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ScienceDirect
Food Chemistry 109 (2008) 408–414
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

# Inactivation of soybean lipoxygenase in soymilk by pulsed electric fields

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Received 29 October 2006; received in revised form 4 December 2007; accepted 3 January 2008

**Abstract**

The inactivation of soybean lipoxygenase by pulsed electric fields (PEF) was studied. Effects of PEF parameters (treatment time, pulse strength, pulse frequency and pulse width) were evaluated. Soymilk was exposed to pulsed strengths from 20 to 42 kV/cm for up to 1036 μs treatment time in square wave pulse of bipolar mode. Moreover, pulse frequency (100–600 Hz) and pulse width (1–5 μs) was also tested at constant pulsed treatment time of 345 μs and strength of 30 kV/cm. Residual activity of soybean lipoxygenase decreased with the increase of treatment time, pulse strength, pulse frequency and pulse width. The maximum inactivation of soybean lipoxygenase by PEF achieved 88% at 42 kV/cm for 1036 μs with 400 Hz of pulse frequency and 2 μs of pulse width at 25 °C. Inactivation of soybean lipoxygenase by pulsed electric fields was modeled using several kinetic models. Weibull distribution function was most suitable model describing the inactivation of soybean LOX as a function of pulsed electric fields process parameters. Moreover, reduction of soybean LOX activity related to the electric field strength could be well described by the Fermi model.


*Keywords:* Inactivation; Pulsed electric fields; Soybean lipoxygenase; Kinetics model

## 1. Introduction

In soymilk processing, elimination of enzymic off-flavour is important (Kwok, Qin, & Bang, 1993). The off-flavours of soymilk have been attributed to soybean lipoxygenases. Lipoxygenases (LOX, EC 1.13.11.12, linoleate: oxygen 13 oxidoreductase) are non-heme and nonsulfur iron containing enzymes that catalyze the dioxygenation of polyunsaturated fatty acids with the *cis*, *cis*-1,4-pentadiene system. These enzymes are responsible for the production of rancid off-flavour in many vegetables, particularly in soybean (Eskin, Grossman, & Pinsky, 1977). Soybean LOX activity also relates to the formation of bitter taste (Baur, Groch, Wieser, & Jugel, 1977). Although thermal treatment inactivates effectively soybean LOX, it denatures soybean proteins, results in amino acid degradation and other deteriorative reactions. Moreover, certain flavours, colours, vitamins and nutrients can also be affected by heat treatment (Adams, 1991; Borhan & Snyder, 1979). Therefore, inactivation of soybean LOX by non-thermal treatments becomes interesting to avoid quality loss of soymilk by thermal processing.

Pulsed electric fields (PEF) a non-thermal food preservation method and become increasingly a promising alternative to thermal pasteurization. PEF has been studied for 30 years (Vega-Mercado et al., 1997; Wouters, Alvarez, & Raso, 2001), and developed almost to commercial application (Min, Jin, Min, Yeom, & Zhang, 2003; Min, Jin, & Zhang, 2003). In comparison with traditional thermal pasteurization, PEF not only can kill microorganisms and inactivate enzymes, but also maintain maximally taste, colour, texture, vitamins, nutrients, and heat-labile functional components of foods (Barsotti, Dumay, Mu, Diaz, & Cheftel, 2002; Cortés, Esteve, Frígola, & Torregrosa, 2005; Elez-Martínez, Martín-Belloso, 2007; Jia, Zhang, & Min, 1999; Li, Chen, & Mo, 2007). PEF has been successfully

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doi:10.1016/j.foodchem.2008.01.001

applied to a variety of liquid foods with low viscosity and low electrical conductivity (Qin, Zhang, Barbosa-Cánovas, Swanson, & Pedrow, 1995).

Many studies focused on PEF inactivation of microorganisms and results show that PEF has a significant effect on microorganisms. Some other research groups investigated a wide range of enzyme inactivation by PEF (Martín, Bendicho, Elez-Martínez, & Barbosa-Cánovas, 2004). PEF treatment time, electric field strength, pulse width, pulse frequency, and pulse number are important processing parameters in the inactivation of enzymes (Bendicho, Barbosa-Cánovas, & Martín, 2003a; Bendicho, Barbosa-Cánovas, & Martín, 2003b; Espachs-Barroso, Elez-Martínez, Barbosa-Cánovas, & Martín-Belloso, 2003; Giner, Grouberman, Gimeno, & Martín, 2005; Min, Min, & Zhang, 2003). Moreover, some research groups applied some kinetic models to explain successfully the relation between inactivation of enzymes and PEF processing parameters (Bendicho et al., 2003a; Bendicho et al., 2003b; Espachs-Barroso, Van Loey, Hendrickx, & Martín-Belloso, 2006; Giner et al., 2000; Giner et al., 2005; Min et al., 2003).

