Application of Pulsed Electric Field for Milk Processing

Arjun Chaudhari, A. H. Jana and J. P. Prajapati
Department of Dairy Technology, SMC College of Dairy Science, AAU, Anand-388110.
E-mail: ary10592@gmail.com

The food industry is continuously investing for new preservation techniques to replace conventional food preservation techniques owing to increasing consumer demand for foods which are natural in taste, nutrient-rich and safe for consumption. Pulsed electric field (PEF) is considered as one of the most promising non-thermal food processing technology, alternative to the traditional pasteurization. Very little heat is generated during PEF processing due to the short treatment time. Application of PEF technology in food processing may avoid the detrimental effects on flavor, freshness, nutrition and bioactive functional components (Yeom *et al.,* 2000; Li *et al.,* 2003). Besides PEF, other promising non-thermal processing techniques includes ionizing radiation, high intensity light pulses, high pressure processing (HPP), electric or magnetic fields, high power ultrasound, oscillating magnetic field, high voltage arc discharge, etc. However, scientists have laid down greater focus on HPP and PEF processing.

Pulsed Electric Field

Pulsed Electric Field (PEF) is an innovative non-thermal technology which could be used as an alternative to the traditional thermal process to inactivate the microorganisms and enzymes in liquid foods such as milk (Sherkat *et al.,* 2009). Thermal processes alter the nutritional and sensory qualities of the food. The conventional HTST processing technique can affect the organoleptic and nutritional properties of milk to varying degrees (Steele, 2000; Mertens and Knorr, 1992). As 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 for a couple of microseconds (Barbosa-Canovas *et al*., 2000; Gaudreau *et al*., 2008; Jose *et al.,* 2010).

Principle of PEF

The basic principle of the PEF technology is the application of short pulses of high electric fields with duration of micro seconds to milliseconds and intensity in the order of 10- 80 kV/cm. The processing time is calculated by multiplying the number of pulses times with effective pulse duration. The applied high voltage results in an electric field that causes microbial inactivation. When an electrical field is applied, electrical current flows into the liquid food and is transferred to each point in the liquid because of the charged molecules present (Zhang *et al*., 1995).The electric field may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, sub-ambient, or slightly above-ambient temperature. After the treatment, the food is packaged aseptically and stored under refrigeration.

Mechanisms of microbial inactivation

Two mechanisms have been proposed for the mode of PEF action on microbial membrane: electroporation (cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes) and electrical breakdown (normal resisting potential difference across the bacterial membrane is 10 mV which leads to the build-up of a membrane potential difference due to charge separation across the membrane). In both cases, the phenomena starts by electroporation, by which the cell wall is perforated and cytoplasm contents leak out resulting in cell death (Tsong, 1990; Martin-Bellosso and Elez-Martinez, 2005).

Parameters affecting microbial inactivation by PEF

Various parameters that can affect the microbial inactivation by PEF includes the type of microorganisms, field intensity, pulse wave shape, conductivity of the medium, pH, treatment temperature, treatment time (flow rate and pulse frequency) and energy input.

Type of microorganisms

Gram-positive bacteria are more resistant to PEF treatment than Gram-negative ones (Hulsheger *et al.,* 1983). Yeasts are more sensitive to electric fields than bacteria, although at low electric fields they were more resistant than Gram-negative bacteria. Logarithmic phase cells are more sensitive to stress than lag and stationary phase cells. Microbial growth in logarithmic phase is more susceptible to the applied electric fields; inactivation effects in the logarithmic phase were 30% greater than in stationary phase (Qin *et al.,* 1995).

Electric field intensity

To achieve microbial inactivation, the applied field needs to be greater than the critical electric field for particular microorganisms (Castro *et al.,* 1993). When the cells are exposed to an electric field of sufficient strength, the field induces the accumulation of electric charges at the non-conductive microbial membranes. *Listeria innocua* (smaller cell size) requires a minimum dose of 15 kV/cm to become inactivated, while the larger cells of *S. cerevisiae* are affected at field strength as low as 2-4 kV/cm.

