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Effect of High Intensity Pulsed Electric Fields on Microbial Inactivation of Cow Milk

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Abstract: High Intensity Pulsed Electric Field (HIPEF) processing of food is a nonthermal method alternative to traditional thermal method. Two HIPEF apparatuses were designed and constructed in this experiment for generating bipolar exponential decay and square wave forms. The central components of the pulse generator consisted of a treatment chamber, pulse driver, cooling system flow meter and a microcontroller. Two coaxial and upgraded cylindrical chambers were made. Different electric fields strength (18-30 kv cm^{-1}), pulse no. (200-1500 for coaxial chamber and 5-120 for the upgraded cylindrical chamber) were applied at various pulse width (0.5-4 μs) and temperatures (25-53°C). The Iranian standard conventional plate count method was employed for microbial count as colony forming unit (CFU mL^{-1}). In all raw cow milk samples testing the microbial counts were reduced significantly. The upgraded cylindrical chamber was more effective than the coaxial chamber. Microbial count reduced 5.5±1.1 times following treating milk samples by the cylindrical chamber and square form bipolar pulses. This reduction for the exponential pulses was 1.8±0.08 times. No changes in milk constituents including Solid Not Fat (SNF), protein and fat contents were observed. For commercialization the milk processing by this system (HIPEF) more researches and financial supports are needed.

Key words: High intensity pulsed electric fields, microbial inactivation, milk, nonthermal, Iran

INTRODUCTION

Consumer demand for naturally produced human foods has increased in Iran in recent years. Most of the educated and civilians in big cities prefer to consume high-quality and minimally or artificially processed foods. These consumers are willing to pay more for ingredients that retain original, fresh nutritional and sensory qualities. This had led to the establishment of new non-governmental organization (Fakharzadeh, 2009). Consequently, a significant investment from the research centers and agro-industries has been paid to the new processing methods for providing the safe and high-quality natural foods in the country recently.

Various methods have been undertaken for food safety and microbial inactivation (Crawfoed *et al*., 1996; Erkmen, 2001; Geveke, 2005), but the dominant conventional treatment is thermal processing, which ensures safety and extends the shelf life of foods. It often leads to detrimental changes in the sensory attributes like taste flavor and color, as well as nutritional quality reduction. This has resulted to the development of alternative nonthermal processing for food preservation or microbial inactivation. High Intensity Pulsed Electric Field (HIPEF) treatment as a nonthermal alternative to traditional food processing has gained increasing interest because its some attractive advantages over the old thermal methods currently used in extending the self life of raw ingredients and foods (Grahl and Markl, 1996; Abram *et al*., 2003). HIPEF processing of foods involves the application of high voltage pulses (typically 20-80 KV cm^{-1}) usually for a couple of microseconds to raw ingredients of foods placed between 2 electrodes (USFDA, 2000). This new technology, which is effective in microbial inactivation results in minimal changes in food quality, including flavor, taste, aroma, color, viscosity, nutrition and even appearance (Siquan, 2003), because it preserves foods without using heat.

Electrical food processing began in the early 1900s. In the beginning microorganisms were inactivated by electrical pasteurization and increasing the temperature of samples (Bendicho *et al*., 2002). However, nonthermal pulsed electric field technology was introduced in the 1960s but its different aspects have not been fully elucidated as yet. Although, numerous research works have demonstrated the inactivation of a wide range of microorganisms both in foods and food products using diversity of HIPEF system, but for making this technology a real breakthrough in food processing some important areas and critical factors need to be further investigated (Barbosa-Canovas *et al*., 1999; Barsotti and Cheftel, 1999; Wouters and Smelt, 1997; Wouters *et al*., 2001).

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Microbial inactivation by HIPEF depends on various key factors, which are critical to outcome of this process. Wouters *et al.* (2001) in an excellent review have classified these factors as process parameters, product parameters and microbial characteristics. The electric field strength, pulse length, pulse shape, number of pulses and temperature have been listed under the main process parameters. In Wouters *et al.* (2001) review, the product parameters were chemical and physical characteristics, conductivity ionic strength and pH of the products. They also noted that microbial inactivation by HIPEF depends on the type, species and strain of microorganisms. It seems the primary mechanism of lethality from HIPEF treatments is cell membrane compression of the microorganisms and pure formation (electroporation) resulting in increased membrane instability and permeability leakage of cytoplasmic contents and lysis. A series of short but, high-voltage pulses causes pores to form in the membrane in liquid media. Depending on factors such as the electric field intensity, pulse duration and numbers, pore formation can be reversible or irreversible. The treated cell membranes by HIPEF become permeable to small molecules. The permeation causes swelling and eventually ruptures the cell membrane (Ramaswamy *et al.*, 2002). Many researchers have reported that HIPEF methods can also, decrease the activity of key enzymes such as, lipoxygenase, pepsin, β-glycosidase, peroxidase and protease, similar to the traditional thermal processing (Zhao and Yang, 2008, 2009).