For inactivation of LOX by PEF, there is little information available. Commercial scale PEF processing (40 kV/cm strength, 57 μs treatment time, 2 μs pulse width) inactivated 54% of the LOX in cold break tomato juice. Moreover, LOX in tomato juice is irreversibly inactivated by PEF processing (Min et al., 2003). Min et al. (2003) reported that PEF strength, PEF treatment time, and PEF treatment temperature affect significantly the efficiency of PEF in the inactivation of tomato juice LOX. Moreover, they applied the first-order inactivation models, the Hulsheger's kinetic model, and the Fermi's kinetic model to describe adequately the inactivation of tomato juice LOX by PEF. However, Van Loey, Verachtert, and Hendrickx (2002) found LOX in pea juice was not inactivated after PEF treatment at field strengths from 2.5 to 20 kV/cm, a pulse width of 1 μs, a pulse frequency of 1 Hz and number of pulses from 100 to 400.

Little is known about the effects of pulsed electric fields on soybean LOX. Therefore, the objectives of this work were to study the effects of PEF treatment parameters on the inactivation of soybean LOX and apply several kinetics models to evaluate the feasibility of soybean LOX inactivation by PEF.

## 2. Materials and methods

### 2.1. Preparation of soymilk

Soybeans (provided by Shandong Academy of Agricultural Science) were washed and soaked in distilled water (bean:water 1:10) for 24 h at room temperature (25 °C) to reach complete hydration. Soaked beans, along with the soaking water were wet ground at room temperature (25 °C) for 5 min in a stainless steel blender (MSE ATOMIX, Crawley, England). The slurry was then filtered through 200 mu nylon cloth sieve. The filtrate was designated as soymilk. The prepared soymilk had a total solids content of 5.7%. Soymilk was treated by PEF under different PEF parameters.

### 2.2. PEF treatment system

The bench scale continuous OSU-4L PEF unit (The Ohio State University, Columbus, OH) was used for PEF treatment of soymilk. A high voltage generator was used to generate high voltage from 0 to 15 kV and provide a bipolar or a unipolar pulse. A trigger generator was used to control pulse duration (pulse width) from 0 to 8 μs, pulse delay time (time between the positive and negative pulses) and pulse frequency from 0 to 1500 pulses per second (Hz). The automatic digital gear pump fluid handling system (Cole Parmer Instrument Company) was used to pump fluid food for controlling sample flow rate. A two-channel digital storage oscilloscope (TDS 1012, Tekeroix, Inc., Beaverton, OR, USA) monitored the voltage and the current of PEF treatment. There were six co-field flow tubular chambers used in this study with a gap distance of 0.292 cm and an inner diameter of 0.23 cm. Each PEF treatment chamber consisted of an insulator and two stainless tubular electrodes. Two chambers were connected with two stainless coils, those were submerged into a water bath (Fisher Scientific, Inc. USA) for controlling the temperature. The type K thermocouples at the inlet and outlet of each pair of the chambers measured the sample temperature. Bipolar square pulses were applied.

### 2.3. PEF treatment conditions

In experimental design, when considering effects of PEF treatment time and strength on soybean LOX activity, PEF pulse frequency and pulse width were kept constant at 400 Hz and 2 μs. The maximum of pulse frequency and pulse width were limited at 600 Hz and 5 μs during PEF treatment, because PEF equipment produced undesirable sparking phenomenon when higher pulse frequency (>600 Hz) and pulse width (>5 μs) were tried. During PEF treatment, temperature was controlled at about 25 °C by water bath.

