Effects of High Intensity Pulsed Electric Field and Thermal Treatments on a Lipase from Pseudomonas fluorescens

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ABSTRACT

Milk and dairy products may contain microorganisms capable of secreting lipases that cause sensory defects and technological problems in the dairy industry. In this study, the effects of thermal and high-intensity pulsed electric field (HIPEF) treatments on an extracellular lipase from Pseudomonas fluorescens, suspended in a simulated skim milk ultrafiltrate (SMUF) have been evaluated. Heat treatments applied were up to 30 min from 50 to 90°C. HIPEF treatments were carried out using pilot plant facilities in a batch or continuous flow mode, where treatment chambers consisted of parallel and coaxial configuration, respectively. Samples were subjected to up to 80 pulses at electric field intensities ranging from 16.4 to 37.3 kV/cm. This resulted in a lipase that was quite resistant to heat and also to HIPEF. High (75°C-15 s) and low pasteurization treatments (63°C-30 min) led to inactivations of 5 and 20%, respectively. Using the batch-mode HIPEF equipment, a 62.1% maximum activity depletion was achieved after 80 pulses at 27.4 kV/cm. However, when HIPEF treatments were applied in the continuous flow mode, an inactivation rate of just 13% was achieved, after applying 80 pulses at 37.3 kV/cm and 3.5 Hz. The results of both heat and HIPEF treatments on enzyme inactivation were adjusted with good agreement to a first-order kinetic model (R² > 62.3%).

(Key words: high-intensity pulsed electric fields, lipase, enzymes, milk)

Abbreviation key: HIPEF = high-intensity pulsed electric fields, RA = relative activity, SMUF = solution similar to skim milk ultrafiltrate.

INTRODUCTION

The widespread practice of storing raw milk at refrigeration temperatures before processing prevents spoilage by lactic acid-producing mesophilic bacteria (Fairbairn and Law, 1986). However, this practice has resulted in the selective growth of psychrotrophic microorganisms (Thomas, 1974; Cousins et al., 1977; Law, 1979).

The psychrotrophs found in refrigerated bulk milk are mainly gram-negative bacteria of the genera Pseudomonas, Achromobacter, Alcaligenes, and Enterobacter (Thomas and Druce 1963, 1969; Lück, 1972; Murray and Stewart, 1978). Pseudomonads, especially strains of Pseudomonas fluorescens are the most commonly encountered psychrotrophs in dairy products (Muir et al., 1979; 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 due to the liberation of butyric acid after the hydrolysis of triglycerides (Andersson et al., 1981).

Milk preservation is currently accomplished by heating the milk, but most enzymes secreted by psychrotrophic microorganisms are resistant to thermal treatments (Lawrence, 1967; Cogan, 1977). The storage stability of UHT-sterilized milk becomes dependent on the content of heat resistant quality-degrading microbial enzymes in the raw milk (Andersson et al., 1981). Kishonti (1975) observed that several extracellular lipases from psychrotrophic bacteria in milk were able to maintain at least 75% of its initial activity following pasteurization treatment (63°C-30 min). Thus, rancid flavor may also appear in milk products that have been subjected to sterilization or pasteurization treatments (Andersson et al., 1981).

Because of the resistance of microbial lipases to heat treatment, nonthermal processes such as high-intensity pulsed electric fields (HIPEF) could be tried to inactivate them. HIPEF treatment involves applying very short electric pulses (µs) at high electric field intensities. Temperature is kept moderate during the process, to destroy the microorganisms, thus maintaining the food’s fresh-like qualities and causing little depletion in the hidro or liposoluble vitamin content (Calderón-Miranda et al., 1999; Bendicho et al., 1999).
Table 1. Composition of simulated skim milk ultrafiltrate (Jeness and Koops, 1962).

