Food Sci. Biotechnol. Vol. 16, No. 6, pp. 1082 ~ 1084 (2007)

RESEARCH NOTE

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Application of Pulsed Electric Fields with Square Wave Pulse to Milk Inoculated with *E. coli*, *P. fluorescens*, and *B. stearothermophilus*

Jung-Kue Shin, Kwan-Jae Jung[1], Yu-Ryang Pyun[1], and Myong-Soo Chung[2]*

Department of Korean Traditional Food Culture, Jeonju University, Jeonbuk 560-759, Korea
[1]Department of Biotechnology, Yonsei University, Seoul 120-749, Korea
[2]Department of Food Science and Technology, Ewha Womans University, Seoul 120-750, Korea

Abstract Ultra-high temperature (UHT) processed full fat milk inoculated with *Escherichia coli, Pseudomonas fluorescens,* and *Bacillus stearothermophilus* was exposed to 30-60 kV/cm square wave pulsed electric field (PEF) with 1 μsec pulse width, and 26-210 μsec treatment time in a continuous PEF treatment system. Eight log reduction was obtained for *E. coli* and *P. fluorescens* and 3 logs reduced for *B. stearothermophilus* under PEF treatment conditions of 210 μsec treatment time, 60 kV/cm pulse intensity at 50°C. There was no significant change in pH and titration acidity of milk after PEF treatment. The electrical energy required to achieve 8 log reduction for *E.coli* and *P. fluorescens* was estimated to be about 0.74 kJ/L.

Keywords: milk, pulsed electric field, electrical energy

Introduction

High voltage pulsed electric field (PEF) processing is an emerging non-thermal technology for food preservation. The PEF is usually applied to the fluid food in the form of short pulses with a pulse duration ranging between a few microseconds and milliseconds. Food may be processed in a short period of time at the ambient temperature, and the energy lost due to heating is minimal. Much research has been proved that both spoilage organisms and pathogens are very effectively killed by PEF treatment (1-5).

It has been shown that high levels of reduction of microorganisms in milk can be achieved by the PEF treatment (6-8). The lethal effect of the PEF treatment for milk is mainly dependent on the electric field strength and treatment time (9-11). Treatment temperature and the type of the medium, in which the bacteria are suspended, are also critical factors affected to the lethal rate of microorganisms (9).

The objective of this research was to investigate the effects of PEF on the inactivation of inoculated *Escherichia coli, Pseudomonas fluorescens,* and *Bacillus stearothermophilus* into ultra-high temperature (UHT) milk. We also analyzed the energy saving effect of this PEF milk pasteurization comparing with conventional thermal process.

Materials and Methods

Microorganisms Shaking cultures of *E. coli* (ATCC 10536), *P. flourescens* (ATCC 13525), and *B. stearothermophilus* (ATCC 7953) were centrifuged. The harvested cells were washed once with 0.85% saline, and inoculated into 200 mL of UHT processed milk (3.4% fat) at the level of ca. 10^8, 10^8, and 10^6 cells/mL for *E. coli*, *P. flourescens,* and *B. stearothermophilus*, respectively. The viable cell counts in inoculated milks before and after PEF treatments were assayed by counting the colony forming units (CFU) on nutrient agar. The mean CFU of at least 3 plates was reported for each sample.

PEF treatments Square wave pulse was generated by the apparatus designed and manufactured by Jaepae Hi-Tec (Incheon, Korea), which supplied 50 kW of the maximum power at a frequency of 5-50 kHz. A continuous treatment chamber, consisting of 7 units with 0.025 mL capacity each and 0.2 cm gap, was used to apply the high voltage PEF treatment with a constant flow rate for the inactivation of microorganisms. The pulse rate was adjusted to achieve 26-210 μsec treatment time as function of electric field intensity (30-60 kV/cm) and pulse width of 1 μsec. Treatment temperature was varied from 10 to 50°C. The pulse treatment was started when the sample was equilibrated at the desired temperature.