Although, understanding the mode of HIPEF action on the enzymatic systems in living organisms is necessary for the further technology development and commercialization of this new process, the mechanisms, which are involved in enzyme inactivation are not fully understood at present.

Despite the fact that many studies have been done on critical factors determining microbial inactivation in food materials in liquid or semi-liquid forms by pulsed electric fields and its commercialization internationally, no reports was found carried out by the Iranian researchers even at the initial design level or laboratory scales. The objective of this research was therefore, designing and making a local HIPEF system for reducing the microbial count of raw cow milk at laboratory scale.

MATERIALS AND METHODS

The exponential electric field pulses were applied with a high intensity pulsed electric field apparatus designed and exclusively constructed by the researchers in this experiment. The system was made and set-up to release bipolar exponential and square wave pulses at 0.5-2 μs each. The central components of the pulse generator outlined schematically in Fig. 1, consisted of a treatment chamber, pulse driver, cooling coil, flow meter and two tanks for material (raw milk) and the product (processed milk) with a capacity of 200 cc. Two hundred and twenty volts ac power was turned to 30 kV ac by a set-up transformer and then rectified to a high voltage dc. The whole system was under the control of a microcontroller (At meago 64 from Atmel).

The flow rate of cow milk in this system was variable between 1-100 cc sec^{-1} and controlled by a flow pump (50 W) and the microcontroller. Treatment time ranged from 0.5-2 μs. The required samples were collected after each treatment.

The treatment charmer (coaxial chamber) with capacity of 20cc was constructed of polished stainless steel electrodes by an insulator and plastic supporters at the bases (Fig. 2). Raw cow milk was pumped in order to

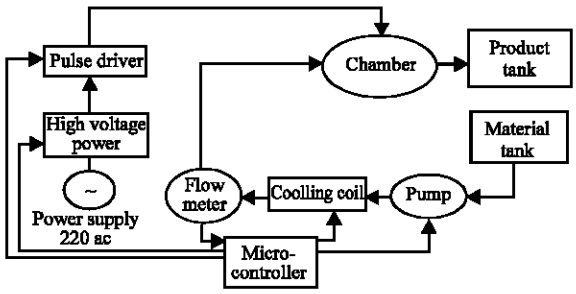

Fig. 1: Block diagram of HIPEF circuit designed and constructed in this experiment

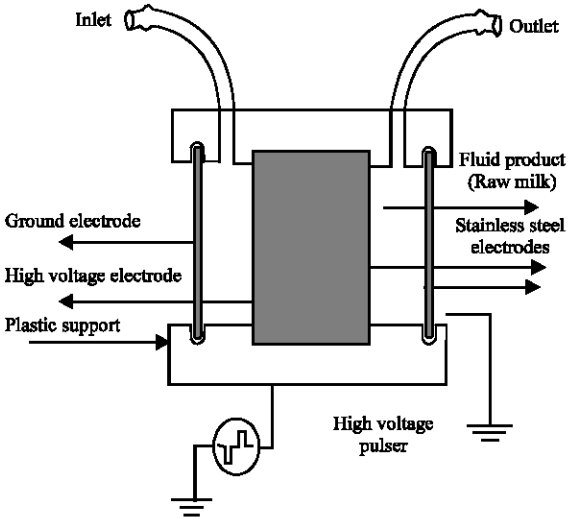

Fig. 2: Schematic of the laboratory-scale coaxial chamber designed and constructed in this experiment

