Modeling High-Intensity Pulsed Electric Field Inactivation of a Lipase from *Pseudomonas fluorescens*

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ABSTRACT

The inactivation kinetics of a lipase from *Pseudomonas fluorescens* (EC 3.1.1.3.) were studied in a simulated skim milk ultrafiltrate treated with high-intensity pulsed electric fields. Samples were subjected to electric field intensities ranging from 16.4 to 27.4 kV/cm for up to 314.5 μ s, thus achieving a maximum inactivation of 62.1%. The suitability of describing experimental data using mechanistic first-order kinetics and an empirical model based on the Weibull distribution function is discussed. In addition, different mathematical expressions relating the residual activity values to field strength and treatment time are supplied. A first-order fractional conversion model predicted residual activity with good accuracy ($A_f = 1.018$). A mechanistic insight of the model kinetics was that experimental values were the consequence of different structural organizations of the enzyme, with uneven resistance to the pulsed electric field treatments. The Weibull model was also useful in predicting the energy density necessary to achieve lipase inactivation.

Key words: high-intensity pulsed electric field, lipase, inactivation kinetic, simulated skim milk ultrafiltrate

INTRODUCTION

Pseudomonads, especially strains of *Pseudomonas fluorescens*, are the psychrotrophs most commonly encountered in dairy products (Richard, 1981; Cousin, 1982; Deeth and Fitz-Gerald, 1983). This species secretes a lipase whose presence in milk causes a highly unpleasant rancid flavor, mainly owing to the liberation of butyric acid after the hydrolysis of triglycerides (Andersson et al., 1981). Moreover, most lipases from psychrotrophic microorganisms can resist mild heat treatment, such as pasteurization, which implies a great inconvenience from a commercial viewpoint (Lawrence, 1967; Cogan, 1977).

Because of the resistance of this lipase to heat treatment, emerging nonthermal technologies, such as high-

intensity pulsed electric field (HIPEF) processing, could be used to inactivate the enzyme. In the HIPEF process, very short electric pulses (μ s) at high electric field intensities are applied to destroy microorganisms while treatment temperatures are kept moderate ($<60^\circ\text{C}$), thus maintaining the freshness of the food without depleting the hydro- or liposoluble vitamin content (Calderón-Miranda et al., 1999; Bendicho et al., 2002a).

High-intensity pulsed electric field treatments have been shown to inactivate microorganisms effectively in milk, thus leading to levels of microbial inactivation similar to those achieved with heat pasteurization treatments (Martín-Belloso and Elez-Martínez, 2005). However, different results have been obtained on enzyme inactivation, depending on the treatment applied and the kind and source of the enzyme. Grahl and Märkl (1996) achieved a 25% decrease of peroxidase (POD) activity when processing raw milk using 20 pulses at 21.5 kV/cm, whereas Van Loey et al. (2002) found no effect on milk POD after a treatment of 500 μ s at 19 kV/cm. Vega-Mercado et al. (2001) achieved up to 60% inactivation of a protease from *P. fluorescens* by applying a continuous treatment consisting of 98 pulses of 2 μ s at a field strength of 15 kV/cm (2 Hz). Severe HIPEF treatments (35.5 kV/cm for 866 μ s) were required to achieve different degrees of inactivation of a protease from *Bacillus subtilis*, ranging from 57.1 to 81.1%, depending on whether skim milk, whole milk, or a skim milk ultrafiltrate (SMUF) solution was used (Bendicho et al., 2003).

Castro et al. (2001) reported a 65% inactivation of alkaline phosphatase in skim milk processed at 18.8 kV/cm for 740 μ s, but Van Loey et al. (2002) did not observe significant reductions when applying a treatment of 200 pulses of 2 μ s at 20 kV/cm. Regarding lipoprotein lipase inactivation, Grahl and Märkl (1996) observed about a 60% reduction of the enzyme activity when treating milk with 20 pulses at 21.5 kV/cm. Moreover, Bendicho et al. (2002b) proved that HIPEF application could accomplish high levels of inactivation of a lipase from *P. fluorescens*, although this was done by applying a more intensive treatment than that required for microorganisms.

