INACTIVATION OF *LISTERIA INNOCUA* AND *PSEUDOMONAS FLUORESCENS* BY PULSED ELECTRIC FIELDS IN SKIM MILK: ENERGY REQUIREMENTS

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ABSTRACT

Pulsed electric field energy applied over a short duration of time was effective in the inactivation of Listeria innocua *and* Pseudomonas fluorescens *inoculated into 0.2% skim milk. Additionally, the energy consumption was reasonable for industry applications compared with the alternative of thermal pasteurization. The energy densities required to achieve three log reductions of the microorganisms were 120, 212 and 270 kJ/L for* L. innocua *corresponding to input voltages of 30, 35 and 40 kV, and 88, 105 and 128 kJ/L for* P. fluorescens *under the same input conditions. Treatment times were, respectively, 145* μs *and 290* μs*, and exponentially decaying wave pulses with time duration of 3* μs *were selected. For* L. innocua*, the inactivation of viable cells was significantly different (*P < 0.05*) between energy inputs of 120, 212 and 270 kJ/L. Meanwhile, the inactivation of* P. fluorescens *exhibited significant differences (*P < 0.05*) between energy inputs of 88 and 128 kJ/L, but not between inputs of 105 and 128 kJ/L. These results consistently indicated that microbial inactivation in skim milk increased as the energy intensity and the treatment time increased.*

INTRODUCTION

In milk processing, thermal pasteurization is the most commonly used preservation technique because of its reliability, relative simplicity and long

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history of safety. In general, heat treatment is predominantly used in food pasteurization to inactivate microorganisms for increased shelf life and maintenance of food safety. However, thermal damage to liquid foods can adversely affect the flavor in addition to nutrient content (Zhang *et al.* 1995a). New emerging technologies to pasteurize and sterilize liquid foods without increasing the temperature during the process are receiving increased attention from the food industry. Pulsed electric field (PEF) technology is one of the most promising alternatives available to preserve foods. Its attraction lies in the fact that PEF treatment is conducted at temperatures below those used in thermal pasteurization, for less than 1 s. As a result, the energy loss due to heating of foods is minimized (Barbosa-Cánovas *et al.* 1999). Further, even some of this energy can be saved by introducing thermal recovery into the PEF system, in effect reusing the PEF energy that goes into the food as heat to raise the temperature of the untreated food so that this preheating – at no additional energy cost – can synergistically work with the PEF treatment to improve microbial inactivation (Heinz *et al.* 2003).

PEF technology has successfully pasteurized milk, juices and other liquid foods, as well as inactivated microorganisms suspended in skim milk and model foods (Martín *et al.* 1994; Pothakamury *et al.* 1996; Vega-Mercado 1996; Martín *et al.* 1997; Barbosa-Cánovas *et al.* 1998; Reina *et al.* 1998; Raso *et al.* 2000). Some of the most important microorganisms in milk and dairy products are *Pseudomonas fluorescens*, *Streptococcus thermophilus* and *Listeria monocytogenes*; however, only the last one is commonly found in pasteurized milk. *L. monocytogenes* can survive under a broad range of temperature (1–45C) and pH (4.1–9.6) conditions, and some of the products affected are raw milk, pork, raw poultry, ground beef and some vegetables (Jay 1992). *P. fluorescens* is one of the psychrotrophic microorganisms responsible for the deterioration of refrigerated milk generating undesirable flavors (Villamiel and Jong 2000).

The energy requirements to pasteurize liquid foods with PEF may vary widely depending on factors such as the intensity of the electric field applied, the number of pulses, frequency, treatment time and characteristics of the food material. Qin *et al.* (1995) reported an energy density requirement of 28 J/mL for the treatment of apple juice at 50 kV/cm, and 10 pulses of 2-μs duration. Quass (1997) reported 140 J/mL for liquid whole eggs treated with PEF at 40 kV/cm, 50 pulses of 2-μs duration. Zhang *et al.* (1995b) achieved nine log reductions of *Escherichia coli* suspended in simulated milk ultrafiltrate at an electric field intensity of 70 kV/cm, with 80 pulses of 2-μs duration, and an energy density requirement of 97 kJ/L. By applying thermal regeneration with apple juice in a continuously circulating PEF system, Heinz *et al.* (2003) not only drastically reduced their energy consumption from 100 to 10 kJ/kg and the food's temperature increase from 71 to 2.5C by this method, but also

achieved an additional log reduction of microorganisms by the resulting increase in the untreated food's initial temperature from 45 to 65C.

