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Comparing predicting models for the *Escherichia coli* inactivation by pulsed electric fields

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Abstract

Inactivation of *Escherichia coli* by pulsed electric field treatments (PEF) between 15 and 28 kV/cm in citrate–phosphate McIlvaine buffer (pH 7, 2 mS/cm) was studied. At all electric field strengths investigated the shape of the survival curves was concave upwards. A two-term exponential model for mixed cell populations, a model based on a Weibull distribution of resistances within the bacterial population, a sigmoidal equation also justified by the existence of a resistance distribution, and a purely empirical equation were used to fit the observed survival curves. The three last models were simpler than the first one and allowed to develop secondary models to estimate the influence of the electric field strength on the inactivation of *E. coli*. A validation study showed that the performance of models derived from the Weibull distribution and the empirical equation were better than the derived from the sigmoidal equation.

Keywords: *E. coli*; PEF; Modelling inactivation; Validation

Industrial relevance: The often non-linear inactivation kinetics of observed microorganisms by treatment with non-thermal processes has indicated a world wide debate on the validity of existing inactivation kinetics including thermal processing. In addition, prediction models are an integral part of process design and development and necessary for regulatory approval of any new process. The present contribution compares four prediction models and concluded that survival curves of *E. coli* were concave upwards (high initial inactivation rate) and that simple models (e.g. based on Weibull distribution) can effectively estimate the impact of PEF parameters on microbial inactivation.

1. Introduction

Pulsed electric field technology is a non-thermal process that has been considered as an alternative to thermal pasteurisation of foods (Qin, Pothakamury, Barbosa-Cánovas & Swanson, 1996). The design of effective PEF pasteurisation treatments involves the development of mathematical models to predict microbial inactivation by PEF. These models are an essential component of hazard analysis and critical control point systems, and could allow equipment manufactures and food processor to predict and control the safety and shelf-life of foods at the design state (Linton, Carter, Pierson & Hackney, 1995).

Traditionally, microbial inactivation by different lethal agents is modelled by describing mathematically survival curves. Survival curves are obtained by plotting the Log_{10} of the number of survivors after a treatment at a constant intensity vs. the treatment time. Generally, four types of survival curves can be obtained: linear, concave upwards (curves with tailing), concave downwards (curves with shoulder) and sigmoidal curves.

Commonly, when survival curves cover few Log_{10} cycles, microbial inactivation by PEF follows a linear inactivation (Martín-Belloso, Vega-Mercado, Qin, Chang, Barbosa-Cánovas & Swanson, 1997; Reina, Jin, Zhang & Youself, 1998; Heinz, Phillips, Zenker & Knorr, 1999). However, if the inactivation is extended for more than 3–4 Log_{10} cycles concave upwards curves are observed (Wouters, Álvarez & Raso, 2001). When survival curves follow a logarithmic order of death it becomes very simple to compare results obtained by different authors or to calculate the treatment time to obtain a given level of microbial inactivation because the death rate is constant and independent of the treat-

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ment time. If survival curves are non-linear, death rate depends on the treatment time, and survival curves extrapolated from few experimental values do not permit to describe the microbial inactivation correctly. Therefore, to obtain reliable parameters to model non-linear survival curves multiple experimental data points need to be obtained.

Several approaches have been proposed to explain the reason of the upward concavity of the survival curves. Some authors have considered that this kind of curves are biphasic and reflect the inactivation of two populations of microorganisms which death kinetics follows a first order inactivation (Humpheson, Adams, Anderson & Cole, 1998). This shape of the survival curves has been also attributed to the results of experimental artefacts (Cerf, 1997). Concave upward curves have been also justified by the existence of a distribution of resistances within the microbial population (Augustin, Carlier & Rozier, 1998; Peleg & Cole, 1998).

In this paper, concave upward survival curves corresponding to the inactivation of *E. coli* by PEF have been analysed with different mathematical modelling approaches: a two-term exponential model for mixed cell populations, a model based on a Weibull distribution of resistances within the bacterial population, a sigmoidal equation also justified by the existence of a resistance distribution, and an empirical equation for curves showing an upward concavity. The purpose of this paper was to compare these models in order to establish the one that more effectively describes the inactivation kinetics of *E. coli* by PEF.

