

Evaluation of selected mathematical models to predict the inactivation of *Listeria innocua* by pulsed electric fields

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Abstract

The inactivation of *Listeria innocua* ATCC 51742 by pulsed electric fields was investigated at 35, 40 and 45 kV/cm. Results indicate that at treatment times shorter than 37 μ s at 40 and 45 kV/cm, and 49 μ s at 35 kV/cm, there is a linear relationship between the logarithm of the survivor fraction and the treatment time. However, longer times result in an abrupt increase in the slope of the inactivation curve and in inactivation values greater than six logarithmic cycles. A model based on Weibull's survival function was used to describe microbial inactivation and then compared to a first-order kinetic model. Distribution parameters of Weibull's survival function and kinetic constant for the first-order kinetic model were calculated by fitting experimental data. Calculated mean times for microbial inactivation from Weibull's distribution were 11.55, 8.65 and 5.39 μ s at 35, 40 and 45 kV/cm, respectively. The goodness-of-fit between experimental and predicted values was determined using an accuracy factor. The model based on the Weibull survival distribution provided better accuracy factors than first-order kinetics. The model based on Weibull's survival function seems promising for describing survival curves that exhibit concavity.

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1. Introduction

Food preservation by technologies different from thermal processing such as pulsed electric fields (PEF), ultra high pressure (UHP), high-intensity light pulses, ionizing radiation and ultrasound is currently under extensive research (Góngora-Nieto, Sepúlveda, Pedrow, Barbosa-Cánovas, & Swanson, 2002). To guarantee safety and shelf life, the implementation of such new technologies at an industrial level requires an understanding of the effect that variations in processing conditions can have on microbial populations originally present in food product.

The effectiveness of PEF technology in reducing microbial populations has been widely documented (Castro, Barbosa-Cánovas, & Swanson, 1993; Grahl & Märkl, 1996; Hülsheger, Potel, & Niemann, 1981; Lubicki & Jayaram,

1997; Wouters & Smelt, 1997; Raso, Calderón, Góngora, Barbosa-Cánovas, & Swanson, 1998; Heinz, Phillips, Zenker, & Knorr, 1999; Calderón-Miranda, Barbosa-Cánovas, & Swanson, 1999a, 1999b, 1999a, 1999b; Alvarez, Raso, Palop, & Sala, 2000). However, a fundamental understanding of microbial inactivation or inactivation kinetics is still needed (Góngora-Nieto et al., 2002). Inactivation of microorganisms by PEF depends on several factors, among which the field intensity and treatment time are very important contributors. Several mathematical models have been proposed to explain the decrease in microbial number as a function of the electrical treatment applied. One of the simplest approaches assumes that at sufficiently high electric field strengths, the microbial inactivation follows the kinetics of a unimolecular reaction (first-order kinetics). Therefore, a semi-log plot of inactivation data against treatment time (or number of pulses) would yield a straight line. The survival fraction (S) is defined as the ratio between the number of survivors and

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the number of initial microorganism (N/N_0). According to first-order kinetics, the model has the following form:

$$S = e^{-kt}, \quad (1)$$

where S is the survivor fraction, k is the kinetic constant that depends on the intensity of the electric field, and t is the treatment time. This approach has been used in defining D (decimal reduction time) and z (dependence of D on temperature) values for thermal treatment and has also been used to describe the linear sections of inactivation curves obtained from nonthermal treatments such as high pressure (Musa & Ramaswamy, 1997) and PEF (Castro et al., 1993; Sensoy, Zhang, & Sastry, 1997). Some objections to the first-order kinetics approach have been noted. For example, since the rate is proportional to the number of molecules in a first-order reaction, then if microbial inactivation follows first-order kinetics, cell death would result from the inactivation of a single target molecule or target site per bacterial cell. This single-target theory, however cannot account for initial lags in death rate usually observed in survival curves and cannot account for sublethal injury in microorganisms, a generally recognized phenomenon observed in microbial inactivation (Moats, 1971).

The discovery of the logarithmic order of destruction of microorganisms by lethal treatments dates back to as early as 1907 and is the basis of thermobacteriology currently used in the food industry (Moats, 1971). However, in an attempt to overcome the limitations of assuming typical first-order kinetics, several researchers have proposed new ways to describe microbial inactivation kinetics (Peleg & Cole, 1998). Thus the multiple-site or multiple-target theories, where inactivation occurs due to the action of heat or lethal agent on more than one site were developed. Moats (1971) proposed a model based on the binomial distribution to describe microbial inactivation. This model assumes that microbial death occurs as the result of inactivation of a portion of multiple critical sites.

Inactivation curves obtained from PEF treatments are usually not linear and models different from first-order kinetics have been proposed to overcome this limitation. One such model, proposed by Hülshager et al. (1981), assumes a linear relationship between the logarithm of survivor microorganisms and the logarithm of treatment time for a given electric field intensity, and a linear relationship between the logarithm of survivor microorganisms and the electric field intensity for a given treatment time. According to this model the inactivation is given by

$$S = \left(\frac{t}{t_c}\right)^{-(E-E_c)/k}, \quad (2)$$

where S is the survivor fraction, t is the treatment time, t_c is the critical treatment time, E is the electric field intensity, E_c is the critical electric field, and k is a constant. The parameters E_c , t_c , and k are proposed to be dependent on the microorganism when limits on experimental conditions are observed (Hülshager et al., 1981).