Results and Discussion

Inactivation of microorganisms Figure 1 shows the killing effects of PEF treatment in UHT milk on 3 test microorganisms. The inactivation of all inoculated bacteria increased with increasing treatment time and temperature. *E. coli* and *P. fluorescence* were reduced by 8 log cycles at 60 kV/cm and 50°C after 200 μsec of treatment, which means that her inocula were inactivated nearly completely. However, *B. stearothermophilus*, which is known as a representative thermostable microorganism, was reduced by only 3 log cycles. The electrical energy required obtaining the 8 log reduction of *E. coli* or *P. fluorescence* was approximately 0.74 kJ/L by applying the equation as follows:

$$E = v \cdot I \cdot t / V \quad (1)$$

*Corresponding author: Tel: +82-2-3277-4508; Fax: +82-2-3277-4508
E-mail: mschung@ewha.ac.kr
Received June 11, 2007; accepted August 20, 2007

where, E: electrical energy per treated volume in kJ/L, v: voltage in kV, I: current in A, t: total treatment time in sec, V: treated volume in L.

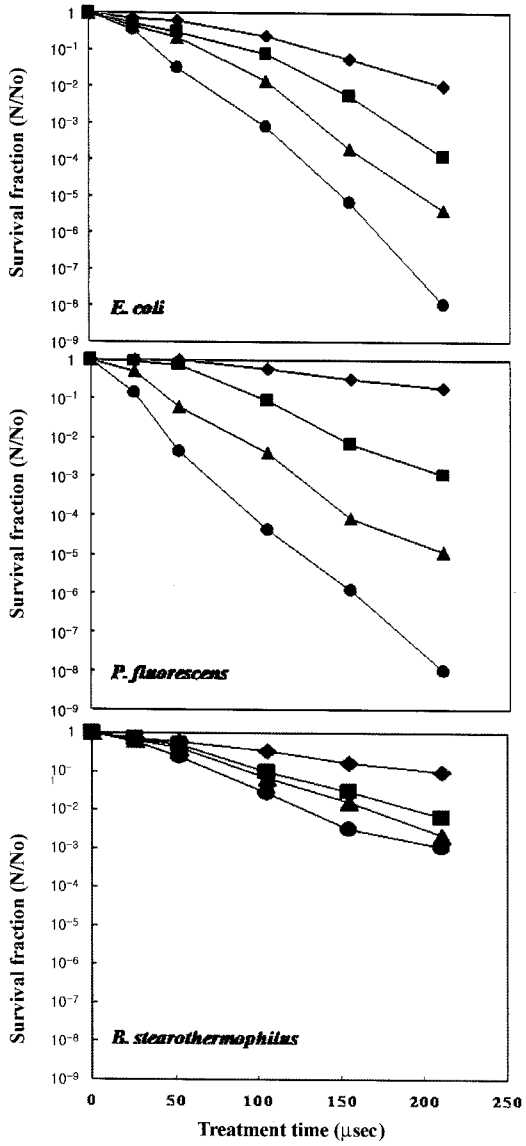

Fig. 1. Effects of PEF treatment time and temperature on the inactivating of microorganisms suspended in full fat UHT milk (60 kV/cm). ◆ 10°C, ■ 30°C, ▲ 40°C, ● 50°C.

Table 1. pH and titration acidity of milk treated with PEF at 50°C and 60 kV/cm

Treatment time (μsec)	pH	Titration acidity (%)
0	6.6	0.15
26.25	6.59	0.152
52.5	6.58	0.153
105	6.58	0.155
155	6.58	0.16
210	6.57	0.166

This indicates that continuous PEF pasteurization technique is a great energy efficient process comparing with conventional thermal process for pasteurization of raw milk with a similar sterility. Biziak *et al.* (12) reported that the energy required for manufacturing processed milk with a continuous pasteurization system at 138-149°C was estimated from 357.9 to 697.6 kJ/kg. Chandarana *et al.* (13) also estimated required energy as 217-667 kJ/kg when milk was processed at 120-140°C. Furthermore, titration acidity and pH were not affected by the PEF treatment conditions dealt in this study (Table 1). These results indicate that PEF treatment of milk has no effect on milk quality and is suitable to milk pasteurization.