<table>
<thead>
<tr>
<th>Item</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>50.00</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>1.58</td>
</tr>
<tr>
<td>Tri-potassium citrate</td>
<td>0.98</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>0.18</td>
</tr>
<tr>
<td>Tri-sodium citrate</td>
<td>1.79</td>
</tr>
<tr>
<td>Calcium chloride dehydrate</td>
<td>1.30</td>
</tr>
<tr>
<td>Magnesium citrate</td>
<td>0.38</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>0.3</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The HIPEF process destroys microorganisms in both milk and simulated milk ultrafiltrate (SMUF) (Zhang et al., 1994; Pothakamury et al., 1995a,b; Dunn and Pearlman, 1987; Martín et al., 1997; Michalac et al., 1999). But there are few studies about the effect of HIPEF effect on milk enzymes. With the electrohydraulic treatment, Gilliand and Speck (1967) achieved significant inactivation of a protease from Bacillus subtilis in a buffer solution. The inactivation mechanism proposed for that enzyme was an oxidative reaction of key components by electric fields as a function of treatment time. Vega-Mercado et al. (1995a) achieved the inactivation of a protease from Pseudomonas fluorescens in milk until up to 60% by HIPEF. Vega-Mercado et al. (1995b) and Castro et al. (1994) reported reductions higher than 90% for plasmin and alcaline fosfatase after a HIPEF treatment of milk. However, Grahl and Märkl (1996) only observed a slight depletion of alcaline fosfatase in milk, similar to that obtained by Ho et al. (1997) in a buffer solution. This latter author also studied the behavior of other milk enzymes when subjected to HIPEF in buffer solutions and obtained variable results. For lipase and peroxidase, up to 85 and 30% inactivation was achieved, respectively (Ho et al., 1997). Grahl and Märkl (1996) obtained similar results with lipase and peroxidase in milk. Other enzymes such as lysozyme and pepsine showed an increase in activity, the former a rise of activity in certain ranges of voltage, whereas the latter reached up to double its initial activity (Ho et al., 1997).

The purpose of this work was to study the effect of batch and continuous HIPEF treatments on the destruction of a thermoresistant lipase from P. fluorescens in SMUF and also to compare the effect of HIPEF to heat treatments.

**MATERIALS AND METHODS**

**Sample Preparation**

This study was conducted with SMUF (Table 1), a salt solution with composition encountered in milk ultrafiltrates. It was proposed by Jeness and Koops (1962) and widely used in nonthermal processing research. The conductivity of SMUF was adjusted to 5.20 mS/cm, in order to be electrically similar to milk.

Lipase from P. fluorescens [9001-62-1] in powder form (42 U/mg) was obtained from Aldrich (Steinheim, Germany). The enzyme activity was expressed in mU/ml after we verified the existence of a linear correlation between enzyme activity in mU/ml (from commercial information) and enzyme concentration in mg/ml by the analytical method (Bendicho et al., 2001) described in the section “enzyme determination.”

Before running the treatments, we added the enzyme lipase to SMUF to a concentration level of 300 mU/ml.

**Thermal Treatments**

Several heat treatments including high (75°C-15 s) and low (63°C-30 min) pasteurization were applied to SMUF for use as reference values (Table 2). Samples were placed in 5-ml test tubes and heated at preselected temperatures 50, 55, 63, 75, and 90°C in a thermostatic bath (Clifton ND-4, Nickel Electron LTD, Weston-Super-Mare, England). Temperature was measured with a temperature probe (H9043, Hanna Instruments, Guipúzcoa, Spain) immersed in the solution. Aliquots of the sample were removed at specified intervals (Table 2) and cooled with ice until activity determination.

**Pulsed Electric Field Treatments**

HIPEF treatments were carried out at two different pilot plant facilities, a batch (University of Lleida) and a continuous one (Washington State University), both manufactured by Physics International (San Leandro, CA). In both cases, the delivered pulses had an exponential decaying waveform.