Deviations from linearity, particularly in the case of nonthermal inactivation of microorganisms, are becoming more evident as research progresses. Hence, the need for developing models that accurately describe survival curves is encouraged (Peleg & Cole, 1998). Modern approaches for modeling microbial inactivation are based on the vitalistic conception, that individuals in a population are not identical. This approach assumes that characteristics such as resistance to a lethal agent are possessed by individuals in various degrees and that the difference in degree of resistance is permanent (Cerf, 1977), hence, microbial inactivation is viewed as a probabilistic process due to the natural variability in microbial populations (Peleg & Cole, 1998) and can be described by distribution functions.

In 1995, Peleg proposed a model to describe microbial inactivation by PEF based on Fermi's function, which is the inverted form of the logistic equation that has been used to model population growth over time. Fermi's function applied to PEF treatment is given by

$$S(V) = \frac{1}{1 + \exp((V - V_c)/a)}, \quad (3)$$

where S is the survivor fraction, V is the electric field strength, V_c is a critical level of V where the survivor fraction is 0.5, and a is a parameter related to the steepness of the curve around V_c . Fermi's equation successfully modeled the inactivation of microorganisms by PEF (Peleg, 1995) and has been suggested for modeling dose–response curves for microbial inactivation (Peleg, 1996).

More recently, use of the Weibull distribution function has been found effective in modeling the curvature of survival curves for *Clostridium botulinum* spores during heat treatments (Peleg & Cole, 2000) and for microorganisms such as *Salmonella typhimurium* or *Listeria monocytogenes* exposed to lethal agents like chlorine or potassium sorbate (Peleg & Penchina, 2000). The equation for estimating the survival fraction, based on Weibull distribution function, is given by

$$\log S = -\left(\frac{t}{b(E)}\right)^{n(E)}, \quad (4)$$

where S is the survivor fraction (N/N_0), b and n are constants that depend on the electric field intensity (E), and t is the treatment time.

In this work, *Listeria innocua* was chosen as a surrogate microorganism for the pathogen *Listeria monocytogenes*, a microorganism of concern in food safety due to its ability to grow at refrigeration temperatures. The objectives of the present work were to study the inactivation of *Listeria innocua* ATCC 51742 suspended in diluted McIlvaine buffer at pH 7.0 under high electric field intensity and to compare the adequacy of several models describing the survival curve. Based on the data obtained from this study the last objective is to build a database for estimating resistance of different microorganisms to PEF treatment.

2. Materials and methods

2.1. Microorganism

Listeria innocua (ATCC 51742, Manassas, VA) was obtained from the American Type Culture Collection and rehydrated with 0.4 mL of tryptic soy broth (TSB) (Difco) enriched with 0.6% yeast extract (YE) (Difco) at room temperature. After 30 min, the broth was inoculated into a 250 mL Erlenmeyer flask containing 50 mL of TSB-YE and incubated at 37 °C for 18 h at 225 rev/min in an orbital shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL). After incubation cells were centrifuged, supernatant was poured and cells were resuspended into 50 mL of fresh broth. One milliliter portions of the culture were stored in sterile glass vials containing 1 mL of 20 mL glycerol/100 mL water solution and stored at –70 °C until used. A growth curve was determined for the previously prepared vials as follows: 1 mL of thawed initial culture was inoculated into a 250 mL Erlenmeyer flask containing 25 mL of TSBYE and incubated at 37 °C and 225 rev/min. Fig. 1 shows the increase in microbial population as a function of time for the initial culture of *L. innocua*. The stationary phase was determined would start after 5 h of incubation under previous conditions. A fresh grown culture of *L. innocua* in early stationary phase was used for each experimental run.

2.2. Buffer solution

One liter of Mc Ilvaine (citric-phosphate) buffer solution at pH 7.0 was prepared mixing 178 mL of 0.1 mol/L citric acid solution (21.01 g $C_6H_8O_7 \cdot H_2O L^{-1}$) and 822 mL of 0.2 mol/m³ Na_2HPO_4 (28.40 g $Na_2HPO_4 L^{-1}$) (Perrin & Dempsey, 1974). Electrical conductivity of the buffer solution was reduced by diluting 1 part buffer with 7 parts

deionized water (1:7 B:W), reaching a final conductivity of 4.53 mS/cm at 21 °C to resemble the electrical conductivity of foods such as milk products (4.0–10 mS/cm) and liquid eggs (5–10 mS/cm). Temperature of the buffer solution was 4 °C at the beginning of each PEF run.

2.3. Inoculation and enumeration of samples

Bacterial cells used for inactivation studies were cultured prior to each run as follows: 25 mL of TSB-YE were inoculated with 1 mL of thawed culture obtained from American Type Culture Collection. The culture was incubated for 5 h at 37 °C and 225 rpm in an orbital shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL) and immediately used. Diluted buffer solution was inoculated at a rate 1 mL of culture per 1 L of buffer solution to yield the initial counts of the untreated sample $\sim 1.4 \times 10^6$ CFU/mL.

Bacterial concentration in treated and untreated samples was determined by the pour-plate method. Serial decimal dilutions in sterile peptone solution (1 g peptone (Difco)/1 L water) were plated on triptic soy agar (TSA) (Difco) enriched with 0.6% YE (Difco) and incubated at 37 °C for 48 h before enumeration.