According to Zhang *et al*. (1995b), the energy density (Q) for exponential decay waveforms is defined by:

$$Q = \frac{V_0^2 C_0 n}{2v} = \frac{V_0^2 t}{2Rv} \quad (1)$$

where C_0 is the capacitance (μF) of the energy storage capacitor, V_0 the initial charge voltage (Volts), n the number of pulses applied, t the treatment time (μs), R the effective resistance (Ohms) and v the volume of the treatment chamber (mL).

The pulsed energy is discharged to the fluid food as Joule heating (Martín *et al*. 1994). Therefore, the maximum increase in temperature (ΔT) in the treatment chamber can be calculated using basic thermodynamic equations when no refrigeration of the fluid food is required or when the pulse repetition rate is very high:

$$\Delta T = \frac{Q}{\rho C_{\mathrm{p}}} \quad (2)$$

where ρ and C_{p} are the density and specific heat of the fluid food in the treatment chamber, respectively.

According to De Jong and Van Heesch (1998), at least 100–200 kJ/L is required to pasteurize liquid foods with PEF alone. In contrast, 300 kJ/L is generally required for thermal pasteurization, depending on the amount of heat regenerated. As a further comparison, Zhang *et al*. (1994) report that 360 J/mL is consumed in the heating portion of a 100% efficient high-temperature short-time (HTST) pasteurization process, while their comparable PEF pasteurization process required only 77–232 J/mL to inactivate several types of microorganism in potato dextrose agar.

In order to study the inactivation of two target microorganisms in milk such as *Listeria innocua* and *P. fluorescens*, PEF was used as an alternative to thermal pasteurization. Two key objectives of this study were first to determine whether the energy consumption for this PEF treatment of milk would prove economically feasible compared with the results reported in the literature, and second to determine which levels of energy consumption would give the best inactivation of the target microorganisms. Thus, the requirements of energy density for each microorganism suspended in skim milk in a PEF coaxial treatment chamber were determined for three different voltage and number-of-pulse combinations. A recirculation system with three energy inputs for

each microorganism was used at a given number of pulses and different treatment times. A process of thermal regeneration (i.e., preheating of the food sample) was not implemented in this experiment, although such could be set up in future investigations. Instead, to ensure that the method of inactivation was through nonthermal PEF treatment alone, the sample was recirculated through cooling coils in order to keep the temperature stable and below pasteurization levels.

MATERIALS AND METHODS

Microbial Preparation

L. innocua (ATCC 51742, Rockville, MD) and *P. fluorescens* (ATCC 17926) were used in this study. Both microorganisms were grown according to ATCC procedure. Tryptic soy broth (DIFCO, Detroit, MI) enriched with 0.6% yeast extract (TSBYE) was used as the growth medium for *L. innocua* and *P. fluorescens*. One milliliter of frozen culture of *L. innocua* was thawed and inoculated in 50 mL of TSBYE with continuous agitation at 190 rpm in a temperature-controlled shaker (Model BSB-332A-1, GS Blue Electric, Blue Island, IL) at 37C for 12 h to reach the early stationary phase. Similar procedure was followed for the growth of *P. fluorescens* in reaching the early stationary phase by incubating the organism at 37C for 10 h. Harvested cultures were stored at –70C with 1 mL of 20% glycerol for later use in PEF experiments.

Microbial Inoculation

Raw whole milk containing 3.5% protein and 3.3% fat was provided by Washington State University's Creamery (Pullman, WA). The milk was separated into skim milk (0.2% fat) and cream at 4C using a De Laval separator, Model No. 614 (De Laral, Lund, Sweden). One pellet was added to 2 L of skim milk at 7C, 30 min prior to treatment, to reanimate *L. innocua* and *P. fluorescens* cells. The initial microbial concentration during experiments was 2.5×10^7 and 1.3×10^6 cfu/mL for *L. innocua* and *P. fluorescens* cells, respectively. To access the cfu/mL before and after PEF, serial dilutions were performed in sterilized 0.1% peptone. For PEF inactivation of *L. innocua* and *P. fluorescens*, dilutions were performed in TSAYE and *Pseudomonas* agar F. The plates were incubated at 37 $\pm$ 1C/24 h and 30 $\pm$ 1C/48 h, respectively.