The batch-mode equipment had a generator with a capacitance of 0.1 µF. The treatment chamber was a parallel plate with a 1.5-cm electrode gap and a volume of 12.4 ml (Figure 1). The peak electric field intensities reached in the chamber were 16.4, 18.5, 22.7, and 27.4 kV/cm. The number of pulses applied was 10, 20, 40, 60, and 80. The temperature of the sample was measured...
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before and immediately after the treatments with a temperature probe (H9043, Hanna Instruments, Guipúzcoa, Spain). The temperature, initially at 18 to 20°C, never exceeded 34°C.

The continuous HIPEF system had a 0.5-µF capacitor and a coaxial treatment chamber containing two stainless steel electrodes with a gap of 0.67 cm and a volume of 30.56 cm³ (Figure 2). In this case, the electric field intensities applied were 26.1, 30.5, and 37.3 kV/cm. Frequencies from 2 to 3.5 Hz were tested up to 80 pulses. We attempted higher field strengths and frequencies, but at those conditions, dielectric breakdown occurred, which caused an undesirable arching phenomenon. The flow rate of SMUF was 500 ml/min, controlled by a variable speed pump (Masterflex 7654-00, Cole Palmer Instruments Co., Chicago, IL). A cooling coil immersed in iced water was used to cool the SMUF at the entrance and exit of the treatment chamber. The temperature of the entrance and exit was recorded with a digital thermometer (John Fluke Mfg. Co., Everett, WA). The inlet temperatures were between 3 and 6°C and the maximum outlet temperature was 35°C.

In both cases, the input energy supplied per volume unit in each pulse (Q, J/l) can be computed:

\[
Q = \frac{V_0^2 \cdot C}{2 \cdot V}
\]

where \(V_0\) is the peak voltage (V), \(C\) the capacitance of the capacitor (F), and \(V\) the volume of the treatment chamber (l).

Enzyme Determination

Solutions. Before we ran the analysis to determine lipase activity, we prepared a p-NPC solution (0.005 M) adding 1326.50 mg of p-NPC (Sigma Chemical Co., St Louis, MO) to 1000 ml of DMSO (Riedel de Haën, Seelze, Germany). A pH 8.5 buffer was prepared mixing 250 ml of THMA 0.2 M (Prolabo, Fontenay S/Bois, France) with 173 ml of HCl 0.1 M (Prolabo) and diluting it with distilled water to 1000 ml.

Lipase activity. Lipase activity was quantified by a spectrophotometric method described and validated by Bendicho et al. (2001). To run the analysis in SMUF, we placed 50 µl of p-NPC solution, 3 ml of pH 8.5 buffer, and 400 µl of the SMUF sample containing the enzyme in a test tube. This mixture was incubated at 37°C for 30 min in a thermostatic bath (Clifton ND-4, Nickel Electron LTD, Weston-Super-Mare, England). Finally, the mixture was transferred into cuvettes to read the absorbance at 412 nm in a spectrophotometer UV/Visible (CE 1021, CECIL, Cambridge, England).

In both HIPEF and thermal treatments, lipase activity of control and processed samples was measured at the same time and immediately after processing to avoid the effects of time in storage.

Statistics

Each processing condition was assayed in duplicate and enzyme determination was also performed in duplicate. Therefore, the results were averages of four measurements. Enzyme activity was expressed as relative activity [RA(%)] and computed using the following expression:
RA = 100 \cdot \frac{A_t}{A_0}

where A_t is the enzyme activity in the sample following treatment and A_0 the enzyme activity of the untreated sample.

Analysis of variance was used to determine significant differences among treatments (P = 0.05) and was performed by “Statgraphics plus” version 2.1 for Windows package (Statistical Graphics Co., Rockville, MD).