2.4. PEF treatment

The electric field was generated using a pilot plant size pulser manufactured by Physics International (San Leandro, CA). A continuous concentric cylindrical treatment chamber with stainless-steel electrodes and 25 mL treatment volume was used. Exponential decay pulses were generated with a 0.5 μ F capacitor and pulse rate was controlled with a gas spark switch. Pulse duration was set at 2 μ s. Capacitor charging voltages were set at 30, 35, and 40 kV to yield maximum electric field strengths of 35, 40 and 45 kV/cm, respectively. The pulse wave form, input voltage and current delivered to and through the chamber were recorded using high-voltage and current probes connected to a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO), which in turn was connected to a PC. The computer had an interactive program that allowed for real-time data collection. The program was developed using HP VEE LAB 5.0TM (Computer BoardTM, Inc., Middleboro, MA), a graphical programming tool that allows interactive data collection and user interface (Góngora-Nieto, 2000).

The number of pulses delivered to the product was fixed at 5, 10, 15, 20, and 25. Flow rate was fixed at 1200 mL/min and controlled by a variable speed pump (Masterflex Model 7654-00, Cole Parmer Instruments Co., Chicago, IL). Pulsing frequency was adjusted to deliver the desired number of pulses and controlled by the computer. The temperature of the product was measured at the inlet and outlet of the treatment chamber and at the outlet of the heat exchanger used to cool down the treated product. Inlet temperature in all cases was between 3 and 4 °C, and the

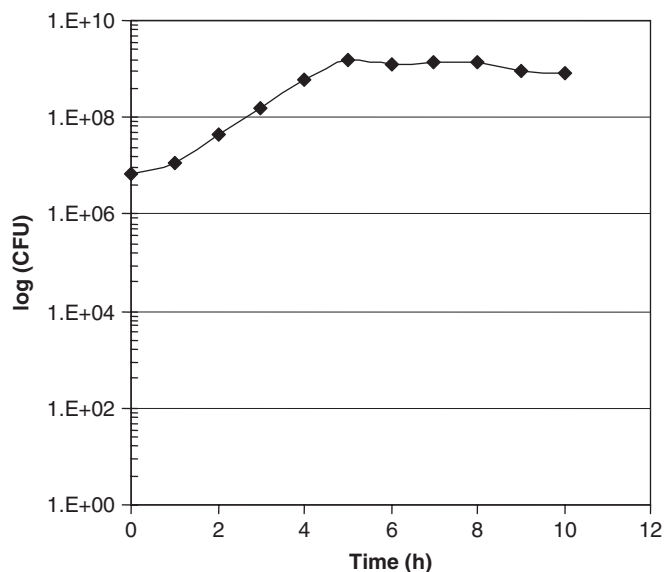


Fig. 1. Growth curve for frozen culture of *Listeria innocua* ATCC 51742.

maximum temperature on the product leaving the treatment chamber ranged from 15 to 65 °C, depending on the electric field intensity and number of pulses delivered. However, the product only remained at outlet temperature for a maximum of 5 s before entering the heat exchanger, which brought the temperature down to values ranging between 17 and 30 °C. Samples were plated in duplicate immediately after PEF treatment.

2.5. Statistical analysis

The experimental design was a randomized complete block design, blocked by capacitor charging voltages. Model parameters were obtained by non-linear regression using Kaleidagraph software (Synergy software, Reading PA) and gamma function was evaluated using Mathematica software (Wolfram Research Inc., Champaign, IL). Analysis of variance of the data were analyzed by the general linear model using SAS^R software (SAS, 1999) and significant differences were established at $\alpha = 0.05$ using Tukey's test.

3. Results and discussion

3.1. Inactivation of *L. innocua*

It has been previously reported that medium composition does not influence the inactivation of microorganisms by PEF (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999; Dutreux et al., 2000; Reina, Jin, Zhang, & Yousef, 1998). Therefore, the experiments for the present work were done using a buffer solution, where the electrical conductivity could be adjusted. Previous reports indicate that the decrease in a microbial population exposed to PEF treatments depends on the number of pulses applied and on the electric field intensity (Calderón-Miranda et al., 1999a, 1999b; Hülshager et al., 1981; Jayaram & Castle, 1992; Martín, Qin, Chang, Barbosa-Cánovas, & Swanson, 1997). Inactivation of *L. innocua* as a function of treatment time is shown in Fig. 2. Capacitor charging voltages of 30, 35, and 40 kV for the current experimental setup resulted in maximum peak electric fields of 35, 40, and 45 kV/cm for the lowest number of pulses (5 pulses), as measured by voltage and current probes on chamber electrodes. Since electrical conductivity is a function of temperature, increase in the number of pulses, so does the temperature, decreases average peak electric field at a higher number of pulses. Variation in electric field strength as a function of number of pulses is shown in Fig. 3. Exponential decay pulses possess a long tail at low electric field strengths, in which case energy delivered at field intensities lower than critical electric field strength will increase the temperature of the sample, causing no inactivation (Zhang, Barbosa-Cánovas, & Swanson, 1995). Hence, the inactivation results presented in this study include the coupled effect of temperature increase due to sample heating. The increase in temperature observed for each number of

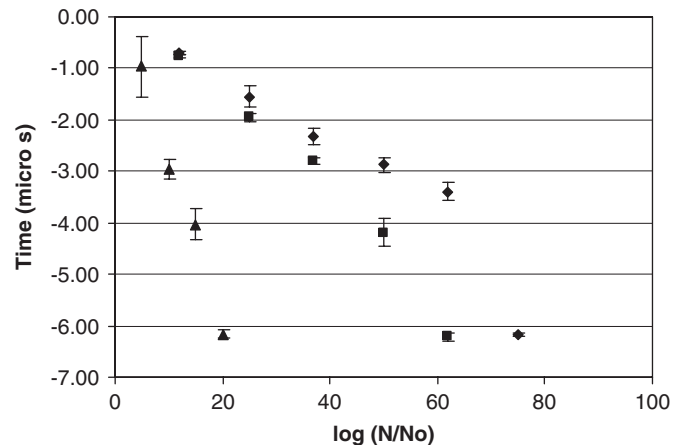


Fig. 2. Inactivation of *Listeria innocua* ATCC 51742 as a function of treatment time and electric field. 35 kV/cm: (◆), 40 kV/cm: (■), 45 kV/cm: (▲).