2. Material and methods

2.1. Microorganism and growth conditions

During the study, the strain of *E. coli* K 12 DH5α (DSMZ-German strain collection-6897) was maintained on slants of nutrient agar (NA;Biolife, Milan, Italy). A broth subculture was prepared by inoculating, with one single colony from a nutrient agar plate, a test tube containing 5 ml of sterile nutrient broth (NB;Biolife). After inoculation, the tube was incubated overnight at 30 °C. With this subculture, flasks containing 50 ml of sterile nutrient broth were inoculated to a final concentration of 10^6 CFU/ml. The cultures were then incubated under agitation (130 rpm; Selecta, Rotabit, Barcelona, Spain) at 30 °C during 24 h.

2.2. PEF equipment

PEF equipment used in this investigation was previously described by Heinz et al. (1999). Microorganisms were treated in a parallel-electrode treatment chamber with a distance between electrodes of 0.25 cm and an area of 2.01 cm^2. The circuit configuration generated square waveform pulses at different frequencies, pulse widths, specific energies and electric field strengths. Pulse frequency of 1 Hz, pulse width of 2 μs, specific energies per pulse from 0.18 to 3.53 kJ/kg, and electric field strengths from 5.5 to 28 kV/cm were used. In all experiments, the initial temperature of the treatment medium was 24 ± 1 °C, and the final was kept under 35 °C. The temperature of the treatment medium was measured as previously described by Raso, Álvarez, Condón and Sala (2000). Actual electric field strength applied was measured in the treatment chamber with a high voltage probe (Tektronix, P6015A, Wilsonville, Oregón, EE.UU.) connected to an oscilloscope (Tektronix, TDS 220, Wilsonville, OR, USA). Treatment time was calculated by multiplying the pulse width (τ) by the number of pulses applied.

2.3. Microbial inactivation experiments

Before treatment, microorganisms were centrifuged at $6000 \times g$ for 5 min at 4 °C and resuspended in citrate-phosphate McIlvaine buffer of pH 7.0 (Dawson, Elliot, Elliot & Jones, 1974) which concentration was adjusted to an electrical conductivity of 2 mS/cm.

The microbial suspension at a concentration of 10^9 CFU/ml was placed into the treatment chamber with a sterile syringe. After filling, the hole of the treatment chamber was sealed with tape. After treatment, appropriate serial dilutions were prepared in sterile Tryptic Soy Broth with 0.6% Yeast Extract and plated into NA. Plates were incubated at 37 °C for 24 h and, after incubation, colonies were counted with an improved image analyser automatic counter (Protos, Analytical Measuring Systems, Cambridge, UK) as previously described elsewhere (Condón, Palop, Raso & Sala, 1996).

2.4. Description of the mathematical models

2.4.1. Model 1

Model 1 is an extension of the exponential model that assumes that there are two populations of microorganisms which differ on their sensitivity to PEF (Pruitt & Kamau, 1993). Model 1 can be expressed as:

$$S(t) = pe^{-\kappa_1 t} + (1-p)e^{-\kappa_2 t} \quad (1)$$

where S(t) is the fraction of total survivors; t, the treatment time (μs); p, the fraction of survivors in population 1 (PEF-sensitive); $(1-p)$, the fraction of survivors in population 2 (PEF-resistant); κ_1, the specific death rate of subpopulation 1; κ_2, the specific death rate of subpopulation 2.

2.4.2. Model 2

Model 2 was proposed by Augustin et al. (1998). This model is justified by a distribution of resistances

within the bacterial population. Survival curves are described by the following sigmoidal equation:

$$\mathrm{CFU}(t) = \mathrm{CFU}(0) \cdot (1 + e^{(t-m)/s^2})^{-1} \quad (2)$$

where CFU(t) is the concentration of survivors; CFU(0), the initial concentration of the population; t, the Log_{10} of the treatment time (μs); m, peak of the PEF resistance or the Log_{10} of the time necessary to destroy the 50% of the population (μs); s, parameter proportional to the standard deviation of the PEF resistance ($\mu s^{0.5}$).

2.4.3. Model 3

Model 3 is a mathematical equation based on the Weibull distribution (Peleg et al., 1998; Van Boekel, 2002). If the microbial PEF resistance follows a Weibull distribution the survival function is:

$$\log_{10} S(t) = -\left(\frac{1}{2.303}\right)\left(\frac{t}{b}\right)^n \quad (3)$$

where $S(t)$ is the survival fraction; t, the treatment time (μs); and b and n are the scale and shape parameters, respectively. The b value represents the time necessary to inactivate 0.434 Log_{10} cycles of the population (μs).