Pulse wave shape

Exponentially decaying and square wave pulses (bipolar or mono polar form) are the most common wave shapes used in PEF systems. An exponentially decaying wave is a unidirectional voltage that rises rapidly to a maximum value and decays slowly to zero. Square wave pulses maintain a peak voltage above the critical field strength for a longer period of time and are more lethal than exponentially decaying pulses. Bipolar pulses are reported to be more lethal than mono polar pulses (Shamsi *et al.,* 2009).

Treatment temperature

When the electric field is applied to the flowing liquid between the electrodes, its temperature rises. Therefore, proper cooling of the product (by using cooling water bath) between the treatment chambers is necessary to avoid heat damage and to limit microbial inactivation only to the PEF effect. PEF treatment at moderate temperatures (50 to 60°C) exhibits additive effects on the inactivation of microorganisms and their spores (Shamsi *et al.*, 2009).

Treatment time and frequency

The inactivation of microorganisms increases with an increase in the pulse number up to a certain extent (Hulsheger *et al*., 1983). The electric field pulses between 1 and 5 µs produced the best results for microbial inactivation (Sepulveda-Ahumada, 2003).

Application of PEF in milk processing

Michalac *et al*. (2003) observed that raw skim milk when subjected to PEF treatment (intensity of 35 kV/cm; pulse with width 3 µs; and time of 9 µs) did not show any significant difference in color, pH, proteins, moisture and particle size. Application of PEF treatment (35 kV/cm; 2.3 µs of pulse width at 65ºC for <10 s immediately after HTST pasteurization extended the shelf life of milk to 78 days at 4ºC (Sepulveda-Ahumada, 2003). PEF treatment of bovine immunoglobulin enriched soymilk at dose of 41 kV/cm for 54 µs did not cause any significant change in bovine IgG activity but resulted in a 5.3 log reduction of initial microbial flora (Li *et al.,* 2003).

Microbial inactivation and shelf life enhancement of milk

Significant reduction in the count of spore forming bacteria in milk is possible with PEF treatment. PEF treatment results in less flavor degradation of milk than equivalent heat treatments, and does not exert any chemical or physical changes in milk fat, protein integrity, and casein structure. Hence, PEF may be used to preserve heat-sensitive dairy products such as whey protein concentrates. An extension of raw milk shelf life to 2 weeks at refrigeration temperature using PEF (two steps of seven pulses, and one step of six pulses at 40 kV/ cm) was noted. There was no difference in the sensory quality of PEF treated and heat pasteurized milk.

High intensity PEF (HIPEF) treatment of whole milk at 35.5 kV for 1000 µs with 7 µs bipolar pulses at 111 Hz reduced the mesophilic aerobic count from 3.2 log to 1 log cycle ensuring microbial stability for 5 days at 4ºC without significant change in acidity, pH and FFA; proteolysis and lipolysis was not observed (Odriozola-Serrano *et al.,* 2006). Floury *et al.* (2006) reported that combination of PEF and heat treatment reduced the *salmonella enteritidis* count in skim milk by 2.3 log cycle compared to 1.2 log cycle obtained by PEF treatment alone.

The shelf of skim milk subjected to HIPEF treatment (40 kV/cm, 60 µs; 36 kV/cm, 84 µs) was enhanced to 14 days when stored at 4ºC (Fernandez-Molina *et al.,* 2005). Odriozola-Serrano *et al.* (2006) reported that whole milk processed with HIPEF (35.5 kV/cm, 1000 µs) had a shelf life of 5 days at 4ºC and change in acidity was not observed during storage. Subjecting chocolate milk to HIPEF (30 kV/cm, 45 µs) prior to heating at 105ºC and 112ºC for 31 s resulted in product having shelf life of 119 days at ambient (37ºC) storage temperature (Sobriono-Lopez and Martin-Bessoso, 2010).