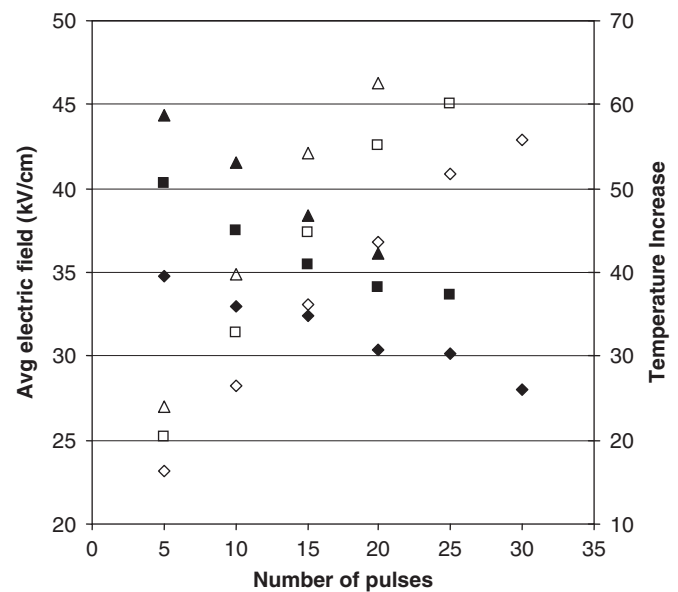


Fig. 3. Average peak electric field decrease as a function of applied pulses for capacitor charging voltages of (■) 30 kV (●) 35 kV and (▲) 40 kV. Temperature increase as a function of applied pulses for capacitor charging voltages of (□) 30 kV (◇) 35 kV and (△) 40 kV.

pulses at each electric field is shown in Fig. 3. The drop in electric field strength at a high number of pulses is sharper at 40 kV than at 30 or 35 kV. It follows that PEF treatments can be optimized for the intensity of the applied electric field, since higher capacitor charging voltages would not necessarily result in higher electric fields but would increase the temperature of the sample.

Inactivation results of the present study are agree with previous reports by other researchers where, within a given voltage, inactivation increases with increasing treatment time, and for a given treatment time, inactivation increases as electric field increases (Alvarez et al., 2000; Calderón-Miranda et al., 1999a, 1999b; Castro et al., 1993; Grahl & Märkl, 1996; Heinz et al., 1999; Hülshager et al., 1981;

Lubicki & Jayaram, 1997; Raso et al., 1998; Wouters & Smelt, 1997). Fig. 2 shows a semi-log plot of inactivation against treatment time. At short treatment times, the relationship between the inactivation and treatment time is more or less linear. However, for longer treatment times, at each electric field, there is an abrupt increase in the slope of the curve, which holds true for the three voltages tested, suggesting a critical time above which inactivation is faster than that at shorter treatment times.

Figs. 4–6 show the inactivation of *L. innocua* as a function of the energy input for electric field strengths 35, 40 and 45 kV/cm. Inactivation results at 35 kV/cm indicate that at energy inputs lower than 200 J/mL a linear response is obtained. However, at energy inputs slightly higher than 200 J/mL, an abrupt increase in the slope of the curve is observed. At 40 kV/cm, the initial response is not as linear as for 35 kV/cm. There is no abrupt change in the slope of the curve, but instead a more gradual transition that results in a slightly downward concave curve. At 45 kV/cm, the response is more or less linear along the range of energy values tested, with a slight change in slope after 200 J/mL. Energy inputs close to 200 J/mL corresponded to 15, 20, and 25 pulses at electric field strengths of 45, 40, and 35 kV/cm, respectively, resulting in a temperature increase of

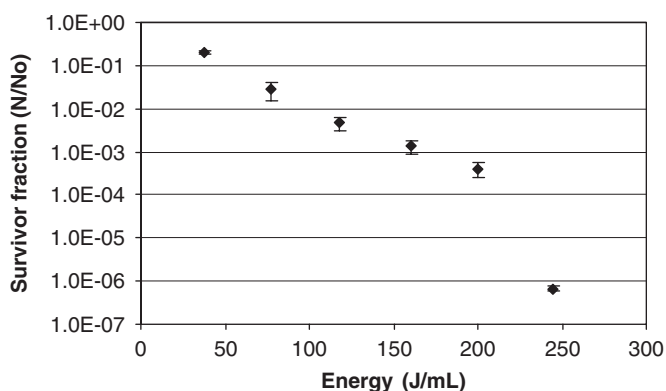


Fig. 4. Reduction of *Listeria innocua* as a function of input energy at 35 kV/cm (◆).