The reduction of enzyme activity as a function of treatment time for each thermal treatment may be adjusted to a first-order kinetic model (equation 2). The Arrhenius model was used to adjust the k_T-values to the treatment temperature (equation 3).

\[
RA = RA_0 \cdot e^{-k_Tt}
\]

\[
k_T = k_0 \cdot e^{\frac{-E_a}{RT}}
\]

where RA_0 is the intercept of the curve, k_T the first-order constant for each evaluated temperature (min^{-1}), t the treatment time (min), E_a the energy of activation (J/mol), R the constant of the ideal gases (8.31 J/(°K⋅mol)), and T the treatment temperature (°K).

Also, an exponential decay model, was used to describe the inhibition of enzyme by HIPEF. Thus, lipase activity depletion might be expressed mathematically by.

\[
RA = RA_0 e^{-k_EN}
\]

where RA_0 is the intercept of the curve, N the number of pulses, and k_E the first-order kinetic constant for each electric field intensity evaluated (pulses^{-1}).

RESULTS

Thermal Treatments

Lipase from P. fluorescens showed a high resistance to heat. Results indicated that after any thermal treatment there was a significant reduction (P < 0.05) in lipase activity with increasing temperature or treatment time (Figure 3). However, neither a low (63°C-30 min) nor a high thermal pasteurization treatment (75°C-15 s) could totally inactivate the enzyme. Only 20 and 5% depletions were reached, respectively. To achieve a near complete depletion, 30 min were required at 75°C or 5 min at 90°C.

The values of RA(%) as a function of heat treatment time were adjusted to a first-order kinetic model (equation 2). The model displayed a high determination coefficient (R^2), which varied from 0.623 to 0.978 (Table 3). The first-order kinetic constant values (k_T) ranged from 0.0024 to 0.0931 min^{-1} and the increase in treatment temperature involved an exponential raise in the first-order k_T values. Under the evaluated conditions, the influence of temperature (T) on k_T could be described by the Arrhenius equation (equation 5) (R^2 = 0.978) (Figure 4). The activation energy (E_a) obtained in this range (50 to 90°C) was 134.48 kJ/mol.

Pulsed Electric Field Treatments

Batch-mode HIPEF. When the SMUF samples containing lipase were treated with batch-mode HIPEF, it was observed that the enzyme activity lowered as the electric field intensity and number of pulses increased (Figure 5). The inactivation obtained at 16.4 and 18.5 kV/cm showed analogous behavior patterns (P < 0.05), reaching up to a 33.5% depletion after an 80-pulse treat-

<table>
<thead>
<tr>
<th>Treatment temperature (°C)</th>
<th>k_T (min^{-1})</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0,0024</td>
<td>0.623</td>
</tr>
<tr>
<td>55</td>
<td>0,0071</td>
<td>0.958</td>
</tr>
<tr>
<td>63</td>
<td>0,0093</td>
<td>0.960</td>
</tr>
<tr>
<td>75</td>
<td>0,0931</td>
<td>0.978</td>
</tr>
</tbody>
</table>
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Figure 4. Effect of the temperature on the rate constant (k_T) in first-order kinetic model used to describe the inhibition of lipase activity by heat treatments. Plotted line is an exponential decay fit.

Figure 5. Inhibition of lipase activity after several batch mode high-intensity pulsed electric field (HIPEF) treatments. Treatments were performed at 16.4 (●), 18.5 (■), 22.7 (▲), and 27.4 kV/cm (●). Plotted lines correspond to the fit of experimental data to a first-order kinetic model.

Figure 6. Effect of the electric field intensity (E) on the rate constant (k_E) in first-order kinetic model used to describe the inhibition of lipase activity by batch-mode pulsed electric field treatments. Plotted line is an exponential decay fit.

Table 4. Exponential decay model rate constants (k_E) for inhibition of lipase activity in simulated milk ultrafiltrate by pulsed electric fields in batch mode at different electric field intensities.