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Table 1. Composition of the simulated skim milk ultrafiltrate¹

Item	Amount, g/L
Lactose	50.00
Potassium phosphate	1.58
Tripotassium citrate	0.98
Potassium sulfate	0.18
Trisodium citrate	1.79
Calcium chloride dehydrate	1.30
Magnesium citrate	0.38
Potassium carbonate	0.30
Potassium chloride	1.00

¹Jeness and Koops (1962).

The factors that mainly influence enzyme inactivation are field strength and treatment time (Martín-Beloso and Elez-Martínez, 2005). So far, no kinetic studies have been conducted on lipase enzymes as affected by HIPEF processing parameters, but most kinetic works on enzyme inactivation have described a log-linear decrease throughout time. Moreover, different empirical models, and among them, especially a model based on the Weibull distribution function, have been used with different degrees of success to describe enzyme inactivation by HIPEF.

The purpose of this work was to model the inactivation of a thermoresistant lipase from *P. fluorescens* in SMUF exposed to HIPEF treatments. We therefore studied whether first-order kinetics is able to describe the data obtained experimentally. The suitability of using an empirical mathematical model to predict enzyme inactivation as a function of field strength and treatment time is also discussed.

MATERIALS AND METHODS

Sample Preparation

An SMUF (Table 1) was used to assess the effectiveness of the HIPEF treatments. The solution was proposed by Jeness and Koops (1962) and has been widely used in HIPEF studies. Lipoprotein lipase enzyme (42 U/mg) from *P. fluorescens* (EC 3.1.1.3.) was obtained from Aldrich (Steinheim, Germany) in powdered form. Enzyme activity was expressed in mU/mL after verifying the existence of a linear correlation between the enzyme activity in mU/mL (from commercial information) and enzyme concentration in milligrams per milliliter using the analytical method. Prior to treatment, the enzyme was added to the SMUF to a concentration level of 300 mU/mL.

Pulsed Electric Field Treatments

High-intensity pulsed electric field treatments were conducted using a pilot plant unit manufactured by

Physics International (San Leandro, CA) equipped with a treatment chamber consisting of parallel plates with an electrode gap of 1.5 cm and a total sample volume of 12.4 mL. Samples were exposed to different electric field intensities (16.4, 18.5, 22.7, and 27.4 kV/cm) using exponential-decay waveform monopolar pulses by means of a 0.1-μF capacitor. The numbers of pulses used for experimental purposes were 20, 40, 60, and 80, representing treatment times of 84.8, 154.7, 241, and 314.5 μs, respectively. Treatment times (*t*) and pulse width (*τ*) were calculated by means of Equations [1] and [2]:

$$t = \Sigma(\tau_i \cdot n_i), \text{ and} \quad [1]$$

$$\tau = R \cdot C. \quad [2]$$

The resistance of the product in the treatment chamber (*R*) varied from 42.4 to 39.3 Ω during treatment because of the temperature increase. The temperature of each HIPEF treatment was controlled by a temperature probe (H9043; Hanna Instruments, Guipúzcoa, Spain) before and after treatment and never exceeded 35°C (from an initial value of 18 ± 1°C). Provided that the shape of the pulses was an exponential decay, the electrical energy density input (*Q*, J/m³) supplied to the samples was computed by the following equation:

$$Q = N \cdot \frac{U_0^2 \cdot C}{2 \cdot v}, \quad [3]$$

where *N* is the number of pulses delivered to the products, *U*₀ is the peak voltage (V), *C* is the capacitance of the capacitor (F), and *v* is the combined volume of all treatment chambers (m³).

Enzyme Determination

Solutions. A *p*-nitrophenyl caprylate (***p*-NPC**) solution (0.005 *M*; Sigma Chemical Co., St. Louis, MO) was prepared by adding 1,326.50 mg of *p*-NPC to 1,000 mL of dimethyl sulfoxide (Riedel de Haën, Seelze, Germany). A pH 8.5 buffer was obtained by mixing 250 mL of 0.2 *M* Tris-hydroxymethyl aminomethane (Prolabo, Fontenay S/Bois, France) with 173 mL of 0.1 *M* HCl (Prolabo) and diluting with distilled water to 1,000 mL.