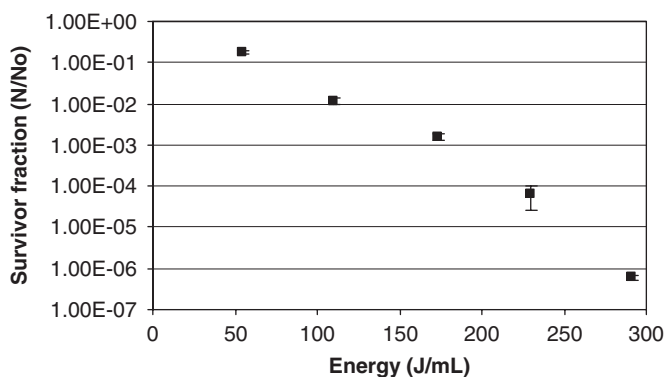


Fig. 5. Reduction of *Listeria innocua* as a function of input energy at 40 kV/cm (■).

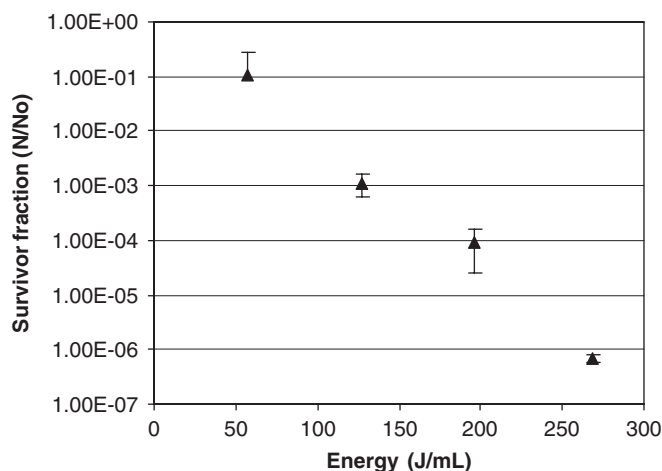


Fig. 6. Reduction of *Listeria innocua* as a function of input energy at 45 kV/cm (▲).

approximately 55 °C in all three cases. Corresponding average electric field strengths for 15 pulses at 40 kV, 20 pulses at 35 kV and 25 pulses at 30 kV were 38.3, 34.1 and 30.1 kV/cm, respectively. No viable cells of *L. innocua* were detected after treatments at the highest number of pulses: 25 pulses at capacitor charging voltage of 30 kV, 25 pulses at 35 kV and 20 pulses at 40 kV.

The inactivation effect observed on *L. innocua* depended on whether the desired number of pulses was applied immediately or in steps. Results of this experiment, showed no tail in the inactivation curve when the desired number of pulses was delivered all at once. However, at maximum electric field of 35 kV/cm, when the electrical treatment was delivered in steps of 15 pulses until reaching 30, 45 and 60 pulses (i.e., in steps 1, 2, 3 and 4), the curve showed a tailing effect at 45 and 60 pulses (data not shown). In treatment by steps, the maximum increase in temperature was 27 °C, i.e., sample temperature was no greater than 31 °C. The maximum reduction observed for 45 and 60 pulses was 3.2 log cycles. A similar tailing effect was observed by Calderón-Miranda et al. (1999a, 1999b). Their results showed that maximum inactivation of *L. innocua* ATCC 51742 suspended in skim milk and liquid whole egg were 2.4 and 3.4 log cycles, respectively, for 32 pulses delivered in three steps with maximum outlet temperature of 34 °C. Although no additional sets of pulses were tested, survival curves obtained by Calderón showed upward concavity confirming the existence of a tail. Hence, the temperature increase due to PEF treatment drastically increases the effectiveness of the treatment and should not be overlooked when selecting a PEF treatment.

To evaluate the adequacy of mathematical models for predicting inactivation, the total number of pulses (5, 10, 15, 20, and 25) was delivered in only one step by increasing pulsing frequency. Therefore, the observed decrease in microbial population is the result of the electric field strength, temperature increase and their interaction.

3.2. Traditional first-order kinetics model

Inactivation data were correlated to treatment time, as shown in Fig. 2. Data showed that at 45 kV/cm inactivation occurs exponentially, thus a semi-log plot of experimental points yields a straight line with a correlation coefficient of 0.98. However, as the voltage decreases and consequently the electric field, and final temperature of the product increases, deviations from linearity due to long treatment times are evident. Correlation coefficients improved with increase in electric field strength. Calculated decimal reduction times were 13.48, 10.29 and 8.09 μs for 35, 40 and 45 kV/cm electric field strengths. Decimal reduction times as a function of electric field strength and first-order kinetic constants for *L. innocua* are shown in Table 1. At lower electric field strength (35 kV/cm) the correlation coefficient for the semi-log plot is very low (0.88) showing that inactivation does not proceed in a linear fashion; therefore, additional models should be considered to describe microbial inactivation by PEF.

3.3. Empirical model proposed by Hülshager et al. (1981)

This model assumes a linear relationship between the log of the survivor fraction and the electric field strength above a critical level. The critical electric field strength can be obtained by extrapolating the linear region of the curves to the intersection with $\log S = 0$. The model also assumes a linear relationship between the log of survivor fraction and the log of treatment time, and establishes the existence of a critical treatment time for each voltage. Critical treatment times are obtained by extrapolating the straight line to $\log S = 0$. Critical electric field values for each treatment time as well as critical times for 35, 40, and 45 kV/cm are given in Table 2. Correlation coefficients for data used to calculate critical electric fields were lower at short treatment times and increased to 0.98 at the longest treatment times. Deviations from linearity in survival curves, in a plot of $\log(S)$ against electric field strength, cause the slope of the lines for each treatment time to change in a way such that the obtained critical electric fields do not follow a trend. Since the calculated critical electric field for 37.4 μs is smaller than the E_c value for 25.12 μs , the model is not adequate for describing our experimental data, probably due to differences in experimental conditions. Therefore, no further analysis of the model was conducted.