### 2.4. Measurement of lipoxygenase activity

LOX activity was measured using a continuous spectrophotometric rate method based on the enzymatic oxidation of linoleic acid to the hydroperoxide of linoleic acid, following a modified procedure described by Indrawati, Van Loey, Ludikhuyze, and Hendrickx (1999). Non-treated and PEF-treated soymilks were centrifuged at 10,000*g* and 4 °C for 15 min. The filtered supernatant was designated as the enzyme extract and used for measurement of LOX activity. One millilitre supernatant was diluted with 19 ml distilled water before use. Substrate linoleic acid solution (0.017%) was prepared by combining 0.05 ml 95% (v/v) ethyl alcohol (Quantum Chemical Company)

with 0.05 ml linoleic acid (No. L-1376, Sigma) into a suitable vortex to dissolve completely; slowly adding 50 ml 0.2 M borate buffer (prepared by using boric acid, (No. B-0252, Sigma) and adjusting to pH 9.0 at 25 °C with 1 M NaOH; followed by stirring until the solution is homogenous. Then 5 ml linoleic acid/ethyl alcohol/borate buffer solution was mixed with 20 ml 0.2 M borate buffer and 5 ml distilled water completely by stirring. Substrate linoleic acid solution (2 ml 0.017%) and 0.95 ml 0.2 M borate buffer were transferred to quartz cuvettes (Unic Instruments, Inc. Shanghai, China) and mixed by inversion and equilibrated at 25 °C. Then 0.05 ml diluted enzyme extract was added into the quartz cuvettes and immediately mixed by inversion. The absorbance was measured immediately at 234 nm for 3 min at 25 °C by a spectrophotometer (UV-2800, Unic Instruments, Inc. Shanghai, China) after mixing. A blank was prepared with 1 ml borate buffer and 2 ml substrate solution. The spectrophotometer reading was recalibrated with a fresh substrate solution after each time the substrate solution was prepared. The rate of the reaction was automatically computed from the maximum linear portion of the absorbance curve. One unit of LOX activity was defined as a change of 0.001 units of absorbance per minute per millilitre of enzyme extract. The percentage of residual activity RA(%) of soybean LOX was defined as

$$\mathrm{RA}(\%) = 100 \times \frac{A}{A_0} \tag{1}$$

where $A$ is the soybean LOX activity of PEF-treated and $A_0$ is the initial soybean LOX activity of un-treated soymilk.

### 2.5. kinetic inactivation models by PEF

Experimental data were adjusted to the inactivation models in Eqs.(2)–(5). Eq. (2) represents the classical first-order kinetic models. The first-order fractional conversion model of Eq. (3) (Espachs-Barroso, Bendicho, Barbosa-Canovas, & Martın-Belloso, 2002) and the Weibull distribution function of Eq. (4) relate to the PEF parameters such as treatment time, strength, pulse frequency and width. Fermi's model of Eq. (5) has been tested to describe the relation between the relative activity of enzyme and pulse strength

$$\mathrm{RA} = \mathrm{RA}_0 \times \mathrm{e}^{-k_t \times E} \tag{2}$$

RA is the residual activity (%) of soybean LOX PEF-treated, $\mathrm{RA}_0$ the initial enzyme activity of un-treated soymilk (100%), $t$ PEF treatment time (μs), $E$ PEF strength (kV/cm), and $k_t$ (cm/kV) the first-order kinetic rate constant.

$$\frac{\mathrm{RA} - \mathrm{RA}_\infty}{\mathrm{RA}_0 - \mathrm{RA}_\infty} = \mathrm{e}^{-k \times p} \tag{3}$$

$\mathrm{RA}_\infty$ is the residual enzyme activity stabilization value, $k$ the first-order rate constant, $p$ the PEF parameters (time, strength, pulse frequency and width).

$$\mathrm{RA} = \mathrm{RA}_0 \times \mathrm{e}^{-(p/\alpha)^\gamma} \tag{4}$$

$\alpha$ is the scale factor and $\gamma$ the shape parameter.

$$\mathrm{RA} = \frac{\mathrm{RA}_0}{(1 + \mathrm{e}^{(E - E_h)/k_F})} \tag{5}$$

$E_h$ is the critical level of $E$ where RA is 50% and $k_\mathrm{F}$ is the steepness parameter of the curve around $E_h$.