2.4.4. Model 4

Model 4 is a purely empirical equation that describes upward concave curves (Peleg & Penchina, 2000):

$$\log_{10} S(t) = -aLn(1 + ct) \quad (4)$$

where $S(t)$ is the survival fraction; t, the treatment time (μs); a and c are parameters characteristics of the equation.

To fit the models to the experimental data the GraphPad PRISM® (GraphPad Software, Inc., San Diego, CA, USA) was used.

2.5. Model validation

Randomly selected combinations of electric field strengths and treatment times not used for the generation of the models were used to validate the models.

For the validation study, the following 27 experiments were performed: 250, 600 and 1000 μs at 15.0, 17.0, 19.0, 20.5, 22.0, 23.5, 25.0, 26.5 and 28.0 kV/cm. These experiments were performed eight months after obtaining the data used in the generation of the model.

Bias and accuracy factors were used as a quantitative way to measure the performance of the different models (Roos, 1996). The bias factor indicates by how much, on average, a model overpredicts (bias factor >1) or underpredicts (bias factor <1) the observed data. The accuracy factor indicates by how many the predictions differ from the observed data.

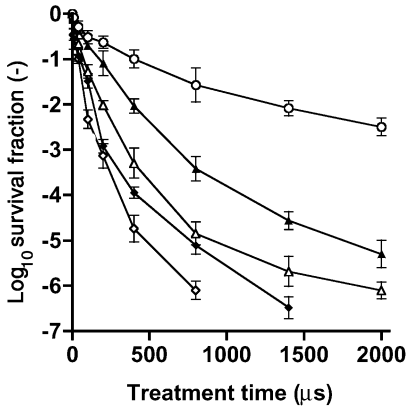

Fig. 1. Survival curves of *E. coli* at different electric field strengths: (○) 15 kV/cm, 1.08 kJ/kg/pulse; (▲) 19 kV/cm, 1.69 kJ/kg/pulse; (△) 22 kV/cm, 2.20 kJ/kg/pulse (♦) 25 kV/cm, 2.86 kJ/kg/pulse; (◇) 28 kV/cm, 3.56 kJ/kg/pulse. Treatment conditions: McIlvaine buffer pH 7, 2 mS/cm; 2 μs; 1 Hz. 95% confidence limits are indicated.

3. Results

Fig. 1 shows the influence of the treatment time on the inactivation of *E. coli* by pulsed electric fields treatments of different intensities. Treatments at electric field strengths of 12 kV/cm or lower scarcely affected viability of *E. coli* cells (data not shown). Inactivation of *E. coli* increased by increasing the electric field strength and the treatment time at electric field strengths of 15 kV/cm or higher. Survival curves of this microorganism did not show a linear behaviour. Therefore, the traditional first order kinetics did not accurately describe them.

Survival curves obtained at different electric field strengths were fitted by the corresponding primary models [Eqs. (1)–(4)] in order to estimate the parameters of each model. An example of the fit of the four models to the inactivation of *E. coli* by a PEF treatment at 28 kV/cm is depicted in Fig. 2. As it is shown by this figure the four models were capable of fitting the experimental data very reasonably. The estimated parameters of each model with their 95% confidence limits for the four proposed models are listed in Table 1. To test the fitness of the models to the individual survival curves, the determination coefficients (R^2) and the root mean square errors (RMSE) are also included. Overall the four models were effective in modelling the inactivation of *E. coli* by PEF in all the range of electric field strengths investigated. In terms of RMSE, the model 4 fitted better the survival curves obtained at three electric fields (22, 25 and 28 kV/cm) and the model 3 fitted better the survival curves obtained at 15 and 19 kV/cm.