PEF Processing

A pilot scale continuous PEF system at Washington State University was used to treat the inoculated skim milk in a recirculation mode. High-intensity

PEFs were produced by discharging a 0.5-μF capacitor via a gas spark–gap switch in a treatment chamber. This chamber has two coaxial stainless steel electrodes with a gap of 0.66–0.69 cm and a treatment volume of 29.96–30.96 cm^3. Two cooling coils with a total volume of 49 mL were connected in front of and behind the treatment chamber, with the ends submerged in water baths to maintain the treatment temperature below 35C. Actual temperature was monitored by digital thermocouples, and predictions of temperatures were calculated by Eq. (2).

The pulse generator used in this research has already been described by Barbosa-Cánovas *et al*. (1999) where input voltages of 30, 35 and 40 kV were supplied from the power source to produce average electric field intensities ranging from 32.5 to 38.9 kV/cm, while the number of applied pulses ranged from 5 to 100. An exponential decay wave pulse was set at 2- to 3-μs pulse width with a pulse repetition frequency of 3 Hz. The flow rate of the milk was maintained constant at 8.33 mL/s throughout the PEF experiment. Detailed process conditions used in this research are listed in Table 1.

The number of pulses (n) applied to skim milk was calculated using Eq. (3), where f is the repetition frequency (Hz), F the volumetric flow rate of the milk (mL/s) and v the volume of the treatment chamber (mL):

TABLE 1.
PROCESSING CONDITIONS DURING PULSED ELECTRIC FIELD INACTIVATION OF *LISTERIA INNOCUA* AND *PSEUDOMONAS FLUORESCENS* SUSPENDED IN SKIM MILK

Parameters	Input voltage (kV)		
	30	35	40
Average electric field (kV/cm)	32.5	37.8	38.9
Peak voltage (kV)	22.4	26.1	25.6
Peak current (Amps)	4,300	8,460	8,900
Effective pulse width (μs)	2.9	3.0	2.9
Number of pulses	50–100	50–100	50–100
Chamber volume (cm^3)	30.97	30.97	29.86
Electrode gap (cm)	0.69	0.69	0.66
Chamber resistance (Ohms)	3.81	2.96	2.98
Treatment time (μs)	145–290	145–290	145–290
Treatment temperature (C)	13 ± 1	25 ± 1	33 ± 1
Predicted treatment temperature (C)	15 ± 1	26 ± 1	33 ± 1
Peak volumetric energy (kJ/L)	120/88	212/105	270/128
Skim milk conductivity (S/m)	0.41	0.53	0.49

$$n = \frac{fv}{F} \quad (3)$$

Voltage and Current Measurements

During processing, voltage and current waveforms were recorded using a high-voltage probe connected to an oscilloscope (Hewlett Packard 5422A, Colorado Springs, CO). Five hundred data points were collected. Plots of voltage and current versus time were made, and the peak voltage and current read directly from the curve. The electric field intensity between coaxial electrodes, E_{co} (kV/cm), was calculated in Eq. (4), where V_p is the peak or average voltage, r_{ave} is the average radius at which the electric field is measured (Eq. 5) and R_1 and R_2 are the radii of inner and outer electrodes, respectively:

$$E_{co} = \frac{V_p}{r_{ave} \ln \frac{R_1}{R_2}} \quad (4)$$

$$r_{ave} = R_2 + \frac{R_1 - R_2}{2} \quad (5)$$

The electric field intensity in the treatment chamber is calculated halfway between the two electrodes, reporting a mean electric field distribution instead of a single value at R_1 or R_2. The uniformity of the electric field in the treatment chamber with coaxial electrodes can be improved if $(R_1 - R_2)/2 < R_2$.