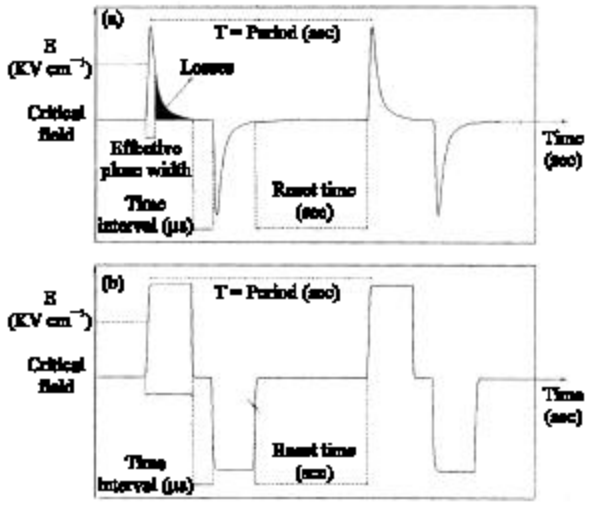

Fig. 3: Forms of the pulsed waves, a): Exponential decay, b): Square pulse

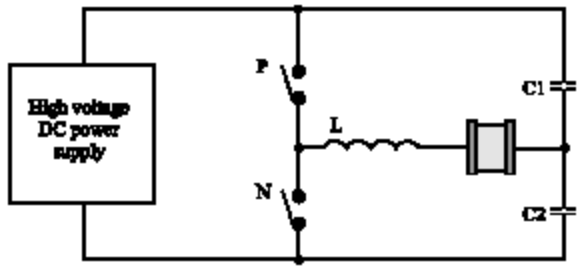

Fig. 4: Schematic of the charge and discharge process applied to the treatment chamber. P: Positive pulse discharge switch, L: Inductor, C1 and C2: Capacitors, N: Negative pulse discharge switch

pass through the electrodes of this chamber. Microbial inactivation was happen following this process. The milk samples were collected, aseptically and chilled immediately for further analysis.

Both exponential decay and square bipolar waves were applied in this system. The efficiency of the square waves was 48% higher than the exponential decay pulses. Because of this higher efficiency the square waves were applied in most of circumstances (Fig. 3).

The 220 ac electricity was transformed to 30 kV high voltage by a rectifier bridge and set-up transformer. This high voltage pulses were applied to the raw milk in the treatment chamber within 0.3-4 µs and electrical field at 18-30 kV cm^{-1}. The numbers pulses were 200-1500 with resting time of 0.1-0.2. sec (Fig. 4). Whole processing system was controlled by the microcontroller.

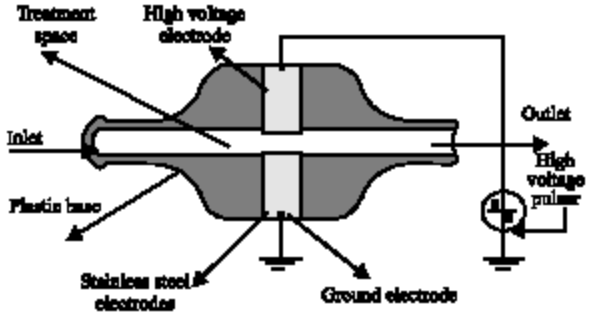

Fig. 5: Schematic of the upgraded cylindrical chamber exclusively designed and made in this experiment

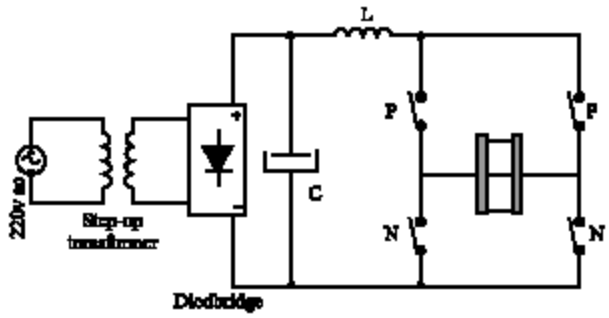

Fig. 6: Diagram of the electrical orbit for the upgraded cylindrical chamber

Since, the electrical field in the coaxial chamber was not uniform an upgraded chamber (Fig. 5) was constructed. In this unique chamber electric pulses with width of 0.5-2 µs in forms of bipolar square waves were applied to the raw milk. Strength of the electric fields was between 18-30 kV with a resting time of 0.9-1.3×10^{3} sec. Its related electrical orbit is shown in Fig. 6.

The Iranian standard conventional plate count method was carried out by the Jehad-Daneshgahi Microbiological Laboratory for measuring colony forming units (CFU mL^{-1} of raw or processed milk).