Lipase Activity Measurement. Lipase activity was quantified using a method described and validated by Bendicho et al. (2001). A mixed solution of 50 μL of *p*-NPC solution, 3 mL of pH 8.5 buffer, and 400 μL of the SMUF sample containing the enzyme was incubated at 37°C for 30 min in a thermostatic bath (Clifton ND-4; Nickel Electron Ltd., Weston-Super-Mare, UK). Finally, the mixture was transferred into a quartz cuvette

to read the absorbance at 412 nm in a UV–visible spectrophotometer (CE 1021; CECIL, Cambridge, UK).

Enzyme activity was expressed as relative activity [RA (%)] and computed using Equation [4]:

$$RA = 100 \cdot \frac{A_t}{A_o}, \quad [4]$$

where A_t is the enzyme activity in the treated sample and A_o is the enzyme activity of the untreated sample.

Statistical Analysis

Each processing condition was assayed in duplicate, as was each enzyme determination. Therefore, the results were averages of 4 measurements. Analysis of variance was used to determined differences between treatments (LSD method) using Statgraphics Plus, version 5.1 for Windows (Statistical Graphics Co., Rockville, MD).

Experimental data were fitted to first-order and to Weibull models by nonlinear regression procedures using the Statgraphics Plus package, version 5.1. Estimated parameters are given with their respective confidence intervals, product of the standard error of the estimates by Student's t adjusted at the degrees of freedom. Fitting accuracy of the models was evaluated through the analysis of R^2 coefficients and the accuracy factor A_f . The accuracy factor was proposed by Ross (1996) to evaluate the performance of models. Equation [5] computes A_f from J experimental observations of RA and their respective J values predicted by the model of concern. Thus, the nearer the A_f -value to the unit, the better the accuracy:

$$A_f = 10^{\frac{\sum_{j=1}^J \left| \log \left(\frac{\text{predicted } RA_j}{\text{observed } RA_j} \right) \right|}{J}} \quad j = 1, 2, 3, \dots, J \quad [5]$$

Mechanistic Models. First-order models are usually used to describe enzyme kinetics. Many enzymes have exhibited an exponential decreasing trend as a function of time (Equation [5]):

$$RA = RA_0 \cdot \exp(-k \cdot t), \quad [6]$$

where RA is the enzyme activity at time t , RA_0 is the initial enzyme activity, k is the first-order kinetic constant, and t is the treatment time.

A first-order fractional conversion model can be used when a fraction of the enzyme is not destroyed after exposure to prolonged treatments (RA_∞). The rate of inactivation can be expressed as

$$f = \frac{RA_0 - RA}{RA_0 - RA_\infty}. \quad [7]$$

Assuming that the enzyme is inactivated following a first-order reaction, fractional conversion can be arranged in a log–linear model in the following form:

$$\ln \left(\frac{RA_0 - RA}{RA_0 - RA_\infty} \right) = -k \cdot t. \quad [8]$$

Equation [7] can be rearranged, resulting in Equation [8], so it is clear that when RA_∞ approaches 0, the equation turns into a simple first-order model (Equation [5]):

$$RA = RA_\infty + (RA_0 - RA_\infty) \cdot \exp(-k \cdot t) \quad [9]$$

Empirical Models. Among the different empirical models that have been used to model enzyme inactivation, Weibull's distribution has recently been proposed to describe enzyme inactivation by HIPEF (Giner et al., 2005, Elez-Martínez et al., 2006). The model is a 2-parameter function (Equation [9]), where α is a scale factor and γ a shape parameter that indicates concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below and above 1, respectively:

$$RA = RA_0 \cdot \exp \left[- \left(\frac{t}{\alpha} \right)^\gamma \right] \quad [10]$$

Derived from the Weibull distribution function parameters (α , γ), t_m is defined as the mean time to inactivate the enzyme by HIPEF and can be used as a measurement of the resistance of the enzyme to HIPEF treatments:

$$t_m = \alpha \cdot \Gamma \left(1 + \frac{1}{\gamma} \right) \quad [11]$$

where Γ is the gamma function.