Energy Determination

The energy absorbed during PEF inactivation of *L. innocua* and *P. fluorescens* in skim milk was determined by integrating the pulse voltage and current traces and then plotting the energy in Joules per pulse versus time (μs). The following expression was used to calculate the energy in Joules per pulse:

$$Q = \int_0^t P(t)\mathrm{d}t = \int_0^t v(t)i(t)\mathrm{d}t \quad (6)$$

where Q (energy in Joules) is the integral over time (t) of the power P in Watts, which is the product of the instantaneous pulse voltage (v) and the current (i) (Barbosa-Cánovas *et al.* 1999). The total energy absorbed during the PEF

inactivation of microorganisms is calculated by multiplying the energy per pulse times the total number of pulses applied.

Time Constant and Treatment Time

The time constant (τ) is 37% of the maximum peak voltage measured from the plot of peak voltage versus time. The treatment time t (μs) is given by:

$$t = \tau * n \tag{7}$$

where n is the number of pulses applied.

Statistical Analysis of Data

Data were analyzed using the general linear model procedures (SAS Institute, Cary, NC, 1999). Comparisons of means were performed using Tukey's studentized range test. Statistical significant effects were at $P < 0.05$ levels.

RESULTS AND DISCUSSION

Results are presented in Tables 1–3 and Figs. 1 and 2. Approximately three log cycle reductions of *L. innocua* and *P. fluorescens* in inoculated skim milk were reached by PEF treatments (Figs. 1 and 2). The total energy input required to attain three log reductions of viable *L. innocua* were 120, 212 and 270 kJ/L for the three different electric field intensities applied, and 88, 105 and 128 kJ/L in the case of *P. fluorescens* (Figs. 1 and 2). The mean electric

TABLE 2.
ANALYSIS OF VARIANCE (ANOVA) FOR ENERGY INACTIVATION OF *LISTERIA INNOCUA* AND *PSEUDOMONAS FLUORESCENS* SUSPENDED IN SKIM MILK

Source of variation	*L. innocua*			*P. fluorescens*		
	df	MS	*P*	df	MS	*P*
Energy	2	3.32	0.0001	2	1.71	0.0008
Pulse (interaction)	10	1.94	0.0001	6	4.03	0.0001
Energy * pulse	20	0.15	0.0001	12	0.34	0.0764
Error	33	0.03	–	21	0.17	–
Total	65	–		41	–	

df, degrees of freedom; MS, mean square; *P*, probability.

TABLE 3.
TUKEY'S STUDENTIZED RANGE TEST FOR LOG (cfu/mL) OF *LISTERIA INNOCUA* AND *PSEUDOMONAS FLUORESCENS* INACTIVATION IN SKIM MILK AT THREE DIFFERENT TOTAL ENERGY INPUTS

L. innocua		*P. fluorescens*	
Mean energy (kJ/L)	Mean survivors*	Mean energy (kJ/L)	Mean survivors*
120	6.74 ± 0.03^{a}	88	5.21 ± 0.17^{a}
212	6.30 ± 0.03^{b}	105	4.83 ± 0.17^{ab}
270	5.96 ± 0.03^{c}	128	4.52 ± 0.17^{b}

* Means with the same letter within column are not significantly different at $P \leq 0.05$.

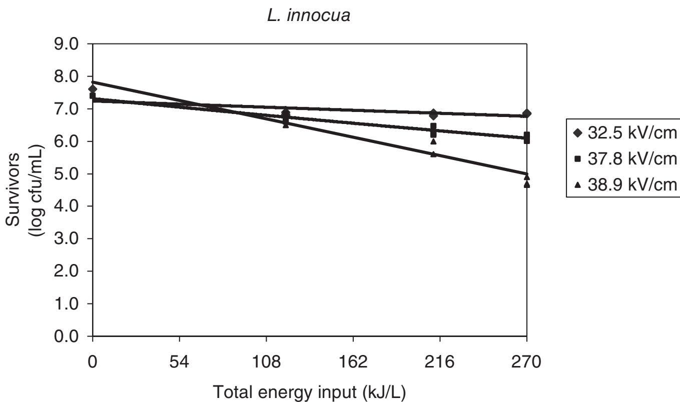

FIG. 1. PULSED ELECTRIC FIELD INACTIVATION OF *LISTERIA INNOCUA* IN SKIM MILK TREATED AT TOTAL ENERGY INPUT OF 120, 212 AND 270 kJ/L
The electric field corresponding to those energy levels are 32.5, 37.8 and 38.9 kV/cm, respectively. Each data point corresponds to the mean of three replicates.