RESULTS AND DISCUSSION

Table 1 shows, the results of treating raw milk with the HIPEF system designed and constructed in this study. According to these results, electric fields between 18-30 kV cm^{-1} with a pulse numbers of 200-1500 have led to a significant reduction in microbial counts after treatment. The highest reduction in microbial counts (69.2%) was detected after applying 500 pulses in electric field strength of 28 KV cm^{-1}. The lowest reduction equal to 12.8% was obtained under treating raw milk with

200 pulses in the electric field of 18 kV cm^{-1}. Although, the highest reduction was detected in microbial count of the milk sample with treatment temperature of 50°C, but considerable reductions were recorded for the samples in lower temperatures. For example, this reduction for the milk sample with temperature of 38 and 40°C were 56 and 30%, respectively. Therefore, no linear correlation between the microbial count reductions and temperature of the treated milk samples was observed in this study.

It means more studies are needed in this subject. However, generally more pulses led to higher microbial count reduction with some exceptions (Table 1).

Qin *et al.* (1995) have stated the microbial redaction of up to 9 logs had been achieved in laboratory scale system using treatment of liquids (water, milk and juices) by pulsed electric field for 2-300 sec.

Ramaswamy *et al.* (2002) have noted that pulsed electric field processing has lethal effects on various vegetative microorganisms such as bacteria, mold and yeasts. A series of short, high-voltage pulses breaks the cell membranes of microbes in liquid media by electroporation. Small molecules can penetrate the membranes of pulsed electric field treated cells. Permeation led to swelling and rupturing the cell membrane. Microbial reduction may have resulted from this path way in this study.

Data presented in Table 2 clearly show that the upgraded chamber had been more effective than the firstly made chamber (Fig. 2). In this chamber the bipolar square waves had higher reduction effect in microbial count in comparison with the bipolar exponential waves. Microbial counts reduced 5.5±1.1 times after applying the square waves, while this figure for the exponential waves was 1.8±.08 times. It means the effectiveness of the square waves were 3.1 times higher. The most effective electric field was 30 kV cm^{-1} for 2 μs, 11 pulses at 43°C in temperature (Table 2). The least value in microbial inactivation was detected in test no 17, when 30 kv cm^{-1} electric field, was used at 0.6 μs, pulse no of 100 and milk temperature of 31°C. In this testing the microbial count was 9.1×10^5 before treatment and it was reduced to 2.2×10^5 after processing in the upgraded chamber with exponential waves. Reduction rates for the tested samples (Table 2) were 3.0, 4.4, 1.6, 4.1, 5.1, 6.5, 12.7, 3.5, 13.0, 10.1, 6.0, 2.0, 1.9, 8.7, 2.5, 12.2, 8.3, 1.7, 2.2 and 4.4 times for the respective test samples no 1-20.

Table 1: The results of treating raw milk with coaxial chamber of HIPEF apparatus designed and constructed in this experiment (microbial count are expressed as CFU mL^{-1})

Test no.	Pulse shape	Type of pulse	Electric field intensity (kv cm^{-1})	Effective pulse width (μs)	Resting time (sec)	Pulse no.	Milk temperature (°C)	Colony forming unit (CFU mL^{-1}) Before treatment	After treatment
1	Exponential	Bipolar	18	3	0.1	200	32	7.8×10^5	6.8×10^5
2	Exponential	Bipolar	18	3	0.1	500	30	10.4×10^5	8.5×10^5
3	Exponential	Bipolar	22	4	0.2	300	40	6.4×10^5	4.5×10^5
4	Exponential	Bipolar	25	2	0.2	400	43	4.8×10^5	3.4×10^5
5	Exponential	Bipolar	26	4	0.3	200	38	7.3×10^5	3.2×10^5
6	Exponential	Bipolar	26	3	0.25	350	30	6.8×10^5	4.4×10^5
7	Exponential	Bipolar	28	4	0.3	300	25	5.6×10^5	3.4×10^5
8	Exponential	Bipolar	28	2	0.3	500	50	12×10^5	3.7×10^5
9	Exponential	Bipolar	30	0.5	0.1	1500	45	6.4×10^5	2.9×10^5

Table 2: The results of treating raw milk with upgraded cylindrical chamber of HIPEF apparatus designed and constructed in this experiment