RESULTS

When the SMUF solution containing lipoprotein lipase was treated by HIPEF, we observed that the enzymatic activity generally decreased with the electric field strength and treatment time (Figure 1). A maximum inactivation of 62.1% was achieved after a 314.5- μ s treatment at 27.4 kV/cm.

First-Order Kinetic Models

The calculated first-order inactivation rate constants (k) for treatments at different electric field intensities

Figure 1. Experimental values (A) and response surface plot (B) relating residual lipase activity (RA) in a simulated skim milk ultrafiltrate to different high-intensity pulsed electric field conditions. Experimental data are shown as the mean $\pm$ standard deviation.

are shown in Table 2. The fit performance of the simple first-order model was good for high electric fields but seemed to decrease for treatments of lower intensity. These determination coefficients ($R^2 = 0.747$ to 0.975) contrasted with those obtained when fitting data to a fractional conversional model, which appeared to be consistently high irrespective of the electric strength ($R^2 = 0.940$ to 0.995 ; Table 2). k -Values of the simple first-order model increased with field strength (Figure 2) following a trend that fit an exponential equation ($R^2 = 0.995$) well in the range of the applied conditions (Equation [12]):

$$k = [(5.5 \pm 0.4) \cdot 10^{-4}] \cdot \exp[(6.8 \pm 0.3) \cdot 10^{-2} \cdot E] \quad [12]$$

Table 2. Simple first-order and first-order fractional conversion kinetic constants¹ for the inactivation of a lipoprotein lipase from *Pseudomonas fluorescens* in a high-intensity pulsed electric field-treated skim milk ultrafiltrate solution at different field strengths

First-order model, kV/cm	$k \cdot 10^3 / \mu s$	R^2
16.37	1.72 ± 0.18	0.747
18.52	1.90 ± 0.17	0.823
22.67	2.66 ± 0.19	0.905
27.42	3.59 ± 0.15	0.975

Fractional conversion, kV/cm	$k \cdot 10^3 / \mu s$	$RA_{\infty}, \%$	R^2
16.37	11.9 ± 2.3	64.6 ± 2.0	0.962
18.52	9.0 ± 2.3	59 ± 4	0.940
22.67	8.1 ± 1.4	46 ± 4	0.971
27.42	6.4 ± 0.5	28 ± 3	0.995

¹Constant values $\pm$ asymptotic standard error. R^2 = determination coefficient adjusted for degrees of freedom; RA_{∞} = residual activity after exposure to prolonged treatments.

On the other hand, the kinetic rates of enzyme inactivation (k) estimated by nonlinear regression of the fractional conversion first-order model took values from $6.4 \cdot 10^{-3} / \mu s$ to $1.19 \cdot 10^{-2} / \mu s$ and decreased with the field strength ($P < 0.05$). The effects of E on k and RA_{∞} are shown in Figure 3. The electric field dependence of k could be described by a potential equation ($R^2 = 0.942$; Equation [13]). On the other hand, the residual activity after prolonged treatments (RA_{∞}) ranged from 28 to 64.6% and decreased when the intensity of the treatment increased. A second-degree polynomial was found to describe ($R^2 = 0.999$) the field strength dependency of RA_{∞} (Equation [14]):

Figure 2. Effect of electric field strength on the rate constant (k) estimated by a simple first-order model used to describe the high-intensity pulsed electric field-inactivation of a lipase from *Pseudomonas fluorescens* in a simulated skim milk ultrafiltrate. Results are expressed as the mean $\pm$ standard deviation.