field intensities applied to the inoculated skim milk (Table 1) were 32.5, 37.8 and 38.9 kV/cm and corresponding input voltages 30, 35 and 40 kV, respectively. Peak voltage and peak current accelerated with increasing energy intensity (Table 1). The effect of energy on the viability of both microorganisms is illustrated in Figs. 1 and 2. Significant differences ($P < 0.05$) between energy and number of pulses were found in the inactivation of *L. innocua* and *P. fluorescens* (Table 2). As the process temperature increased from 13 to 33C (Table 1), the inactivation of both organisms accelerated with the increasing total energy input and electric field intensity (Figs. 1 and 2).

The inactivation of viable cells of *L. innocua* was highly significant ($P < 0.05$) at the three energy intensities applied (Table 3), whereas the inac-

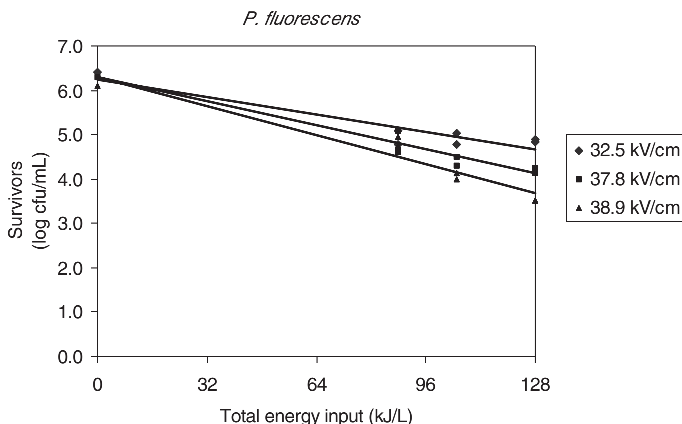

FIG. 2. PULSED ELECTRIC FIELD INACTIVATION OF *PSEUDOMONAS FLUORESCENS* IN SKIM MILK TREATED AT TOTAL ENERGY INPUT OF 88, 105 AND 128 kJ/L
The electric field corresponding to those energy levels are 32.5, 37.8 and 38.9 kV/cm, respectively. Each data point corresponds to the mean of three replicates.

tivation of viable cells of *P. fluorescens* was significantly different ($P < 0.05$), ranging between 88 and 128 kJ/L, but not between 88 and 105 kJ/L, or between 105 and 128 kJ/L (Table 3). This indicates that 88 kJ/L of total energy input was sufficient to inactivate cells of *P. fluorescens*. The trend toward energy efficiency, in terms of increasing inactivation, was 120 → 212 → 270 kJ/L for *L. innocua* and 88 → 105 → 128 kJ/L for *P. fluorescens*, respectively (Table 3).

Gram-positive (*L. innocua*) bacteria were more resistant to PEF treatment than Gram-negative (*P. fluorescens*) bacteria. Application of half the number of pulses with the same electric field intensities to *P. fluorescens* achieved the same log reductions as for *L. innocua* (Figs. 1 and 2). These differences are due to the varying morphological and biochemical properties of the respective cell parts, particularly the cell membranes (De Jong and Van Heesch 1998).

The effect of energy intensity is related to treatment time and process temperature (Table 1, Figs. 1 and 2). As the treatment time and temperature were increased, the applied treatment resulted in higher inactivation of viable cells of both organisms. This temperature rise depends on the total electric energy supplied and on the pulse frequency, pulse width and electric field intensity (Raso *et al*. 2000). Heating could lead to significant changes in the actual electric field intensity and conductivity of the sample and, consequently, to changes in the actual electric field intensity applied to the sample during processing (Raso *et al*. 2000). In this research, both conductivity and pulse

width varied with treatment temperature (Table 1). At elevated temperature, the pulse width is reduced because of the decreased resistivity of the liquid medium. Thus, the temperature effect should cause higher survivability because of the decreased time constant. Experimental observations, however, have shown a decreased survival rate with increased temperature at comparable field strengths (Jayaram *et al.* 1992). This statement is in agreement with the results obtained in this experiment. The liquid medium in which the cells are suspended can exhibit a wide range of conductivity (S/m), depending on the type of medium and process temperature (Jayaram *et al.* 1992).

