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Application of Pulsed Electric Field to Process Milk

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Abstract: Milk is a nutritious food consumed by people of all ages. It is highly nutritious and thus is highly perishable unless and until preserved by some heat treatment like sterilization, ultra-heat treatment or boiling. In the present article, scope of utilizing pulsed electric field (PEF) has been discussed as an emerging technique of preservation to provide safe, fresh and shelf-stable milk with minimum or no nutrient losses. It covers details about the equipment, process and its effects on concerned microorganisms, enzymes and nutrients.

Keywords: Pulsed electric field, food, milk, sterilization, pasteurization.

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Introduction

Milk supplies energy and essential nutrients to nourish body. However, it is highly perishable and is, therefore, normally preserved with the application of heat. Various thermal techniques like pasteurization and sterilization are widely employed to process milk which however, also result in undesirable browning, development of cooked flavour, reduction of nutritional properties like losses in vitamins particularly B-complex and impairment of rennet-ability. To overcome such effects of thermal processing techniques on milk, several new and emerging methods of milk preservation are under scientific exploration. In the present paper pulsed electric field (PEF) is discussed to understand whether it can be used as a promising processing technique for milk. Indian dairy is growing at a CAGR of 6.4% and has produced 198.4 million metric tonnes of milk in 2019-20 maintaining its number one position in World Milk Production. Exploitation of novel technologies like PEF to process and extend the shelf-life of milk will offer new opportunities to the young Indians who are exploring dairy sector to venture into start-ups which in turn will give an added thrust to dairy industry.

In PEF, short pulses of high intensity electric fields (1-100 kV/cm) are applied for a period of microseconds to milliseconds to the food product flowing between two electrodes at ambient or moderately elevated temperatures. Depending on various process parameters, PEF treatment inactivates microorganisms and enzymes to varying degree. However, PEF brings minimum changes in the sensory attributes, nutrition of the treated food products and helps maintain 'fresh-like' quality (Upadhyay *et al.* 2019). PEF is considered as a superior method in comparison to thermal processing and preservation (Reineke *et al.* 2015). In India, Defence Food Research Laboratory (DFRL, Mysore) has conducted studies on the effect of PEF in combination with heat or sonication and have been able to extend the shelf-life of milk (flavoured) to 120 days at 5 ^{0}C and 90 days at ambient condition and that of curd to 180 Days at 5 ^{0}C and 21 days at ambient condition, respectively (Kumar *et al.* 2019).

In the early 1900s, ohmic heating (rise in temperature due to electrical resistance) was used to inactivate micro-organisms and to obtain pasteurized milk. Beattie and Lewis (1925) designed the equipment with 3000–4000 V to process milk electrically and Fetterman (1928) processed milk using the ''Electro Pure Process'', where milk at 70^{0}C was passed through carbon electrodes in an electric heating chamber to inactivate *Mycobacterium tuberculosis* and *Escherichia coli*. In the last decade, research is focused on studying the potential of PEF to inactivate microorganisms and enzymes in milk due to the growing importance of dairy industry (Bendicho *et al.* 2002a; Sharma *et al.* 2014). Globally various research scientists are working on industrial applications of PEF in the different parts of the World.

2. PEF Equipment and Process

The main components of typical PEF equipment are a high voltage pulse generation system, a treatment chamber assembly (continuous or static), a pump for subjecting liquid food for example, milk, to enable continuous PEF treatment along with necessary monitoring and controlling devices and an aseptic packaging system to avoid post-processing contamination (Kumar *et al.* 2019). High voltage pulse generator supplies electrical energy of selected voltage. Heat exchangers can be installed for pre-heating and cooling of the food before and after PEF treatment. (Sharma *et al.* 2014). Processing time can be calculated by multiplying the number of pulses by effective pulse duration (Upadhyay *et al.* 2019). Barbosa-Canovas *et al.* (1999) reported several designs of PEF treatment chambers; out of which parallel plates, coaxial and co-linear configurations are the most commonly used. Bellebna *et al.* (2017) have tried different configurations comprising of stainlesssteel electrodes and square shape treatment chamber made of Plexiglas to achieve savings in electricity as well as higher processing output.

3. Microbial Inactivation in Milk by PEF

Knowledge about the mechanism of microbial inactivation helps in designing efficient PEF equipment. Based on microbial and physicochemical studies using phospholipid vesicles and planar bilayers model systems, PEF induced microbial inactivation is believed to be due to dielectric breakdown and electroporation of the cell membrane (Ho and Mittal, 2000). In 1967, Sale and Hamilton described membrane damage as the direct cause of cell inactivation. Some authors have suggested that it starts by electroporation wherein there is electrical breakdown of the cell wall 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, cell poration and cell inactivation resulting from osmotic imbalance across the cell membrane (Tsong, 1990). Large pores can be obtained by increasing the intensity of the electric field and pulse duration or by reducing the ionic strength of the medium (Schoenbach *et al.* 1997).