Table 1
Decimal reduction time (*D*-value) for *L. innocua* ATCC 51742 in diluted Mc Ilvaine buffer solution

Electric field strength (kV/cm)	<i>D</i> -value (μs)	<i>k</i> (μs^{-1})	R^2
35	13.48	0.17088	0.8862
40	10.29	0.22385	0.9705
45	8.09	0.28651	0.9846

Table 2

Constants for the model proposed by Hülshager, Potel, and Niemann (1981) for inactivation of *Listeria innocua* ATCC 51742 in diluted Mc Ilvaine Buffer Solution

Electric field strength (kV/cm)	t_c (μs)	R^2	Treatment time (μs)	E_c (kV/cm)	R^2
35	11.30	1	12.6	10.02	0.9230
40	14.22	0.9489	25.12	24.74	0.9408
45	8.96	0.9997	37.44 49.89	22.18 26.59	0.9405 0.9867

3.4. Model based on Fermi's equation

The parameters of Fermi's function for *L. innocua* are shown in Table 3 along with parameters previously reported for other microorganisms. In this table, V_c indicates a level of electric field where survival fraction is 50%, and the parameter a refers to the steepness of the survival curve around V_c . Furthermore, V_c and a have been proposed as exponential functions of the number of applied pulses by Peleg (1995). The value of critical electric field (V_c) decreases with increasing number of pulses from 5 to 15; however, at 20 pulses the V_c increases and is similar to the V_c value obtained for 10 pulses. This behavior suggests that the difference in V_c values for 10, 15 and 20 pulses might not be significant but that inactivation proceeds faster at a higher number of pulses as indicated by the parameter a . *Listeria innocua* shows a large value a at a low number of pulses, which implies a wide span in microbial inactivation (Peleg, 1995). However, as the number of pulses increases, the value of a becomes smaller indicating a steep decline in the survival curve. In general, V_c values for inactivation of *L. innocua* are higher than those reported for *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Candida albicans* (Rodrigo, Martínez, Harte, Barbosa-Cánovas, & Rodrigo, 2001), indicating a higher resistance to PEF treatment by *L. innocua* than by the other three organisms. On the other hand, *Lactobacillus plantarum* showed greater V_c values than *L. innocua*, indicating that *L. plantarum* is slightly more resistant to PEF treatment than *L. innocua*. Inactivation data at low number of pulses (5 and 10) as a function of electric field strength adjusted well to the Fermi function, as shown by high correlation coefficients (Table 3). Inactivation was almost complete at a higher number of pulses, so data adjusted well to the tail of the function; however, inactivation data at pulses lower than 5 were required to obtain more accurate correlations since a relationship between parameter V_c as a function of treatment time was not observed, mainly because V_c values for 10, 15, and 20 were similar.

3.5. Model based on Weibull's distribution

The cumulative form of the Weibull distribution (Eq. (4)) has been successfully used to describe nonlinear

Table 3
Survival constants for several microorganisms exposed to PEF treatment using Peleg's model based on the Fermi function

Microorganism	Number of pulses (treatment time)	V_c (kV/cm)	a (kV/cm)	R^2	Reference
<i>Listeria innocua</i> ATCC 51742 (Diluted buffer solution)	5 (12 μ s)	22.2	6.97	0.994	Current work
	10 (25 μ s)	19.1	3.14	0.999	
	15 (37 μ s)	18.1	2.24	—	
	20 (50 μ s)	19.3	1.62	—	
<i>Listeria monocytogenes</i> I	2	14.9	2.8	0.981	Peleg (1995)
	4	12.7	2.0	0.994	
	10	10.3	2.4	0.992	
	30	8.5	2.0	0.999	
<i>Pseudomonas aeruginosa</i>	2	12.9	2.6	0.982	Peleg (1995)
	4	10.6	2.4	0.994	
	10	8.3	2.1	0.993	
	30	6.7	1.8	0.999	
<i>Candida albicans</i>	2	21.2	3.1	0.999	Peleg (1995)
	4	15.3	3.1	0.993	
	10	10.1	1.3	0.997	
	30	7.5	1.2	0.999	
<i>Lactobacillus plantarum</i> (Carrot–orange juice)	10 μ s	31.6	3.13	0.9553	Rodrigo et al. (2001)
	15 μ s	30.6	2.81	0.9529	
	20 μ s	28.8	3.61	0.9133	
	30 μ s	27.6	3.04	0.9599	
	46 μ s	27.1	3.09	0.9405	

survivor curves in thermal and nonthermal food processing (Peleg & Cole, 1998). The cumulative form of the Weibull distribution has the advantage such that when $n = 1$, the survival curve yields a straight line when plotted in semilogarithmic coordinates, resembling first order kinetics. Parameters b and n are the scale and shape factors, and can be used to estimate the mean of the distribution (t_c) if known, which has been suggested to be a measure of the microbial resistance to PEF treatment (Rodrigo et al., 2001). For a population following Weibull's distribution, t_c can be calculated as

$$t_c = b\Gamma(1 + 1/n), \quad (5)$$

where t_c is the mean of the distribution, b and n are the scale and shape parameters, and Γ is the gamma function. Parameters b and t_c vary with the intensity of the applied field and can be used as kinetic parameters. Parameters for Weibull distribution fits, as well as the mean t_c values for *L. innocua* (Table 4), and are comparable to those reported for *L. plantarum* and *Byssochlamys fulva*. Values of b and t_c decreased as electric field increased. If Weibull parameters are used as an indication of bacterial resistance to PEF treatment, *L. innocua* is more resistant to PEF treatment than *B. fulva*, as indicated by greater values of t_c for *Listeria*. *L. plantarum* is slightly less resistant to PEF treatment than *L. innocua*, as shown by their close t_c values.