As observed in Table 1, at temperatures of 13, 25 and 33C, the conductivity of skim milk was 0.41, 0.53 and 0.49 S/m, respectively. This indicates that the conductivity of skim milk increased when treatment temperature was increased from 13 to 25C, but then decreased at 33C because of a decrease in the electrode gap from 0.69 to 0.66 cm (Table 1). The effect of electric field intensity on the survivability of both microorganisms is presented in Figs. 1 and 2. Survivability declined rapidly at low field strength and more gradually at higher field strengths. The treatment temperatures were always below the predicted temperatures calculated in Eq. (2) (Table 1). The predicted temperatures were 15, 26 and 33C. A positive correlation, r^2 (0.997), was obtained (data not displayed) between the experimental and the predicted treatment temperatures (Table 1). Reina *et al.* (1998) reported that increasing the treatment temperature from 10 to 50C, and the treatment time from 300 to 600 μs during inactivation of *L. monocytogenes* in whole milk, increased the reduction of viable cells of this organism to four log cycles. In the present experiment, as treatment temperatures increased from 13 to 33C, the PEF energy increased from 120 to 270 kJ/L, and also from 88 to 128 kJ/L. Consequently, the inactivation of *L. innocua* and *P. fluorescens* also increased by 2.7 and 2.6 log cycles (Table 1, Figs. 1 and 2). Zhang *et al.* (1995b) reported that increasing the temperature from 7 to 20C significantly increased the PEF inactivation of *E. coli* in simulated milk ultrafiltrate, but additional increase in temperature (from 20 to 33C) did not result in increased PEF inactivation. According to Hülsheger *et al.* (1981), there is a synergistic effect with PEF treatment from the temperature of the medium on the inactivation of microorganisms. Thus, increasing the medium temperature decreases the transmembrane potential of the cell membrane, causing thermal injury and resulting in higher inactivation (Jeyamkondan *et al.* 1999).

The results obtained in this research suggest that the inactivation of viable cells of *L. innocua* and *P. fluorescens* was due to the intensity of PEF energy applied to skim milk and not to the heating effect. The maximum temperature of the most severe treatment was 33C, which could not affect the viability of *L. innocua* and *P. fluorescens*, and which only minimally affected the actual value of the electric field intensity applied to the milk.

Energy consumption is a very important issue concerning the industrial application of the PEF process. In this study, the total amount of energy used for the inactivation of *L. innocua* and *P. fluorescens* in skim milk averaged 201 and 107 kJ/L, respectively. These total energy inputs are lower than the energy used for the heating portion of the HTST milk pasteurization process, which requires more than 300 kJ/L to batch pasteurize 1 L of milk without heat regeneration (De Jong and Van Heesch 1998).

CONCLUSIONS

High-intensity PEF energy effectively reduced the population of *L. innocua* and *P. fluorescens* in skim milk by approximately three log cycles, using an average energy input of 201 and 107 kJ/L, respectively, for the respective microorganisms, which is lower than the energy typically supplied for HTST milk pasteurization. The energy required to inactivate both organisms depended on the electric field intensity, number of pulses and type of microorganism. The inactivation of gram-positive *L. innocua* required more treatment time than gram-negative *P. fluorescens*. Increasing the energy intensity induced microbial inactivation with PEF treatment. The results of PEF inactivation of *L. innocua* and *P. fluorescens* demonstrate the feasibility of the technology for relatively low energy preservation of liquid foods.

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REFERENCES

BARBOSA-CÁNOVAS, G.V., POTHAKAMURY, U.R., PALOU, E. and SWANSON, B.G. 1998. High intensity pulsed electric fields: Processing, equipment and design. In *Nonthermal Preservation of Foods* (G.V. Barbosa-Cánovas, U.R. Pothakamury, E. Palou and B.G. Swanson, eds.), Marcel Dekker, New York, NY.

BARBOSA-CÁNOVAS, G.V., GÁNGORA-NIETO, M.M., POTHAKAMURY, U.R. and SWANSON, B.G. 1999. *Preservation of Food with Pulsed Electric Fields*, pp. 4–31, Academic Press, London, U.K.