Effect of PEF treatment on sensory and nutritional quality of milk

The vitamin content including thiamine (B_1), riboflavin (B_2), cholecalciferol (D), and tocopherol (E) of PEF treated milk remained unchanged after treatment of up to 400µs at field strength of 18.3 to 27.1 kV/cm for 400µs; 93% of the ascorbic acid was retained. This is significantly higher retention of vitamins as compared to LTLT and HTST pasteurized milks. The pH, titratable acidity, fat content, fat and protein integrity, starter growth, rennet clotting time, yield, cheese production, calcium distribution, color, moisture and particle sizes remained unaffected by the PEF treatment at field strength of 20-80 kV/cm. Fox *et al.* (2000) showed that heat treatment of milk at a temperature > 65ºC adversely affected its rennet coagulation property. PEF treatment up to 35 kV/cm for 73µs did not exert any significant effect on the bovine IgG in a protein-enriched soymilk. Flavored milk, yogurt, and yogurt drink treated with PEF coupled with heat had lower yeast and mold count, showed increased shelf life and did not exhibit any change in the sensory score of resultant product (Yeom *et al.,* 2007)

Inactivation of enzymes by PEF

The alkaline phosphatase enzyme activity in simulated milk ultrafiltrate (SMUF) subjected to electric field strength of 22 kV/cm and seventy pulses using static chamber was reduced by 65% (Castro, 1994). A 90% reduction in plasmin activity in SMUF was attained by use of PEF at strength of 30 kV/cm. Destruction of plasmin by non-thermal PEF is of significance since its activity can cause bitterness and gelation in UHT milk. PEF treatment at a dose of 21.5 kV/cm achieved 60% reduction in the milk lipase activity too. Inactivation of protease and lipases produced by psychrotropic microorganism such as *Bacillus* and *Pseudomonas* by use of PEF is of significance in dairy industry since these enzymes are heat stable and some remain active even after high-temperature treatment. The inactivation of enzymes increased with field strength, treatment time, input energy, and pulse frequency and was dependent on the composition of milk. The inactivation of enzymes achieved in skim milk was higher than that of whole milk. Vega-Mercado *et al.* (1995) reported a 90% reduction in plasmin activity in SMUF at 30 kV/cm after 50 pulses. PEF treatment at 21.5 kV/cm and high energy input reduced the lipase and peroxidase activity of raw milk by 65% and 25% respectively with negligible effect on alkaline phosphatase activity (Grahl and Markl, 1996).

Conclusion

PEF technology has considerable potential for use in the dairy industry. Its major application is non-thermal inactivation of vegetative microorganisms in foods. It causes little or no change in color, flavor and nutritive value as opposed to the impact by thermal treatments. Other applications such as inactivation of enzymes may show promise for improving the product quality. PEF is effective for extending the shelf life of milk. PEF treatment is largely restricted to liquid foods that can endure high electric fields and is not suitable for fluids containing particulate or gases, without pretreatment. PEF may be useful for treatment of products containing heat sensitive components such as whey proteins and immunoglobulins.

References

Barbosa-Canovas GV, Pierson MD, Zhang QH and Schaffner DW. (2000). Pulsed electric fields. Special supplement: kinetics of microbial inactivation for alternative food processing technologies. *J Food Sci*,65:65-79.

Castro AJ. (1994). Pulsed electric field modification of activity and denaturation of alkaline phosphate. Ph.D Dissertation, Washington State University, Washington.

Castro A, Barbosa-Canovas GV and Swanson BG (1993). Microbial inactivation of foods by pulsed electric fields. *J Food Proc Preserv*, 17:47-73.

Fernandez-Molina JJ, Fernandez-Gutierrez SA, Altunakar B, Bermudez-Aguirre, Swanson, B G and Barbosa-Canovas GV. (2005). The combined effect of pulsed electric fields and conventional heating on the microbial quality and shelf-life of skim milk. *J Food Process Preserv*, 29:390-406.