Concavity of the curve depends on the n value and its practical implication is related to the existence of tails. When $n < 1$, the distribution has a strong right skew and a semilogarithmic plot of the survival curve shows upward

concavity (existence of a tail). Alternatively, when $n > 1$, the semilogarithmic survival curve has a pronounced downward concavity (Peleg & Cole, 1998). The value of parameter n , which indicates the shape of the curve, is greater than 1 for *L. innocua* but smaller than 1 for *L. plantarum* and *B. fulva*. Parameter n should be useful when defining PEF processing conditions, since in the case of *L. innocua*, it implies that at all three voltages tested, there is a certain treatment time beyond which inactivation will increase drastically (downward concavity). Whereas for *L. plantarum* and *B. fulva*, parameter n , indicates the opposite, i.e., there is a treatment time beyond which no further reduction due to PEF treatment is expected (upwards concavity or tail). Correlation coefficients improved from 0.955 at 35 kV/cm to 0.996 at 40 and 45 kV/cm, suggesting the adequacy of the Weibull distribution function to estimate microbial inactivation at high electric fields and long treatment times, as opposed to the traditional use of first-order kinetics to describe the linear region of survival curves, which usually takes place at low voltages and short treatment times. Fig. 7 shows experimental and predicted inactivation values for *L. innocua* at 35, 40 and 45 kV/cm.

3.6. Accuracy factor for Weibull distribution

The accuracy factor (A_f) suggested by Ross (1996) was used to evaluate the performance of obtained predictive models. The A_f is defined as:

$$A_f = 10^{\frac{\sum |\log(pred/obs)|}{n}}, \quad (6)$$

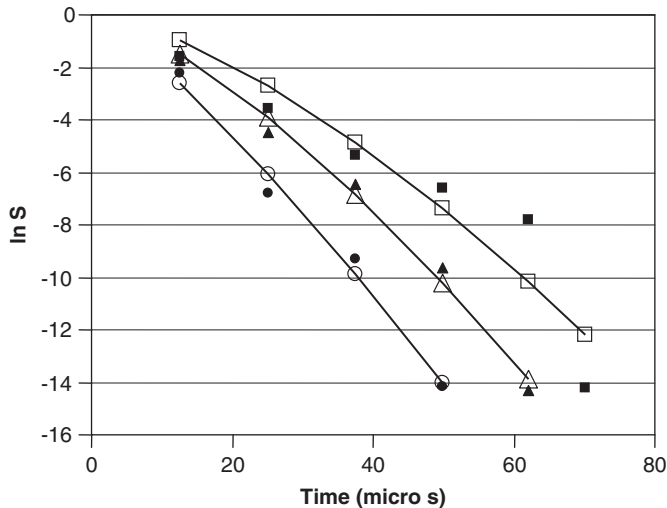


Fig. 7. Experimental and Weibull's survival function predicted inactivation values for *L. innocua* ATCC 51742 in diluted Mc Ilvaine buffer. Experimental: (■) 35 kV/cm (●) 40 kV/cm and (▲) 45 kV/cm. Predicted: (□) 35 kV/cm (△) 40 kV/cm and (○) 45 kV/cm.

where Af is the accuracy factor, $pred$ is the predicted value, obs is the observed value, and n is the number of observations. The Af value is equal to 1 when there is perfect agreement between predicted and observed values. Accuracy factors calculated for the first-order kinetic model were 2.23, 2.86, 2.18 and 1.25, 1.09, 1.09 for the model based on Weibull Distribution for 35, 40 and 45 kV/cm, respectively. Weibull distribution had a lower accuracy factor, indicating better agreement between predicted and experimental values than traditional first-order kinetics. Similar Af values close to 1 were reported by Rodrigo et al. (2001) for the inactivation of *L. plantarum* using Weibull's distribution function to model inactivation by PEF treatment, confirming the adequacy of this function to model microbial inactivation by PEF.

4. Conclusion

The Weibull survival function provided a better model to describe the inactivation of *L. innocua* ATCC 51742 in diluted buffer solution in the experiment conditions tested than traditional first-order kinetics, as shown by lower Af values. Hülshager's empirical model provided accuracy factor values close to 4, due to the fact the model was derived for a specified set of experiment conditions, hence, the applicability of this model to modeling inactivation of *L. innocua* by PEF is not advised. The model based on Weibull distribution proved effective in describing microbial inactivation at high electric field strength and long treatment times, where survival curves tend to show concavity and the linear relationship assumed to exist between treatment time and survivor fraction no longer exists.