Figure 3. Effect of electric field strength on the rate constants (k , RA_{∞}) estimated by a first-order fractional conversion model used to describe the high-intensity pulsed electric field-inactivation of a lipase from *Pseudomonas fluorescens* in a simulated skim milk ultrafiltrate. Results are expressed as the mean $\pm$ standard deviation.

$$k = (0.23 \pm 0.10) \cdot E^{(-1.09 \pm 0.19)}, \text{ and} \quad [13]$$

$$RA_{\infty} = 100 - (1.40 \pm 0.07) \cdot E - (0.045 \pm 0.003) \cdot E^2 \quad [14]$$

Hence, substitution of k by Equation [12] into the simple first-order model (Equation [6]) transforms the equation into a function dependent on the field strength and treatment time (Equation [15]). A similar function (Equation [16]) is obtained when replacing the constants defined in the fractional conversion model (Equation [9]) by Equations [13] and [14], respectively:

$$RA = 100 \cdot \exp(-[(5.5 \pm 0.4) \cdot 10^{-4}] \cdot \exp\{[(6.8 \pm 0.3) \cdot 10^{-2} \cdot E] \cdot t\}, \text{ and} \quad [15]$$

Table 3. Estimated parameters of the Weibull distribution function proposed to describe the inactivation of a lipoprotein lipase from *Pseudomonas fluorescens* in a high-intensity pulsed electric field-treated skim milk ultrafiltrate solution at different field strengths

Field strength, kV/cm	α , μ S	γ	R^2
16.37	$2,817 \pm 1,961$	0.38 ± 0.10	0.947
18.52	$1,171 \pm 532$	0.52 ± 0.13	0.922
22.67	558 ± 90	0.58 ± 0.08	0.971
27.42	305 ± 14	0.78 ± 0.06	0.990

$$RA = [100 - (1.40 \pm 0.07) \cdot E - (0.045 \pm 0.003) \cdot E^2] \cdot \exp\{ -[(0.23 \pm 0.10) \cdot E^{(-1.09 \pm 0.19)}] \cdot t\} \quad [16]$$

As can be deduced from Figure 3B, total inactivation after prolonged treatment can be reached at high field strengths. Therefore, the minimal field strength necessary to achieve a 100% enzyme inactivation ($RA_{\infty} = 0$) can be calculated from Equation [14]. An estimated value of 34.1 kV/cm is obtained through the resolution of the second-degree quadratic equation.

Weibull Distribution Function

The estimated parameters for the Weibull distribution function proposed to describe lipoprotein lipase inactivation at different field strengths are displayed in Table 3. The model described data with good accuracy irrespective of the treatment conditions ($R^2 = 0.922$ to 0.990). Electric field dependence of γ and α is visualized in Figure 4. The shape parameter (γ) took values of 0.38 to 0.78, and field dependency was found to be linear ($R^2 = 0.960$) in the range of applied conditions (Equation [17]). The scale factor (α) decreased when the field strength increased (Figure 4) following a trend that could be described with good accuracy ($R^2 = 0.971$) using a potential model (Equation [18]). Estimated α -values ranged from 305 to 2817 μ S. Uncertainty in the estimated scale factors increased dramatically when the field strength approached zero (Table 3):

$$\gamma = [(0.033 \pm 0.005) \cdot E] - (0.14 \pm 0.10), \text{ and} \quad [17]$$

$$\alpha = [(2.89 \pm 0.23) \cdot 10^8] \cdot E^{(-4.2 \pm 0.5)} \quad [18]$$

By rearranging Equations [17] and [18] in Equation [10], a new function is obtained (Equation [19]) that allows estimation of the activity after treatment as a function of treatment time (t) and field strength (E):

Figure 4. Effect of electric field strength on the rate constants (α , γ) estimated by a Weibull model used to describe the high-intensity pulsed electric field-inactivation of a lipase from *Pseudomonas fluorescens* in a simulated skim milk ultrafiltrate. Results are expressed as the mean $\pm$ standard deviation.

$$RA = 100 \cdot \exp \left[- \left(\frac{t}{(2.89 \pm 0.23) \cdot 10^8 \cdot E^{(-4.2 \pm 0.5)}} \right) \{ [(0.033 \pm 0.005) \cdot E] - (0.14 \pm 0.10) \} \right] \quad [19]$$

The values of mean time to achieve inactivation (t_m), calculated from Equation [11], decreased with increasing field strength, thus varying from 11,216 μ s to 349 μ s for the field intensities applied.