<table>
<thead>
<tr>
<th>Electric field intensity (kV/cm)</th>
<th>k_E (pulse^-1)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4</td>
<td>6.2 × 10^{-3}</td>
<td>0.702</td>
</tr>
<tr>
<td>18.5</td>
<td>6.3 × 10^{-3}</td>
<td>0.855</td>
</tr>
<tr>
<td>22.7</td>
<td>9.8 × 10^{-3}</td>
<td>0.820</td>
</tr>
<tr>
<td>27.4</td>
<td>1.28 × 10^{-2}</td>
<td>0.958</td>
</tr>
</tbody>
</table>

Continuous-mode HIPEF. Lipase activity varied with any increase in number of pulses applied and electric field intensity (P < 0.005) when SMUF samples were exposed to continuous-mode HIPEF treatments. By holding the rest of the variables constant, the application of pulses at 2 or 3.5 Hz showed a different extent of inhibition.

At 2 Hz, it was observed that 26.1 kV/cm led to different results of enzyme inactivation than that obtained with 30.5 and 37.3 kV/cm. The latter showed a similar...
Inhibition of relative lipase activity (RA) exposed to 2 and 3.5-Hz high intensity pulsed electric field treatments. Treatments were performed at 26.1, 30.5, and 37.3 kV/cm. Plotted lines are exponential decay fits.

Figure 7. Inhibition of relative lipase activity (RA) exposed to 2 and 3.5-Hz high intensity pulsed electric field treatments. Treatments were performed at 26.1 (●), 30.5 (■) and 37.3 kV/cm (▲). Plotted lines are exponential decay fits.

Table 5. Exponential decay model rate constants (k_E) for inhibition of lipase activity in simulated milk ultrafiltrate by pulsed electric fields in continuous mode at different electric field intensities.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Electric field intensity (kV/cm)</th>
<th>k_E (pulse⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>26.1</td>
<td>4×10⁻⁴</td>
<td>0.929</td>
</tr>
<tr>
<td></td>
<td>30.5</td>
<td>9×10⁻⁴</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>37.3</td>
<td>7×10⁻⁴</td>
<td>0.882</td>
</tr>
<tr>
<td>3.5</td>
<td>26.1</td>
<td>9×10⁻⁴</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td>30.5</td>
<td>3×10⁻⁴</td>
<td>0.782</td>
</tr>
<tr>
<td></td>
<td>37.3</td>
<td>1.6×10⁻³</td>
<td>0.879</td>
</tr>
</tbody>
</table>

It can be observed that the 3.5-Hz treatments led to higher levels of enzyme inactivation than did the 2-Hz treatments, which could be advantageous, since the higher the process frequency, the faster the achievement of equivalent treatments. Thus, the 3.5-Hz process, besides being faster, is more effective than the 2-Hz one.

The kinetic constants obtained could not be adjusted to any kinetic model because the resulting linear k_E-values not always increased proportionally to the field strength applied.

**Lipase inactivation as a function of supplied energy per unit volume.** Energy densities supplied to the samples in all the different HIPEF experimental conditions were calculated from equation 1 and multiplied by the applied number of pulses. As observed in Figure 8, the RA of lipase as related to the supplied energy for each evaluated electric field intensity could be adjusted with good agreement to exponential models that held k_E-values from 3×10⁻⁴ to 1.6×10⁻³ pulses⁻¹ (R²: 0.782 to 0.952) (Table 5).

Different results were obtained when applying similar conditions at 3.5 Hz. In this case, the maximum extent of inhibition (13%) was achieved when the samples were subjected to 30.5 or 37.3 kV/cm (Figure 7). In these three cases, inactivation increased exponentially (R²: 0.88 to 0.97) with the number of pulses. The first-order k_E-values obtained from each field strength adjustment ranged from 4×10⁻⁴ to 9×10⁻⁴ pulses⁻¹ (Table 5).