Inactivation kinetics The inactivation of 3 test organisms increased with increasing the electrical field strength when the field strength, E, exceeded a critical value E_c (Fig. 2). According to the reports of Grahl and Markl (14), the microbial survival fraction, S, is related to the electric field strength, E, as following equation:

$$\ln S = - k_E (E-E_c) \quad (2)$$

where, k_E: regression coefficient or death rate constant, E: applied electrical field in kV/cm, E_c: critical electrical field obtained by the extrapolated value of E for 100% survival.

Table 2 shows the results for estimating critical electrical field strength at different treatment temperatures. As shown in this Table, the values of E_c decreased with increasing treatment temperature for all tested microorganisms in this study. *B. stearothermophilus* showed slightly higher value of E_c than those of *E. coli* and *P. fluorescens*. Hülsheger *et al.* (15) proposed an inactivation kinetic model that relates microbial survival fraction, S, with total PEF treatment time (t) in the form of:

$$\ln S = k_t (t-t_c) \quad (3)$$

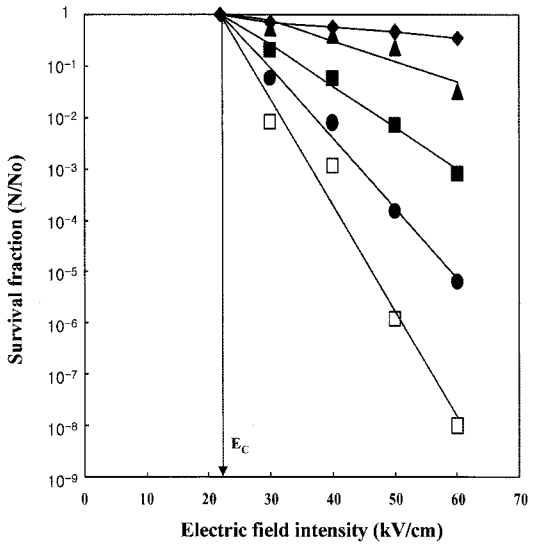

Fig. 2. Dependence of the fractions of surviving cells of *E. coil* on the electric field intensity of PEF treatment at 50°C. ◆ 26.25 μsec, ▲ 52.5 μsec, ■ 105 μsec, ● 155 μsec, □ 210 μsec.

Table 2. Electrical parameters of microorganisms from regressive analysis

Temperature (°C)	E_c, Critical electric field strength (kV/cm)			T_c, Critical treatment time (μsec)		
	E. coli	*P. fluorescens*	*B. stearothermophils*	*E. coli*	*P. fluorescens*	*B. stearothermophils*
10	28	27	28	24	26	40
30	24	25	27	23	22	32
40	22	23	24	22	14.1	28
50	21	21	21	18	8.3	25

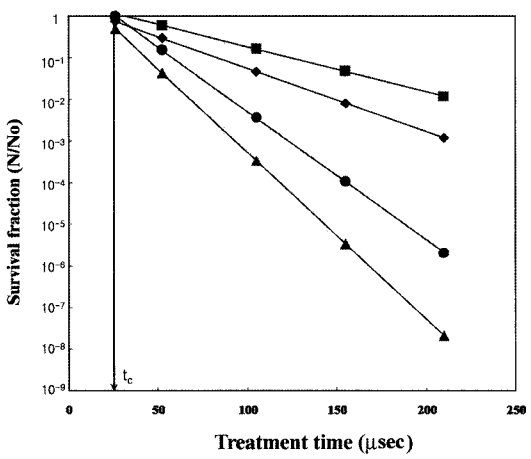

Fig. 3. Dependence of the fractions of surviving cells of *E. coli* on the treatment time of PEF treatment at 50°C. ■ 30 kV/cm, ◆ 40 kV/cm, ● 50 kv/cm, ▲ 60 kV/cm.

where, k_t: regression coefficient or death rate constant, t: treatment time in μsec, t_c: extrapolated value of t for 100% survival or critical treatment time.