DISCUSSION

Lipase inactivation can be achieved in an SMUF solution by applying HIPEF treatments. In general, we observed that the activity decreased with increasing pulse number and electric field strength (Figure 1). Data were fitted to the different models to evaluate the influence of the applied conditions on the residual enzyme activity.

To our knowledge, no specific literature exists in which the inhibition kinetics of milk lipase or related enzymes has been studied. The kinetic behavior of li-

pase after HIPEF treatments related to treatment time agrees with that reported by Giner et al. (2000) and Elez-Martínez et al. (2007) for pectin methylesterase (**PME**) from tomato and orange juice, respectively, and by Giner et al. (2001) for apple and pear polyphenoloxidases (**PPO**). Giner et al. (2000, 2001) used a simple first-order model to describe inactivation of both enzymes. They obtained k -values ranging from $0.36 \cdot 10^{-4}$ to $3.5 \cdot 10^{-4} / \mu$ s for PME using electric field intensities from 5 to 24 kV/cm, and k -values from $0.24 \cdot 10^{-4}$ to $4.40 \cdot 10^{-4} / \mu$ s for PPO using electric field intensities from 5.50 to 24.6 kV/cm. In our study, kinetic rates defined by the simple first-order model ranged from $1.52 \cdot 10^{-3}$ to $3.59 \cdot 10^{-3} / \mu$ s, thus indicating that lipase in an SMUF solution was slightly less resistant to HIPEF processing than were PPO and PME enzymes from vegetable sources. According to our results, the studies performed with other enzymes showed that a plot of the k -values obtained from the first-order adjustment vs. the applied field strength matched an exponential trend with good agreement (Giner et al., 2000, 2001; Elez-Martínez et al., 2007).

As a result of the fitting, a mathematical expression was obtained relating inactivation to electric field strength and treatment time (Equation [15]). The fitting performance of the equation was evaluated by calculating the accuracy factor (A_f). The A_f -value for the simple first-order equation was 1.066, indicating a relatively good performance of the model. However, a lack of randomization was observed in the residual analysis. When a simple first-order kinetic model does not sufficiently approach the experimental data, other first-order expressions, such as biphasic and fractional conversion models, can be used to describe enzyme kinetics.

Enzymes characterized by several isoenzymes can often be grouped into 2 fractions, one more resistant to adverse conditions than the other (Chen and Wu, 1998). In addition, the existence of different enzyme isoforms can also modify the behavior of the enzyme when subjected to different treatments. Fernández-Lorente et al. (2003) found that, at moderate enzyme concentrations, lipase from *P. fluorescens* was able to aggregate into bimolecular structures, which were much more stable than the monomeric forms of the enzyme. This could explain the deviations in the values estimated by a simple first-order model in relation to the observed values (Figure 5).

A fractional conversion model takes into account a nonzero activity after prolonged treatment and could therefore be used to describe lipase inactivation if it is assumed that total inactivation of the enzyme isoforms cannot be reached at low field strength irrespective of the duration of the treatment. In fact, pulse treatments of low and moderate intensity could favor the aggrega-

Figure 5. Lipase inactivation in a high-intensity pulsed electric field-treated simulated skim milk ultrafiltrate solution as modeled by simple first-order, fractional conversion, and Weibull kinetics. Plotted lines correspond to the values obtained from the estimated equations (see Equations 15, 16, and 19 in text). Diamonds, squares, triangles, and circles correspond to experimental residual activity (RA) found after 16.37-, 18.52-, 22.67-, and 27.42-kV/cm treatments, respectively.

tion of lipases into dimeric or even oligomeric forms. In some cases, very large lipase oligomers have been found, usually after treating the lipase under severe conditions, such as during freeze-drying (Liou et al., 1998). As previously estimated, treatments above 34.1 kV/cm would avoid this phenomenon, thus allowing total enzyme inactivation to be reached. Hence, the fractional conversion model estimates a 99% inactivation

with a treatment of 34.1 kV/cm for 925 μ s. With this treatment an energy density input of 9,030 MJ/m³ is supplied, which is a value similar to the 8,067 MJ/m³ that was found to inactivate a 98.6% POD in orange juice (Elez-Martínez et al., 2007).