Figure 8. Relative lipase activity (RA) in simulated milk ultrafiltrate exposed to different inputs of electric energy densities (Q) supplied by high intensity pulsed electric fields (HIPEF) treatments in batch (■) or continuous mode (●). Plotted lines are exponential decay fits.
energy densities (Q) could be successfully described by a first-order kinetic model for both batch (R² = 0.897) and continuous (R² = 0.644) treatments (equations 7 and 8). The k-values obtained were 1.9 × 10⁻³ L/kJ in the batch-mode treatment and 2 × 10⁻⁴ L/kJ in the continuous one.

\[ RA = 94.24 \cdot e^{-1.9 \cdot 10^{-3} \cdot Q} \]  
\[ RA = 99.40 \cdot e^{-0.2 \cdot 10^{-3} \cdot Q} \]

**DISCUSSION**

The evaluated lipase from *P. fluorescens* was quite resistant to currently used thermal treatments (75°C-15 s and 63°C-30 min). High and low pasteurization treatments led to inactivations of 5 and 20%, respectively. Griffiths et al. (1981), in studying the behavior of a lipase from another strain of *P. fluorescens*, also noticed that the enzyme showed resistance to heat; after a thermal treatment of 77°C-17 s, they observed a 35% decrease in activity. Other authors also have highlighted the thermoresistance of extracellular enzymes from milk psychrotrophic bacteria. Kishonti (1975) found that, in general, several lipases were able to maintain at least 75% of its initial activity after a treatment at 63°C-30 min. Driessen (1983) and Andersson (1981) found lipase activity in pasteurized and UHT treated milk that resulted in an off flavor due to milk fat hydrolysis.

The depletion of enzyme activity related to treatment time for each studied temperature followed a first-order kinetic model. The results were consistent with those reported by Driessen (1983) for several thermoresistant extracellular lipases from Gram-negative bacteria when processed at different temperatures (85 to 110°C). Moreover, the kₑ obtained in the range of temperatures studied (50 to 90°C) could be adjusted to the Arrhenius model. The Eₚ for lipase was 134.48 kJ/mol, similar to the value reported by Driessen (1983) in the range of 50 to 60°C for a lipase from *P. fluorescens* 22F (185.362 kJ/mol).

As regards the HIPEF process, it affected the activity of this thermoresistant lipase, although the level of inactivation depended on the treatment applied. When using a batch-mode HIPEF process, up to a 62.1% lipase inhibition was attained after an 80-pulse treatment at 27.4 kV/cm. To reach similar results applying heat, 10 min at 75°C or 1 min at 90°C was needed, whereas heat treatments of 10 to 15 min at 63°C or 1 to 3 min at 75°C would achieve the maximum reduction reported for the continuous treatment mode.

Ho et al. (1997) also studied the effect of HIPEF on a lipase, but in a buffer solution. Its activity was reduced up to 85% after applying 30 pulses at 90 kV/cm with the continuous HIPEF equipment. No more references have been found regarding the inhibition of any lipase by HIPEF. However, several studies exist as to the effect of HIPEF on other milk enzymes. Using a batch-mode HIPEF system, Castro et al. (1994) achieved nearly the same extent of inactivation for alkaline phosphatase than that obtained in the present study for lipase under similar electrical conditions.

The level of lipase inactivation reached when processing the samples in continuous mode (13%) was widely overtaken when plasmin added to SMUF or a protease from *P. fluorescens* added to skim milk, were treated using the continuous-mode HIPEF equipment (Vega-Mercado et al., 1995a,b). Plasmin was reduced 90% after 50 pulses at both 30 and 45 kV/cm, and a 60% reduction was attained for protease when 14 or 15 kV/cm and 98 pulses were applied at pulsing rates of 1 and 2 Hz (Vega-Mercado et al., 1995a,b).