Parameters obtained by fitting the equations to the data were analysed. The p parameter of the two-term

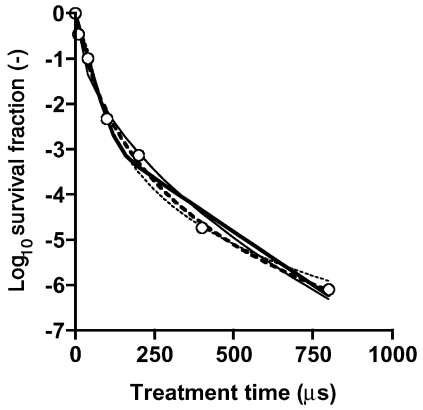

Fig. 2. Fitting of the four models to the survival curve of *E. coli* at 28 kV/cm. (○) Observed values; (—) Model 1; (···) Model 2; (¯) Model 3; (•••) Model 4. Treatment conditions: McIlvaine buffer pH 7, 2 mS/cm; 2 μs; 1 Hz.

exponential model gives an idea about the proportion of PEF-sensitive and resistant subpopulations. The sensitive fraction changed with the electric field strength. Considering the p value obtained by the model and the initial population (10^9 CFU/ml), the resistant fraction appeared after inactivating 0.74, 2.13, 4.00, 2.96 and 2.36 Log_{10} cycles at 15, 19, 22, 25 and 28 kV/cm, respectively. On the other hand, the rate of inactivation of the sensitive population was similar at 15 and 19 kV/cm and then increased at higher electric fields. The rate of inactivation of the resistant population also changed with the electric field strength but the estimated PEF-resistance was higher at 22 than at 19 kV/cm.

In the other three models, a significant correlation was detected between the electric field strength and one of the parameters (m for model 2, b for model 3 and c for model 4). The s, n and a parameters of the corresponding models did not display a relationship with the electric strength, showing random variation. In order to reduce the number of parameters of each model, and as the values of these parameters (s, n and a) were similar at the electric fields investigated, Eqs. (2)–(4) were refitted with the s, n and a values set at their mean values (0.370, 0.526 and 2.092, respectively). The new m, b and c parameters after the second fitting and their 95% confidence limits are shown in Table 2. For the three models, although the refitted models have one less parameter, the examination of the R^2 and RMSE indicates that the goodness of the fit is still good.

In order to obtain a secondary model to estimate the influence of the electric field strength on the PEF-inactivation of *E. coli*, the new parameters m, b and c from models 2, 3 and 4, were related to the electric field strength.

$$\text{Model 2: } m = 0.006E^2 - 0.370E + 6.521 \quad (R^2 = 0.997) \tag{5}$$

$$\text{Model 3: } \mathrm{Log}_{10} b = 130.685E^{-1.576} \quad (R^2 = 0.994) \tag{6}$$

$$\text{Model 4: } c = 0.00005E^2 - 0.0007E + 0.0003 \quad (R^2 = 0.999) \tag{7}$$

where E is the electric field strength (kV/cm). The secondary models were introduced in the primary ones and tertiary models were obtained. To test the predictive performance of the developed tertiary models, they were validated with 27 different treatments that were different from data used for the generation of the models but within the treatment conditions range used for the generation of the models. Values obtained in these experiments were graphically compared to predicted values obtained from the three tertiary models (Fig. 3). The bias factors were 1.053, 1.035 and 1.071 for models 2, 3 and 4, respectively, and the accuracy factors 1.271, 1.154 and 1.122 for models 2, 3 and 4, respectively.

4. Discussion

Survival curves of *E. coli* at all electric fields investigated were concave upwards indicating that the rate of PEF inactivation was higher at the first moments of the treatment and then gradually declined. These curves are similar in shape to others obtained in our laboratory with other microorganisms such as *Salmonella senftenberg* (Raso et al., 2000), *Listeria monocytogenes* (Álvarez, Pagán, Condón & Raso, 2002) and *Yersinia enterocolitica* (Álvarez, Raso, Sala & Condón, 2002). Other authors working with batch or continuous PEF systems have also observed that survival curves of different microorganisms were upwardly concaved (Jayaram, Castle & Margaritis, 1992; Sensoy, Zhang & Sastry, 1997; Simpson, Whittington, Earnshaw & Russell, 1999; Ohshima, Akuyama & Sato, 2002; Periago, Palop, Martínez & Fernández, 2002).

As generally it is assumed that microbial inactivation follows a first order kinetics, survival curves such as those obtained in this investigation are considered as a deviation from the logarithmic order of death called tailing.

