplasm and changes in the cytoplasm were observed.

Rowan *et al.,* [32] investigated the influence of treatment temperature and PEF intensity on the viability of *Mycobacterium paratuberculosis*cells suspended in 0.1% (w/v) peptone water and in sterilised cow's milk. The viability was assessed through direct viable counts and transmission electron microscopy (TEM). Treatment at 50oC with 2,500 pulses of 5 Hz and 30 kV/cm reduced the number of viable *M. paratuberculosis* cells by approximately 5.3 logs in 0.1% peptone water and 5.9 logs in cow's milk, while PEF treatment at 5°C reduced the cells only by 1.6 logs. Heating alone at 50°C for 25 min or at 72°C for 25 s resulted in 0.01 and 2.4 logs reduction, respectively. Thus, under the conditions tested, PEF treatment at 50°C was found to be more effective than thermal pasteurisation for the inactivation of *M.paratuberculosis*.

Evrendilek and Zhang [15] investigated the effects of pulse polarity and "pulse delaying time"(the time elapsed between two consecutive crests passing a given point) on the inactivation of *E. coli* O157:H7 in apple juice and skim milk treated at field strengths of 31 and 24 kV/cm, respectively. Various pulse delaying times of 3 to 1430 µs were applied to both products. The pH and electrical conductivity for apple juice were 3.7±0.24 and 2.3 mS/cm and for skim milk 6.7±0.65 and 6.2±3.4 mS/cm, respectively. The average temperatures of apple juice before and after PEF treatment were 9±1°C and 29±2°C, and for skim milk 7±2°C and 30±3°C, respectively. A significant difference was observed in *E. coli* O157:H7 numbers in skim milk between mono (1.27 logs) and bipolar (1.96 logs) pulses, but not in apple juice (2.6 and 2.63 logs, respectively) at the pulse delaying time of 20 µs. The differences in the inactivation level can be attributed to the difference in pH and ionic composition of skim milk and apple juice.

Sepulveda *et al.*, [35] subjected the pasteurised milk to PEF treatment immediately after pasteurisation and after 8 days storage at 4oC using field intensity of 35 kV/cm and 2 pulses of 2.3 µs duration each. The final temperature was 65oC with a residence time of less than 10 s. It was shown that the application of PEF immediately after pasteurisation could extend the shelf life of milk up to 60 days at 4oC, while PEF processing after 8 day storage resulted in a longer shelf life of 78 days due to further eradication of enteric and psychrotrophic bacteria by PEF.

In all the above studies, PEF has been shown to be partially effective in microbialinactivation, however, to achieve a higher inactivation it may be necessary to combine the PEF treatment with other factors such as heat.

PEF in Combination with Antimicrobials

A number of researchers have demonstrated that the microbial inactivation in milk by PEF treatment can be enhanced by the combined use of low levels of food grade antimicrobials or other hurdle technologies Among the most widely investigated antimicrobials are nisin and lysozyme. Nisin, a peptide bacteriocin from *Lactococcus lactis*is one of the most commonly used food grade antimicrobials. Lysozyme and plant extracts have been used in combination with PEFtreatment [26]. Pol *et al.*, [29] subjected vegetative cells of *B. cereus* to either low doses of nisin (0.06 µg/mL, equivalent to 2.4 IU/mL), mild PEF treatment (16.7 kV/cm, 50 pulses each of 2 µs duration), or the combination of nisin and PEF treatment. The latter treatment resulted in 1.8 logs extra reduction in *B. cereus* numbers than the sum of the reductions obtained from the individual treatments, indicating a synergistic effect.

Calderon *et al.*, [10] combined PEF treatment with nisin addition to inactivate *Listeria innocua* in skim milk. The selected field intensities (and temperatures) were 30 (22^{o}C), 40 (28^{o}C) and 50(34^{o}C) kV/cm and the number of pulses applied were 10.6, 21.3 and 32, respectively. Thesensitization exhibited by PEF treated *L. innocua* to nis in was assessed for 10 or 100 IU nisin/mL.

Listeria innocua count was reduced to 2, 2.7 and 3.4 logs after exposure to the field intensities of

30, 40 and 50 kV/cm in presence of 10 IU nisin/mL while at 100 IU nisin/mL under the same PEF treatment conditions the reduction increased to 2.5, 3 and 3.8 logs. The increase in microbial reduction was attributed to the additive effect of nisin on PEF treatment.