The effectiveness of PEF treatment to inactivate 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). The 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, (1962) and is now widely used in dairy-related research (Bendicho *et al.* 2002). Various studies published on treatment of milk by PEF have proven this technology as an effective method for the inactivation of moulds, yeasts and vegetative bacterial cells. The microorganisms inactivated by PEF belong to the major Gram +ve and Gram -ve 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.* (2000) studied effect of PEF treatment of *E. coli* and *L. innocua* suspended in pasteurised skim milk and in phosphate buffer (inlet and outlet temperatures:

17°C and 37°C; flow rate: 0.5 L/min; frequency: 3 Hz; field intensity: 41 kV/cm). They found leakage of cytoplasm due to the partially broken cell membrane. 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 (Table 1). Rowan *et al.* (2001) investigated the viability of *Mycobacterium paratuberculosis* cells suspended in 0.1% (w/v) peptone water and in sterilised cow's milk as influenced by PEF. While PEF treatment at 5°C reduced the cells only by 1.6 logs, treatment at 50°C 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. Evrendilek and Zhang, (2005) reported 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 (field strengths of 31 and 24 kV/cm, respectively; pulse delaying times: 3 to 1430 μs). The pH and electrical conductivity for apple juice (3.7±0.24 and 2.3 mS/cm) and for skim milk (6.7±0.65 and 6.2±3.4 mS/cm), was found to be related to the significant difference 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. To achieve a higher level of microbial inactivation in milk, PEF can be used in combination with heat or organic acids. Molina *et al.* (2005) investigated the shelf life of various PEF-treated skim milks at room temperature or conventional heating at 60 or 65°C for 21 s as well as the combination of PEF treatment and heat or organic acids (acetic or propionic acids) on total number of aerobic bacteria (including *Pseudomonas fluorescens*). In all the studies, PEF treatment with exponential decaying pulses, field intensities of 30 to 50 kV/cm, pulse frequency of 4 Hz and treatment temperature of 40 to 65°C in combination with organic acids had a greater effect on inactivation of microorganisms than PEF alone or combined with mild temperature. In an attempt to further extend the shelf-life of HTST pasteurised milks by PEF treatment Sepulveda *et al.* (2005) subjected pasteurised milk to PEF treatment immediately after pasteurisation and after 8 days storage at 4°C using field intensity of 35 kV/cm and 2 pulses of 2.3 μs duration each. The final temperature was 65°C 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 4°C, while PEF processing after 8 days storage resulted in a longer shelf life of 78 days due to further eradication of enteric and psychrotrophic bacteria by PEF.

Table 1: Effect of PEF on Milk Microflora

S. No.	*Type of Milk*	*PEF treatment*	*Microorganism studied*	*Log reduction*	*Reference*
1.	Fruit juice–whole or skim milk beverages	35 kV/cm, for 1800 μs	*Listeria innocua*	5 Log cycle	Salvia Trujillo *et al.* (2011)
2.	Milk	40 kV/cm	*Listeria innocua*	4.3 Log cycle	Guerrero-Beltrán at el. (2010)
3.	UHT Milk	31 kV/cm	*Pseudomonas*	>5 Log cycle	Craven *et al.* (2008)
4.	UHT Full Fat Milk	30 – 60 kV/ cmfor 26 – 210 μs	*E. Coli*	8 Log cycle	Shin *et al.* (2007)
			Pseudomonas fluorescens	8 Log cycle	
			Bacillus stearothermophilus	3 Log cycle	
5.	Raw Skim Milk, UHT Skim Milk	35 kV/cm, 188 μs	*Pseudomonas fluorescens, Lactococcus lactis, Bacilluscereus*	0.3 – 3 Log cycle	Michalac *et al.* (2003)
6.	Cow Milk	30 kV/cm, 2500 pulses	*Mycobacterium tuberculosis*	5.9Log cycle	Rowan *et al.* (2003)

To explore the potential of PEF technology. some studies have been undertaken to understand the effect of PEF in combination with presence of food grade antimicrobials like nisin and lysozyme (Mittal and Griffiths, 2005). Pol *et al.*, 2000 reported a synergistic effect on subjecting vegetative cells of *B. cereus* to combination of nisin and PEF treatment with 1.8 logs extra reduction in B. cereus numbers than the sum of the reductions obtained from the individual treatments. Calderon *et al.* (1999) combined PEF treatment with nisin addition to inactivate *Listeria innocua* in skim milk. The selected field intensities (and temperatures) were 30 (22°C), 40 (28°C) and 50 (34°C) kV/cm and the number of pulses applied were 10.6, 21.3 and 32, respectively. *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, respectively 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.