The shape of the survival curves obtained in this investigation could be the result of experimental artefacts. For example, Mañas, Barsotti and Cheftel (2001) observed that the presence of a dead space in the treatment chamber of a PEF equipment resulted in survival curves with a tail with little or not change in tail survival levels. In order to obtain survival curves free from methodological artefacts that could influence their shape, experimental conditions used in this investigation were similar to those described previously by Raso et al. (2000).

The occurrence of tailing has also been attributed to protection resulting from the contents of dead cells, which shield the remaining survivors, or to the microbial

Table 1
Kinetics parameters from the first fitting of the models 1, 2, 3 and 4 to the survival curves of *E. coli* treated by PEF

kV/cm	Model 1					Model 2			
	p (CL 95%)[a]	k_1 (CL 95%)[a]	k_2 (CL 95%)[a]	R^2[b]	RMSE[c]	m (CL 95%)[a]	s (CL 95%)[a]	R^2[b]	RMSE[c]
15	0.8167 (0.6891–0.9443)	0.0128 (0.0054–0.0202)	0.0021 (0.0016–0.0026)	0.992	0.078	1.955 (1.752–2.159)	0.499 (0.447–0.552)	0.981	0.123
19	0.9925 (0.9717–1.000)	0.0139 (0.0092–0.0186)	0.0038 (0.0019–0.0056)	0.991	0.219	1.932 (1.684–2.180)	0.344 (0.299–0.388)	0.973	0.340
22	0.9999 (0.9997–1.0000)	0.0208 (0.0174–0.0242)	0.0025 (0.0009–0.0041)	0.994	0.201	1.690 (1.537–1.848)	0.337 (0.314–0.360)	0.991	0.224
25	0.9989 (0.9970–1.000)	0.0362 (0.0280–0.0444)	0.0058 (0.0039–0.0077)	0.994	0.172	1.533 (1.309–1.757)	0.337 (0.302–0.371)	0.984	0.288
28	0.9966 (0.9894–1.000)	0.0569 (0.0361–0.0776)	0.0108 (0.0068–0.0148)	0.991	0.187	1.416 (1.216–1.616)	0.331 (0.298–0.364)	0.989	0.229
	Model 3				Model 4				
kV/cm	b (CL 95%)[a]	n (CL 95%)[a]	R^2[b]	RMSE[c]	a (CL 95%)[a]	c (CL 95%)[a]	R^2[b]	RMSE[c]	
15	87.026 (70.530–103.500)	0.562 (0.519–0.601)	0.998	0.040	1.100 (0.823–1.376)	0.004 (0.002–0.006)	0.994	0.071	
19	25.291 (14.930–35.650)	0.579 (0.519–0.639)	0.995	0.129	2.568 (1.854–3.280)	0.003 (0.002–0.005)	0.994	0.160	
22	7.576 (−1.239–16.390)	0.488 (0.375–0.600)	0.978	0.353	2.174 (1.800–2.549)	0.009 (0.005–0.012)	0.995	0.159	
25	4.907 (0.672–9.142)	0.483 (0.401–0.564)	0.989	0.224	2.218 (1.763–2.673)	0.012 (0.006–0.018)	0.994	0.161	
28	4.490 (0.520–8.460)	0.517 (0.419–0.614)	0.990	0.206	2.400 (1.973–2.826)	0.015 (0.009–0.021)	0.997	0.106	

[a] CL 95%: Confidence limit.
[b] R^2: Determination coefficient.
[c] RMSE: Root mean square error.

aggregation during the treatment. No aggregation of the cells was observed by microscopic observation of untreated and treated suspensions of *E. coli* and identical survival curves were obtained when the *E. coli* cells were inoculated in a previously inoculated and PEF treated medium (data not shown).

A difficulty that has been attributed to predictive microbiology is that the modelling approach is in many cases not based in the mechanisms involved in the process under study (Baranyi, Ross, McMeekin & Toberts, 1996). Presently, as the mechanisms that govern microbial inactivation by PEF are incompletely understood, it is not possible to develop purely mechanistic models. Therefore, the models that can be used for modelling survival curves are empirical or based on biological assumptions. In terms of R^2 and RMSE, the four models used in this paper, one empirical (model 4) and three based on biological assumptions (models 1, 2,3) accurately described the kinetics of inactivation of *E. coli* by PEF. However, goodness of the fit is not the only criteria that have to be considered in order to choose the best model. Mathematical models should be as simple as possible describing the experimental data using the smallest possible number of parameters. Furthermore, they have to properly accommodate the effect of experimental conditions in order to develop secondary and so tertiary models that describe the influence of different factors on microbial inactivation (Ross, McMeekin & Baranyi, 1999).