References

- Alvarez, I., Raso, J., Palop, A., & Sala, F. J. (2000). Influence of different factor son the inactivation of *Salmonella senftenberg* by pulsed electric fields. *International Journal of Food Microbiology*, 55, 143–146.
- Barbosa-Cánovas, G. V., Góngora-Nieto, M. M., Pothakamury, U. R., & Swanson, B. G. (1999). *Preservation of foods with pulsed electric fields*. San Diego, CA: Academic Press.
- Calderón-Miranda, M. L., Barbosa-Cánovas, G. V., & Swanson, G. V. (1999a). Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin. *International Journal of Food Microbiology*, 51, 19–30.
- Calderón-Miranda, M. L., Barbosa-Cánovas, G. V., & Swanson, G. V. (1999b). Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *International Journal of Food Microbiology*, 51, 7–17.
- Castro, A. J., Barbosa-Cánovas, G. V., & Swanson, B. G. (1993). Microbial inactivation of foods by pulsed electric fields. *Journal of Food Processing and Preservation*, 17, 47–73.
- Cerf, O. (1977). Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, 42, 1–19.
- Dutreux, N., Notermans, S., Wijtzes, T., Gongora-Nieto, M. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (2000). Pulsed electric fields inactivation of attached and free-living *Escherichia coli* and *Listeria innocua* under several conditions. *International Journal of Food Microbiology*, 54, 91–98.
- Góngora-Nieto, M. M., Sepúlveda, D. R., Pedrow, P., Barbosa-Cánovas, G. V., & Swanson, B. G. (2002). Food processing by pulsed electric fields: Treatment delivery, inactivation level and regulatory aspects. *Lebensmittel Wissenschaft und Technologie*, 35, 375–388.
- Góngora-Nieto, M. M. (2000). *Food preservation by pulsed electric fields: Evaluation of critical processing parameters and process optimization*. Ph.D. thesis. Washington State University. 185pp.
- Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology*, 45, 148–157.
- Heinz, V., Phillips, S. T., Zenker, M., & Knorr, D. (1999). Inactivation of *Bacillus subtilis* by high intensity pulsed electric fields under close to isothermal conditions. *Food Biotechnology*, 13(2), 155–168.
- Hülshager, H., Potel, J., & Niemann, E. G. (1981). Killing of bacteria with electric pulses of high field strength. *Radiation and Environmental Biophysics*, 20, 53–65.
- Jayaram, S., & Castle, G. S. P. (1992). Kinetics of sterilization of *Lactobacillus brevis* cells by the application of high voltage pulses. *Biotechnology and Bioengineering*, 40, 1412–1420.
- Lubicki, P., & Jayaram, S. (1997). High voltage pulse application for the destruction of the Gram-negative bacterium *Yersinia enterocolitica*. *Bioelectrochemistry and Bioenergetics*, 43, 135–141.
- Martín, O., Qin, B. L., Chang, F. J., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields. *Journal of Food Protection*, 20, 317–336.
- Moats, W. A. (1971). Kinetics of thermal death of bacteria. *Journal of Bacteriology*, 105(1), 165–171.
- Musa, D. M., & Ramaswamy, H. S. (1997). Ultra high pressure pasteurization of milk: Kinetics of microbial destruction and changes in physico-chemical characteristics. *Lebensmittel Wissenschaft und Technologie*, 30(6), 551–557.
- Peleg, M. (1995). A model for microbial survival after exposure to pulsed electric fields. *Journal of Food Science and Agriculture*, 67, 93–99.
- Peleg, M. (1996). Evaluation of the fermi equation as a model of dose-response curves. *Applied Microbiology and Biotechnology*, 46, 303–306.
- Peleg, M., & Cole, M. B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, 39(5), 353–380.
- Peleg, M., & Cole, M. B. (2000). Estimating the survival of *Clostridium botulinum* spores during heat treatments. *Journal of Food Protection*, 63(2), 190–195.

- Peleg, M., & Penchina, C. M. (2000). Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Reviews in Food Science and Nutrition*, 40(2), 159–172.
- Perrin, D. D., & Dempsey, B. (1974). *Buffers for pH and metal ion control*. London: Chapman & Hall 89pp.
- Raso, J., Calderón, M. L., Góngora, M. M., Barbosa-Cánovas, G. V., & Swanson, G. V. (1998). Inactivation of mold ascospores and conidiospores suspended in fruit juices by pulsed electric fields. *Lebensmittel Wissenschaft und Technologie*, 31, 668–672.
- Reina, L. D., Jin, T. Z., Zhang, Q. H., & Yousef, A. E. (1998). Inactivation of *Listeria monocytogenes* in milk by pulsed electric fields. *Journal of Food Protection*, 61, 1203–1206.
- Rodrigo, D., Martínez, A., Harte, F., Barbosa-Cánovas, G. V., & Rodrigo, M. (2001). Study of inactivation of *Lactobacillus plantarum* in orange-carrot juice by means of pulsed electric fields: Comparison of inactivation kinetics models. *Journal of Food Protection*, 64(2), 259–263.
- Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81(5), 501–508.
- SAS Institute. (1999). *SAS/SAT software* (Version for Windows). Cary, NC, USA: SAS Institute, Inc.
- Sensoy, I., Zhang, H. Q., & Sastry, S. K. (1997). Inactivation kinetics of *Salmonella Dublin* by pulsed electric field. *Journal of Food Process Engineering*, 20, 367–381.
- Wouters, P. C., & Smelt, J. P. P. M. (1997). Inactivation of microorganisms with pulsed electric fields: potential for food preservation. *Food Biotechnology*, 11(3), 193–229.
- Zhang, Q. H., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). Engineering aspects of pulsed electric field pasteurization. *Journal of Food Engineering*, 25, 261–281.