3.1. *Effects of PEF on Milk enzymes*

The impact of PEF on inactivation of enzymesis not so clearas some cases report a high level of inactivation while in other cases no effect was observed (Loey *et al.* 2002; Martin-Belloso *et al.* 2005; Yu *et al.* 2012). The effects of PEF treatment on activities of various milk enzymes including alkaline phosphatase, lipases, lactoperoxidase and proteases commonly present in milk or SMUF has been reported by several researchers (Castro *et al.* 2001; Riener *et al.* 2009; Sharma *et al.* 2014). Sharma *et al.* (2014) reported a reduced activity of enzymes plasmin (12%) and xanthine oxidase (32%) on PEF treatment of 26.1 kV/cm for 34 μs in combination with preheating at 55 ^{0}C for 24 s. Riener *et al.* (2009) found a reduction in activity of lipase, alkaline phosphatase and protease of fresh bovine milk by 14%, 29% and 37 %, respectively on the application of 35 kV/cm for 75 μs. Enzyme inactivation requires a more severe PEF treatment than that needed for inactivating microorganisms (Ho *et al.* 1997). The higher the electric field intensity and temperature, the greater reduction in enzyme activity is achievable. Barsoti and Cheftel, (1999) related unfolding, denaturation, breakdown of covalent bonds and oxidation-reduction reactions in the protein structure to enzyme inactivity.

3.2. *Effects of PEF on the Functionality of Milk Nutrients*

Thermal method of preserving milk affects the physico-chemical and functional properties of milk proteins and fat globules. The processing parameters adopted during PEF treatment determines to large extent the changes that will bring denaturation in protein or modify the milk fat structure or nutrient content, for example, rise in temperature. The content of protein and fat greatly affect the yield and physical characteristics of the products made from the milk therefore, it is important to understand the effect of PEF technology on protein and fat as it can have a direct impact on product economics and consumer liking. Upadhyay *et al.* (2019) reported that PEF treatment of milk at low intensities does not significantly affect the proteins, fats, vitamins and other milk nutrients. Thermal or non-thermal treatment of cheese milk has been known to affect the final properties of cheese directly or indirectly. Floury *et al.* (2006) reported that the PEF treatments (field intensities: 45 or 55 kV/cm; pulse widths: 500 and 250 square monopolar pulses, respectively), decreased the coagulation time. At a total treatment time of just 2.1-3.5 μs, there was a significant drop in casein micelle size along with a decrease in milk viscosity and the coagulation properties were enhanced. Wüst *et al.* (2004) found that increasing the field strength decreased the strength of the cottage cheese gel made from PEF-treated skim milk (bipolar square pulses of 2 μs; field intensities: 25 and 28 kV/cm; pulse frequency: 200 and 400 Hz; and flow rate of 120 mL/min at a treatment temperature of less than 45°C). The yield of cottage cheese was marginally increased compared to cheeses made from raw or pasteurised skim milk. At a frequency of 400 Hz, the "raw milk" odour was also got removed. Yu *et al.* (2012) found better rennetability of PEF treated milk (treatment at 30kV/cm or less) in comparison to thermally treated milk (heat treated at 50 ^{0}C or less).

Unlike thermal processing, PEF treatment (30.76 to 53.84 kV/cm) of skim and whole milk at different temperatures of 20 ^{0}C, 30 ^{0}C, and 40 ^{0}C, brought just minor variations in pH, electrical conductivity, density, colour, solids-not-fat (Bermudez-Aguirre *et al.*, 2011) and Reiner *et al.* (2009) reported not much effect of PEF treatment on retinol, vitamin E, thiamine or riboflavin content of raw milk. Hemar *et al.* (2011) found no significant change in the viscosity of concentrated milk (total solids 18%) on PEF treatment at 45 kV/cm for 20 μs.

4. Limitations of PEF

Processing the milk products having air bubbles with PEF technology results in non-uniform treatment which could affect the operational safety. Further, milk products with suspended particulates could cause system malfunction. There should not be any clumps of particles and the maximum particle size in the milk must be smaller than the gap between the electrodes in the chamber for the uniform processing operation. Also, innovations and engineering advancements are required to adopt the PEF technology in its application to handle milk and milk products at large scale with maximum output and efficiency.

5. Conclusion

PEF, as a non-thermal process, does not largely alter the original composition of milk. It has the potential to serve as an alternative to traditional heat treatment of milk with the advantages of maintaining sensory and nutritional quality as of fresh produce. Intensive studies are required to assure the safety of PEF technology in achieving the desired level of enzymatic and microbial inactivation when used alone or in combination with other processing hurdles like heat, preservatives or acids. Further, in order to make PEF technology applicable in the dairy industry, large scale PEF systems will be required with the equivalent efficiency of currently available bench-top or pilot scale systems.

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