The biological assumption of the two-term exponential model (model 1) is based on the fact that two populations, one PEF-sensitive and other PEF-resistant were present in the suspension of *E. coli*. In comparison with the other models, this one uses four parameters to describe the survival curves. Additionally, when parameters obtained by fitting the equation to the data are analysed from a mechanistic point of view several drawbacks are observed. According to the parameter *p* obtained, the proportion of sensitive and resistant microorganisms was not constant and depended on the intensity of the treatment. On the other hand, the rate of

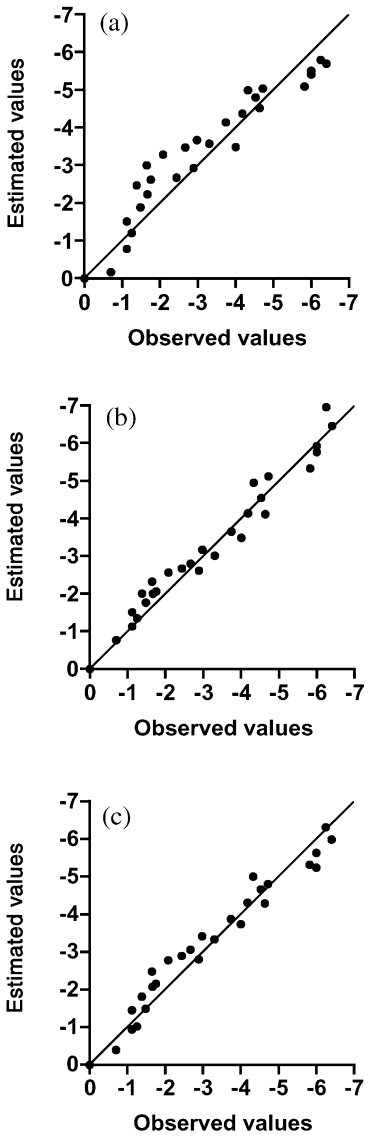

Fig. 3. Correlation between observed and estimated data obtained with the tertiary models 2 (a), 3 (b) and 4 (c) for *E. coli* treated by pulsed electric field strengths (15–28 kV/cm, 250–1000 μs). (a) Model 2; (b) Model 3; (c) Model 4.

Table 2
m, *b*, and *c* values from the second fitting of models 2, 3 and 4 with the *s*, *n* and *a* values set at 0.370, 0.526 and 2.092, respectively, to the survival curves of *E. coli* treated by PEF

kV/cm	Model 2			Model 3			Model 4		
	m (CL 95%)[a]	R^{2b}	RMSE[c]	*b* (CL 95%)[a]	R^{2b}	RMSE[c]	*c* (CL 95%)[a]	R^{2b}	RMSE[c]
15	2.429 (2.308–2.551)	0.960	0.300	70.728 (65.840–75.620)	0.997	0.057	0.001 (0.001–0.002)	0.989	0.144
19	1.793 (1.674–1.911)	0.962	0.367	16.451 (14.810–19.090)	0.993	0.174	0.005 (0.005–0.006)	0.989	0.194
22	1.488 (1.390–1.586)	0.983	0.323	10.387 (8.674–12.100)	0.974	0.366	0.010 (0.009–0.011)	0.995	0.164
25	1.333 (1.204–1.463)	0.970	0.391	7.085 (6.157–8.012)	0.986	0.262	0.014 (0.013–0.016)	0.993	0.169
28	1.190 (1.052–1.328)	0.972	0.369	4.643 (4.045–5.242)	0.989	0.221	0.021 (0.018–0.023)	0.995	0.147

[a] CL 95%: Confidence limit.
[b] R^2: Determination coefficient.
[c] RMSE: Root mean square error.