Floury J, Grosset N, Lesne E and Jeantet R. (2006). Continuous processing of skim milk by a combination of pulsed electric field and conventional heat treatments: does a synergetic effect on microbial inactivation exist? *Lait*, 86:203-211.

Fox PF, Guinee TP, Cogan TM, and Mc Sweeney PLH. (2000). In: Fundamentals of Cheese Science. Aspen Publishers, Gaithersburg, MD.

Gaudreau M, Hawkey T, Petry J and Kempkes M. (2008). Pulsed electric field processing for food and waste streams. *Food Australia*, 60:323-325.

Grahl T. and Märkl H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microb. Biotec.*, 45:148-157.

Hulsheger H, Potel J and Niemann EG. (1983). Electric field effects on bacteria and yeast cells, *Radiation Environ. Biophysics*,22:149-162.

Jose A, Guerrero-Beltran, Sepulveda DR, Maria M, Gongora-Nieto MM, Swanson B and Barbosa-Canovas GV (2010). Milk thermization by pulsed electric fields and electrically induced heat. *J Food Engg*,100: 56-60.

Li SQ, Zhang QH, Lee YZ and Pham TV. (2003). Effetcs of pulsed electric field and thermal processing on the stability of bovine IgG in enriched soymilk. *J Food Sci*, 68:1201-1207.

Martin-Belloso O, Elez-Martinez P. (2005). Food safety aspects of pulsed electric field. In Emerging Technologies for Food Processing. Da-Wen Sun (Ed.), Elsevier Academic Press, London, pp.184-217.

Mertens B and Knorr D. (1992). Developments of non thermal processes of food preservation. *Food Technol*,5:124-133.

Michalac SL, Alvarez VB and Zhang QH. (2003). Inactivation of selected microorganisms and properties of pulsed electric field processed milk. *J Food Process Preser*, 27:137-151.

Odriozola-serrano I, Bendicho-porta S and Martin Belloso O. (2006). Comparative study on shelf life of whole milk processed by high intensity PEF or heat treatment. *J Dairy Sci*, 89: 905-911.

Qin BL, Barbosa-Cánovas GV and Swanson BG, Pedrow PD and Olsen RG. (1995). Inactivating microorganisms using pulsed electric fields continuous treatment system. *IEEE Transactions Dielectrics Electrical Insulation*,34:43-50.

Sepulveda-Ahumada DR (2003). Preservation of fluid foods by pulse electric fields in combination with mild thermal treatments. Ph.D Thesis. Pullman WA: Washington State University.

Shamsi K., Wan J., Versteeg C and Sherkat F. (2009). Effects of pulsed electric field treatment on the rheological and textural properties of rennet-induced curds of bovine milk. Poster presented at the 4th Innovative Foods Centre Conference, 17-18 September 2008, Brisbane, Australia.

Steele J. (2000). History, trends and extent of pasteurization. *J Am Vet Medical Assoc*, 217: 175-178.

Tsong T. (1990). Reviews on electroporation of cell membranes and some related phenomena. *Bioelectrochem Bioenergetics*, 24:271.

Vega HM, Powers JR, Barbosa-Canovas GV, Swanson BG, and Luedecke L. (1995). inactivation of protease from *pseudomonas fluorescens* M3/6 using high voltage pulse electric fields. IFT 1995. *Annual Meeting*. Book of Abstracts, paper no. 89, pp. 267-278.

Yeom HW, Streaker CB, Zhang QH and Min DB. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *J Agric Food Chem,* 48:4597-4605.

Yeom HW, Evrendilek GA, Jin ZT and Zhang QH. (2007). Processing of yoghurt based products with pulsed electric field: microbial, sensory and physical evaluations. *J Food Process Preserv,* 28:161-178.

Zhang QH, Barbosa-Cánovas GV and Swanson BG. (1995). Engineering aspects of pulsed electric field pasteurization. *J Food Engg,*25: 261–281.