DE JONG, P. and VAN HEESCH, E.J.M. 1998. Review: Effect of pulse electric fields on the quality of food products. Milchwissenschaft *53*, 4–8.

HEINZ, V., TOEPFL, S. and KNORR, D. 2003. Impact of temperature on lethality and energy efficiency of apple juice pasteurization by pulsed electric fields treatments. Innov. Food Sci. Emerg. Technol. *4*, 167–175.

HÜLSHEGER, H., POTEL, J. and NIEMAN, E.G. 1981. Killing of bacteria with pulsed electric field strength. Radiat. Environ. Biophys. *20*, 53–65.

JAY, J. 1992. *Modern Food Microbiology*, 4th Ed., Van Nostrand Reinhold, New York, NY.

JAYARAM, S., CASTLE, G.S.P. and MARGARITIS, A. 1992. Kinetic of sterilization of *Lactobacillus brevis* cells by application of high voltage pulses. J. Biotechnol. Bioeng. *40*, 1412–1420.

JEYAMKONDAN, S., JAYAS, D.S. and HOLLY, R.A. 1999. Pulsed electric field processing of foods: A review. J. Food Prot. *62*, 1088–1096.

MARTÍN, O., ZHANG, Q., CASTRO, A.J., BARBOSA-CÍNOVAS, G.V. and SWANSON, B.G. 1994. Pulsed electric field of high voltage to preserve foods. Microbiological and engineering aspects of the process. Span. J. Food Sci. Technol. *34*, 1–34.

MARTÍN, O., VEGA-MERCADO, H., QIN, B.L., CHAN, F.J., BARBOSA-CÍNOVAS, G.V. and SWANSON, B.G. 1997. Inactivation of *Escherichia coli* suspended in liquid egg using pulsed electric fields. J. Food Process Eng. *20*, 317–336.

POTHAKAMURY, U.R., VEGA, H., ZHANG, Q., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1996. Effect of growth temperature on the inactivation of *E. coli* by pulsed electric fields. J. Food Prot. *59*, 1167–1171.

QIN, B., CHANG, F., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1995. Nonthermal inactivation of *Saccharomyces cerevisiae* in apple juice using pulsed electric fields. Food Sci. Technol. *28*, 564–568.

QUASS, D.W. 1997. *Pulsed Electric Field Processing in the Food Industry, A Status Report*, CR-109742, pp. 1–2, Electric Power Institute, Palo Alto, CA.

RASO, J., ALVAREZ, I., CONDÓN, S. and SALA-TREPAT, F.J. 2000. Predicting inactivation of *Salmonella senftenberg* by pulsed electric fields. Innov. Food Sci. Emerg. Technol. *1*, 21–29.

REINA, L.D., JIN, Z.T., ZHANG, Q.H. and YOUSEF, A.E. 1998. Inactivation of *Listeria monocytogenes* in milk by pulsed electric field. J. Food Prot. *61*, 1203–1206.

SAS INSTITUTE. 1999. *SAS/SAT Software*, Version 8.0 for Windows, SAS Institute, Cary, NC.

VEGA-MERCADO, H. 1996. *Inactivation of proteolytic enzymes and selected microorganisms in foods using pulsed electric fields*. PhD Dissertation, Washington State University, Pullman, WA.

VILLAMIEL, M. and JONG, P. 2000. Inactivation of *Pseudomonas fluorescens* and *Streptococcus thermophilus* in trypticase soy broth and total bacteria in milk by continuous-flow ultrasonic treatment and conventional heating. J. Food Eng. *45*, 171–179.

ZHANG, Q., CHANG, F.J., BARBOSA-CANOVAS, G.V. and SWANSON, B.G. 1994. Inactivation of microorganisms in a semi-solid model food using high voltage pulsed electric fields. Lebensm. Wiss. Technol. *27*, 538–543.

ZHANG, Q., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1995a. Engineering aspects of pulsed electric fields. J. Food Eng. *25*, 261–281.

ZHANG, Q., QIN, B., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1995b. Inactivation of *E. coli* for food pasteurization by high strength pulsed electric fields. J. Food Process Pres. *19*, 103–118.