Effects of PEF on Enzymes in Milk or SMUF

The impact of PEF on enzymatic inactivation is a matter of controversy since in several cases a high level of inactivation has been reported while in other cases no effect has been observed.

The mechanism of enzyme inactivation by PEF is unclear, but it is believed to be due to unfolding, denaturation and breakdown of covalent bonds and oxidation-reduction reactions caused by intense electric fields in the protein structure [4].

Not all researchers appear to have taken into account the temperature effects in PEF treatment which is an important variable in enzyme inactivation. The effects of PEF treatment on activities of various milk enzymes including alkaline phosphatase (AlP), lipases, lactoperoxidase (LPX) and proteases (e.g. plasmin) in milk or SMUF has been reported by several researchers [11].

According to Ho *et al.*, [21] enzyme inactivation requires a more severe PEF treatment than that needed for inactivating microorganisms. The higher the electric field intensity and temperature, the greater reduction in enzyme activity is achievable. Various researchers have reported large variations in the inactivation for different enzymes in milk and SMUF by PEF treatment [2]

Effects of PEF Treatment on the Functionality of Milk Proteins and Fat Globules

There are very few studies on the effects of PEF on functionality of fat and proteins in milk as most of the studies have focused on microbial and enzymatic inactivation. However, since the protein and fat functionality affect the yield and physical characteristics of the products made from milk, it is of great importance to expand research in this area. For example, thermal or non-thermal treatment of cheese milk can directly or indirectly affect the final physical and sensory properties of cheese.

Floury *et al.,* [18] found that the PEF treatments at the field intensities of 45 or 55 kV/cm with pulse widths of 500 and 250 ηs (square monopolar pulses) respectively, decreased the coagulation time. At a total treatment time of 2.1-3.5 μs, a significant drop in casein micelle size was observed while the viscosity of milk decreased and the coagulation properties were enhanced.

Wüst *et al.,* [43] assessed the physical attributes of cottage cheese made from PEF-treated skim milk. The treatment was conducted by applying bipolar square pulses of 2μs at field intensities of 25 and 28 kV/cm with pulse frequencies of 200 and 400 Hz and flow rate of 120 mL/min at a treatment temperature of <45°C. It was found that increasing the field strength decreased the strength of the cottage cheese gel and marginally increased the yield of cottagecheese compared to cheeses made from raw or pasteurised skim milk. The “raw milk”odour was also removed from the samples treated at a frequency of 400 Hz.

CONCLUSION

PEF technology can be considered as a potential alternative to traditional thermal pasteurization of milk with the advantages of minimizing sensory and nutritional damage, thus providing fresh-like products. However, more investigation is needed to understand the mechanism of PEF effects and to achieve a maximum level of enzymatic and microbial inactivation in order to make PEF technology applicable in the dairy industry. Most PEF systems used for treatment of dairy or non-dairy products have been limited to bench top or pilot scale systems.

There are currently various research groups in Australia, Belgium, Canada, China, France,Germany, Iceland, Japan, the Netherlands, New Zealand, Scotland, Spain, Sweden, Switzerland, Taiwan, the United Kingdom and the USA working on different industrial applications of PEF. However, no industrial scale PEF plant has so far been established to “pasteurize” milk for public consumption.

The PEF technology has some shortcomings, which must be taken into account in future research. For instance, the presence of air bubbles may lead to non-uniform treatment as well as operational and safety problems. Presently, the PEF application is restricted to liquid food products that can withstand high electric fields. The particle size of the liquid food in both static and flow treatment modes could cause system malfunction. The maximum particle size in the liquid must be smaller than the gap between the electrodes in the chamber in order to maintain a uniform processing operation and there should be no clumping of particles. In addition, there are still technical hurdles to overcome in achieving

successful application of PEF technology at an industrial scale such as designing treatment chambers with maximum output and efficiency, preventing ohmic heating (which can adversely affect heat-sensitive products), need for highly specific electrical pulsing equipment and switches able to handle high voltages and minimizing the electrolysis between the electrodes and product.

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