Typical treatment time effect on the inactivation of *E. coli* at 50°C is shown in Fig. 3. Regression analysis showed the survival fraction followed a first order kinetic of Eq. 3. As shown in Table 2, the critical treatment time, t_c, decreased with increasing treatment temperature. The results also indicated that temperature dependence of death rate constant, k_t, could be explained by the Arrhenius relationship (data not shown). Grahl and Markl (14) reported that the values of t_c decreased with increasing electrical field strength above E_c and suggested that a factor of $1.5 \times E_c$ for determining critical times, because t_c became a constant beyond this multiple. In this study, t_c approached to a constant value at the field strength exceeding 40 kV/cm (Fig. 3). In the PEF treatment microbial death kinetics are very complicated if we consider the effects of treatment time, electric field strength, and medium temperature simultaneously, and further studies are needed.

References

1. Ho SY, Mittal GS, Cross JD, Griffiths MW. Inactivation of *P. fluorescens* by high voltage pulsed electric pulse. J. Food Sci. 60: 1337-1340 (1995)
2. Lubicki P, Jayaram S. High voltage pulse application for the destruction of the Gram-negative bacteria *Yersinia enterocolitica*. Bioelectroch. Bioener. 43: 135-141 (1997)
3. Mazurek B, Lubicki P, Staroniewicz Z. Effect of short duration HV pulses on bacteria and fungi. IEEE T. Dielect. El. In. 2: 418-425 (1995)
4. Amiali M, Ngadi MO, Smith JP, Raghavan GSV. Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg yolk. J. Food Eng. 79: 689-694 (2007)
5. Fleischman GJ, Ravishankar S, Balasubramaniam VM. The inactivation of *Listeria monocytogens* by pulsed electric field (PEF) treatment in a static chamber. Food Microbiol. 21: 91-95 (2004)
6. Knorr D, Guelen M, Grahl T, Sitzmann W. Food application of high voltage electric field pulses. Trends Food Sci. Tech. 5: 71-75 (1994)
7. Zhang Q, Barbosa-Canovas GV, Swanson BG. Inactivation of *E. coli* for food pasteurization by high-strength pulsed electric fields. J. Food Process Pres. 19: 103-118 (1995)
8. Evrendilek GA, Zhang Q, Richter ER. Application of pulsed electric fields to skim milk inoculated with *Staphylococcus aureus*. Biosyst. Eng. 87: 137-144 (2004)
9. Jayaram S, Castle GSP. Kinetics of sterilization of *Lactobacillus brevis* cells by the application on high voltage pulses. Biotechnol. Bioeng. 40: 1412-1420 (1992)
10. Vega-Mercado H, Martin-Belloso O, Barbosa-Canovas GV, Swanson BG. Non-thermal food preservation (pulsed electric fields). J. Food Sci. Tech. Mys. 8: 151-157 (1997)
11. Qin BL, Barbosa-Canovas GV, Swanson BG. Inactivating micro-organisms using a pulsed electric field continuous treatment system. IEEE T. Ind. Appl. 34: 43-50 (1998)
12. Biziak RB, Swartzel KR, Jones VA. Energy use for continuous thermal processing of milk. J. Food Sci. 50: 1607-1614 (1985)
13. Chandarana DI, Frey BC, Stewart LE, Mattick JF. UHT milk processing effect on process energy requirements. J. Food Sci. 49: 977-978 (1984)
14. Grahl T, Markl H. Killing of microorganisms by pulsed electric fields. J. Appl. Microbiol. Biotechnol. 45: 148-157 (1996)
15. Hülsheger H, Pottel J, Niemann EG. Killing of bacteria with electric pulses of high field strength. Radiat. Environ. Bioph. 20: 53-65 (1981)