Test no.	Pulse shape	Form of pulse	Electric field strength (kv cm^{-1})	Pulse width effective (μs)	Resting time (sec)	Pulse no.	Milk temperature (°C)	Colony forming unit (CFU mL^{-1}) Before treatment	After treatment
1	Square	Bipolar	18	2	1.3×10^3	5	45	8.3×10^5	2.7×10^5
2	Square	Bipolar	20	1.5	1.1×10^3	4	41	12×10^5	2.7×10^5
3	Square	Bipolar	22	2	1.3×10^3	3	52	7.2×10^5	4.6×10^5
4	Square	Bipolar	24	1	1×10^3	7	53	9.1×10^5	2.2×10^5
5	Square	Bipolar	26	1.5	0.9×10^3	8	48	8.2×10^5	1.6×10^5
6	Square	Bipolar	28	1	1.1×10^3	12	51	7.1×10^5	1.1×10^5
7	Square	Bipolar	30	2	1.25×10^3	11	43	8.9×10^5	0.7×10^5
8	Square	Bipolar	26	1	1.15×10^3	8	41	9.4×10^5	2.7×10^5
9	Square	Bipolar	28	2	1.05×10^3	10	39	10.5×10^5	0.8×10^5
10	Square	Bipolar	24	1.5	1.2×10^3	10	37	12.1×10^5	1.2×10^5
11	Square	Bipolar	24	1.5	1×10^3	12	35	13.2×10^5	2.2×10^5
12	Square	Bipolar	24	2	1.15×10^3	20	45	7.5×10^5	3.5×10^5
13	Exponential	Bipolar	20	1	1.25×10^3	100	39	9.1×10^5	4.7×10^5
14	Exponential	Bipolar	23	0.5	0.9×10^3	70	29	8.7×10^5	1.0×10^5
15	Exponential	Bipolar	25	1	0.45×10^3	80	43	8.1×10^5	3.2×10^5
16	Exponential	Bipolar	27	0.7	1.1×10^3	75	35	12.2×10^5	0.9×10^5
17	Exponential	Bipolar	30	0.6	0.95×10^3	100	31	9.1×10^5	1.1×10^5
18	Exponential	Bipolar	23	1	1.1×10^3	115	32	6.5×10^5	3.9×10^5
19	Exponential	Bipolar	25	1	1.2×10^3	120	37	8.1×10^5	2.8×10^5
20	Exponential	Bipolar	27	1	1.1×10^3	100	31	11.5×10^6	5.1×10^5

Table 3: The effect of HIPEF apparatus containing coaxial chamber on raw milk chemical composition

	Solid not fat (SNF) content (%)		Protein content (%)		Fat content (%)	
Test no.	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	8.52	8.52	3.08	3.09	3.36	3.31
2	8.81	8.82	3.00	3.00	3.51	3.48
3	8.91	8.89	3.12	3.12	3.12	3.10
4	8.71	8.68	3.17	3.16	3.25	3.24
5	8.61	8.62	3.95	2.94	3.12	3.11
6	8.58	8.59	3.45	3.46	3.15	3.14
7	8.58	8.59	3.41	3.40	3.41	3.40
8	8.65	8.64	3.42	3.41	3.21	3.18
9	8.50	8.51	3.33	3.30	3.25	3.21
$\bar{X} \pm SE$	8.65±0.14	8.65±0.13	3.21±0.19	3.21±0.19	3.26±0.14	3.24±0.14

Again no linear correlation was found between the reduction rate in microbial counts and the temperature of tested milk samples. Applying higher pulses were not necessarily resulted to higher reduction in microbial counts. For example, treating milk sample with a square form wave and pulse no of 11 resulted to 6 time reduction in microbial count, while processing the sample with pulse no of 20 led to a reduction time of 2.2. Such variation was also detected in applying the exponential wave and pulses (Table 2). Data recorded in Table 3 indicated that treating raw milk with the exclusively made HIPEF apparatus in this experiment had no effect on milk constituents including SNF, protein and fat contents. The average figures and standard deviations at the end of the (Table 3) show some values (e.g., protein and fat contents) were quite similar. It means that no adverse effect has been resulted by applying this process at the noted characteristic.

CONCLUSION

The laboratory-scale made HIPEF was an effective and successful in this experiment. Therefore, this process has good prospects for being used in the intensive dairy industry in the area. The treatment could provide an alternative to the traditional methods since it provides lots of advantages as low destruction of flavor or nutrients. It maintains the nutritional quality of milk, which is fundamental objective in Iranian dairy industry. However, for commercialization of this process and making appropriate apparatus more financial supports and experimentation are needed. To make the this technology a real break through in dairy processing some important areas such as finding the correlation between form of waves, pulse numbers, strength and the temperature of row milk have to be studied intensively.

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