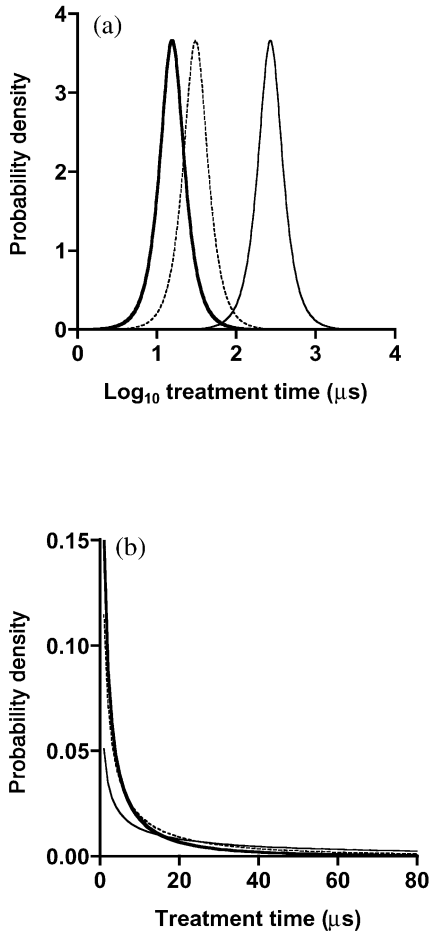

Fig. 4. Probability density distributions from models 2 (a) and 3 (b) of PEF resistance of the PEF-treated bacteria of *E. coli* at 15 (—), 22 (···) and 28 (—) kV/cm.

inactivation of the sensitive population was similar at 15 and 19 kV/cm and increased with the electric field strength at higher electric fields. However, the rate of inactivation of the resistant population was higher at 19 kV/cm than at 22 kV/cm.

Models 2 and 3 assume that there is a PEF resistance distribution within the bacterial population and, as consequence, the survival curves obtained at a constant intensity are the cumulative form of a temporal distribution of lethal events and not an expression of the reaction kinetics (Peleg et al., 2000).

Fig. 4 shows that different types of distributions can describe concave-upwards survival curves of the same shape. While the Weibull probability density distribution is asymmetric, the one derived from the Augustin model is symmetric. At the moment, there is not experimental evidence to be able to discern if this heterogeneity is innate in the population or if it is as consequence of an adaptation of the microorganisms to the treatment that leads to significantly increase their resistance to PEF. According to the mechanisms of microbial inactivation by PEF, variations on the cell size, cell morphology or composition and structure of the microbial membranes could be the cause for the innate distribution of resistances within the microbial population (Wouters et al., 2001). However, similarly to heat, some proteins (shock proteins) that protect microorganisms could be synthesised during the PEF treatment or the PEF treatment could induce changes in size, morphology or membrane structure of the microbial cells that enabled microorganisms survive to the treatment increasing in this way their resistance to PEF.

Models based on a distribution of resistances (models 2 and 3) and also the empirical model (model 4), are simpler than the two-term exponential model (model 1) because it is possible to reduce the number of parameters of the equation from 3 to 2 by fixing one of the parameters to its mean value. In addition from these equations, simple tertiary models that described the influence of the electric field strength and the treatment time on the microbial inactivation could be developed.

Model validation provides information on how well the mathematical model can be used to predict the response to treatments that were not tested in the original treatment.

The bias and the accuracy factors have been suggested to compare objectively the performance of different models (Roos, 1996; Zhao, Chen & Schaffner, 2001). However, it has also been suggested that in addition of calculating the bias and the accuracy factors it is important to plot the predicted and observed values to detect systematic deviations between predicted and observed responses (Roos, 1996). According to the values of these factors obtained in the validation analysis, it is not possible to determine the model with a better performance. Fig. 3 reveals that model 2 systematically overpredicts the observed responses in the region from 1 to 5 Log_{10} cycles and underpredicts in the region above 5 Log_{10} cycles. Overall there is no evidence or a slightly over or under prediction in some region of the plot for the other two models.

Results presented in these investigations show that different primary mathematical model approaches both purely empirical and based on biological assumptions may properly describe the inactivation of *E. coli* by PEF. Simple models derived from the model 3 and model 4 effectively estimated the influence of the electric field strength and the treatment time on the inactivation of this microorganism by PEF. The capability of these models to properly accommodate the effect of environmental conditions and to predict the microbial inactivation in real foods, join with a better understanding of the mechanisms involved on the PEF microbial inactivation, help to establish the superiority of one type of model above the other.

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