The depletion of lipase could be adjusted to the applied number of pulses for all electric field intensities studied, in both batch and continuous treatments. The higher the number of pulses the higher the inactivation level achieved. The results could be adjusted to a first-order kinetic model. Moreover, the batch-mode treatment kₑ-values increased exponentially with the processing field strength. No references have been found concerning the inhibition kinetic constants of any lipase treated with HIPEF in milk or similar medium. But the kinetic behavior of the considered lipase when submitted to batch-mode HIPEF treatments agrees with that reported by Giner et al. (2000, 2001) for vegetable or fruit enzymes. The activity of tomato pectin methyl esterase and apple and pear polyphenoloxidase, processed with batch-mode HIPEF equipment, also decreased exponentially with the number of pulses (Giner et al., 2000, 2001). These authors also adjusted the kₑ to the electric field intensity in a first-order kinetic model.

The effectiveness of both HIPEF treatments can be compared by means of the energy required for each HIPEF treatment. Using the HIPEF batch-mode system, the maximum energy applied was 504.97 kJ/L, which led to a maximum lipase inhibition of up to 62.1%. However, with a similar value of energy (424.36 kJ/L) in the continuous-mode equipment, released when an 80-pulse at 37.3 kV/cm process was applied, only a 13% decrease in activity was observed. In both cases, enzyme depletion increased exponentially with the supplied energy. Giner et al. (2000) also proposed exponential adjustments to model enzyme inhibition by the energy applied, but reported that much higher values of energy (about 35,000 kJ/L) were needed to achieve near-
complete inactivation of tomato pectin methylesterase or apple and pear polyphenoloxidases.

The batch-mode treatment was much more effective than the continuous one. This was unexpected because with the batch HIPEF system, the field strengths applied were lower. So, at the evaluated conditions, we observed that the response of enzymes to HIPEF did not depend as much on the strength of treatment, which is opposite of that reported for microorganisms. Martín et al. (1997) found that continuous-mode treatment is more effective in the inactivation of E. coli than is the batch-mode. These results are consistent with those reported by other authors, who also found that enzymes presented different behavior patterns than microorganisms. Depending on the enzyme itself and on the treatment conditions, the effect of HIPEF can be very different. Ho et al. (1997) observed that depending on the kind of enzyme, results could vary from high depletion to an increase in initial activity. They obtained up to an 85% decrease in peroxidase, whereas pepsin activity showed a raise of up to 250%. Concerning the treatment conditions, Vega-Mercado et al. (1995a) obtained very different results as to the effect of HIPEF on a protease from P. fluorescens in milk. The effects of HIPEF on proteolytic activity varied from a 60% inactivation when processing the samples at low intensity electric fields (14 and 15 kV/cm) and high pulse rates (1 and 2 Hz) to an increase in initial proteolytic activity when exposure to HIPEF was performed at low pulse rates (0.6 Hz) and 25 kV/cm. As for lipase, the inactivation patterns depended on the electrical conditions applied.

Application of HIPEF might have affected the forces involved in maintaining the three-dimensional structure, because denaturation of the enzyme was observed. However, the conformational changes in the molecule that take to an inhibition of the activity better occurred with the batch HIPEF system, possibly due to the superiority of the voltage applied, the slowness of the treatment, or the length of the pulse delay.

CONCLUSIONS

It has been proved that this lipase from P. fluorescens is quite resistant to temperature and also to HIPEF. The effectiveness of HIPEF treatments depends on the electrical conditions and on the treatment mode. The batch-mode HIPEF process inhibits the lipase activity more significantly than the continuous mode. A first-order kinetics properly matches lipase depletion with the number of pulses applied. Moreover, the batch-mode rate constants increase exponentially when the applied electric field intensity goes up.

Decrease of lipase activity with input electric energy density can be adequately modeled by a first-order kinetic model in both treatment modes, although the k-value obtained was much higher for the batch-mode process.

In this work, it has been proved that the activity of a bacterial thermoresistant enzyme can be reduced by HIPEF. However, further research is needed to establish treatments that are more effective, to find the inactivation mechanisms, and to generalize the effect of HIPEF on native enzymes in milk.

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