The first-order fractional conversion model (Equation [9]) fitted the experimental data with good agreement. An excellent correlation was found between observed

Figure 6. Experimental values of residual lipase activity (RA) expressed as a function of the electric energy density (Q) supplied under different high-intensity pulsed electric field treatment conditions. The plotted line corresponds to the adjustment of the Weibull function to the observed data (see Equation 20 in text).

and residual activity values predicted by the model (Figure 6). Other studies on enzyme inactivation as affected by HIPEF conditions have also used the fractional conversion equation with good results. Elez-Martínez et al. (2006, 2007) inactivated orange juice PME and POD with constant rates of $(2.5 \pm 1.3) \cdot 10^{-3}/\mu\text{s}$ and $2.4 \cdot 10^{-3}$ to $1.9 \cdot 10^{-2}/\mu\text{s}$, respectively, with monopolar pulses of 4 μs at 200 Hz, applying field intensities ranging from 5 to 35 kV/cm for 100 to 1,500 μs . These rates are similar to those found in the present study (Table 2). Furthermore, our results, indicating a quadratic correlation between RA_{∞} -values and field strength, in contrast with those of Elez-Martínez et al. (2006, 2007) showing a linear dependence for POD but no dependence for PME.

The Weibull model can be regarded as an alternative to the fractional conversion model, but assuming that zero activity after prolonged treatments can be achieved whenever enough energy is supplied to the system, even with low or moderate field strength. As a comparison of the models, A_f -values for the simple first-order, fractional conversion, and Weibull equations were 1.066, 1.018, and 1.037, respectively. Therefore, from a quantitative point of view, data were accurately fitted by the fractional conversion model (Equation [16]), followed by the Weibull model (Equation [19]) and the simple first-order kinetic (Equation [15]). This is illustrated in Figure 5, where the residual activity estimated by the models is compared with the data. The divergence from the bisector in the plot of observed versus predicted values is closely related to the fitting accuracy of each model, so that the more the measured and estimated values mutually differ, the less successful the model is.

The relationship between residual lipoprotein lipase activity and the energy supplied with the different

treatments is shown in Figure 6. The reduction in lipase activity could be related to the energy density input (Q) by using the Weibull equation (Equation [20]).

$$RA = 100 \cdot \exp \left[- \left(\frac{Q}{2227 \pm 160} \right)^{0.63 \pm 0.04} \right] \quad [20]$$

The Weibull distribution yielded the best accuracy factor ($A_f = 1.118$) compared with a first-order model ($A_f = 4.545$) and to a fractional conversion model ($A_f = 1.280$). The energy density necessary to inactivate a fraction of the enzyme can be obtained from the equation. Thus, the estimated energy densities to be supplied to achieve 50, 90, and 99% inactivation were 1,249, 8,906, and 24,765 MJ/m^3 , respectively. These data show that, to the extent residual activity approaches zero, the amount of extra energy needed to continue to inactivate the enzyme increases.

CONCLUSIONS

Reduction of lipoprotein lipase activity in an SMUF solution can be achieved by applying severe HIPEF treatments, thus supplying large amounts of energy. The kinetics of inactivation was accurately fitted with a first-order fractional conversion equation. The relationship between the nonzero residual activity after prolonged treatment defined by the model (RA_{∞}) and the treatment field strength (E) suggests the existence of different forms of the enzyme, probably resulting from the aggregation of monomers into more complex forms that would have a greater resistance against pulsed electric field damage. Indeed, moderate HIPEF treatments could promote the aggregation of the unimolecular enzyme structures into aggregate structures that, in turn, would be more resistant to the applied treatments. A model based on the Weibull distribution appears to be an alternative empirical tool to describe enzymatic inactivation but specifically to relate the degree of inactivation to the amount of energy density supplied to the system.

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