### 2.6. Statistical analysis

All statistical analysis and graphs were performed with OriginPro 7.0. Analysis of variance (ANOVA) and regression analysis were used to determine the significant difference at 5% confidence intervals ($p$). Experimental data were fitted to model by CurveExpert1.3 procedures using the Mathematica 4.0. Fitting accuracy of the models was evaluated through the analysis of $R^2$ (regression parameters) and $S$ (standard error). The higher the $R^2$ value and the lower the $S$ value, the better the adequacy of the model is to describe the data. All experiments were done in triplicate. The mean values of triplicate experiments were used.

## 3. Results and discussion

### 3.1. Effects of PEF treatment time and strength on soybean LOX activity

Fig. 1 shows effects of PEF time and strength on soybean LOX inactivation. The RA of soybean LOX decreased as PEF time and strength increased at constant pulse frequency 400 Hz and pulse width 2 μs at 25 °C. The inactivation of soybean LOX was achieved solely by PEF treatment because no thermal inactivation could happen at 25 °C. About 88% of the soybean LOX activity was inactivated when soybean LOX was exposed up to 42 kV/cm for 1036 μs at 25 °C, achieving the maximum

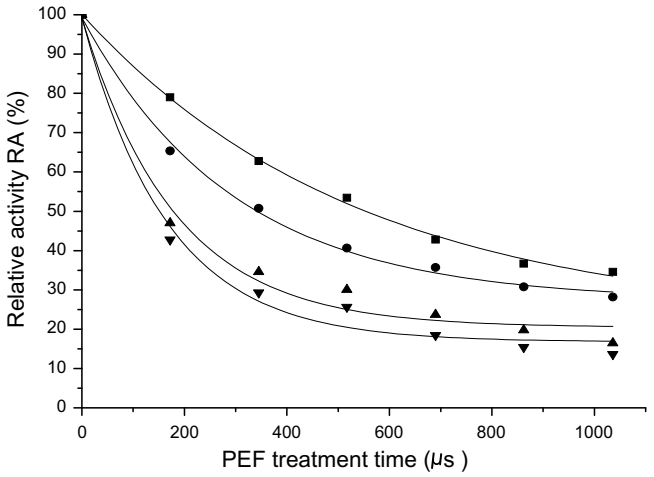


Fig. 1. The residual activity (RA) of soybean LOX as a function of the treatment time $t$ (mean ± SD, $n = 3$) after several PEF strength treatments. PEF strength (kV/cm): (■) 20, (●) 30, (▲) 35, (▼) 40.

inactivation of soybean LOX. ANOVA results indicated that PEF time and strength had significant effects on the residual activity of soybean LOX ($p < 0.05$).

This result agreed with that of inactivating tomato LOX by PEF reported by Min et al. (2003). PEF time and strength affect significantly the efficiency of inactivation of tomato juice LOX (Min et al., 2003). Commercial scale PEF processing (40 kV/cm pulse strength, 57 μs treatment time, 2 μs pulse width) inactivates 54% of the LOX in cold break tomato juice. Moreover, LOX in tomato juice is irreversibly inactivated by PEF treatment (Min et al., 2003). The inactivation extent of soybean LOX by PEF was within the range of previous studies (Giner et al., 2000; Giner et al., 2005). There are 97.9% and 93.8% inhibitions of commercial pectinesterase (8 ms, 40 μs pulse width and 24 kV/cm) and tomato pectin methylesterase (400 pulses, 20 μs pulse width and 24 kV/cm), respectively (Giner et al., 2000; Giner et al., 2005). The trend of greater soybean LOX inactivation due to longer treatment time and higher pulse strength was also found for peroxidase and polyphenol oxidase as well as horseradish peroxidase (Zhong, Hu, Zhao, Chen, & Liao, 2005; Zhong et al. 2007). Whereas Van Loey et al. (2002) reported that high pulsed electric fields has little or no effect on the activity of soybean LOX in distilled water or pea juice within the range of experimental conditions. These different results are probably due to the differences of PEF equipment system, experiment conditions, medium and enzyme kinds.

### 3.2. Kinetic inactivation model – PEF treatment time and strength

The experimental RA values of soybean LOX as a function of treatment time and strength were adjusted to the classical first-order kinetic models of Eq. (2), the first-order fractional conversion model of Eq. (3), Weibull distribution function of Eq. (4), and Fermi's model of Eq. (5), respectively. The first-order kinetic model of Eq. (2) appeared to be insufficient to model the inactivation of soybean LOX by PEF as a function of the treatment time. The calculated inactivation constants, the regression parameters $R^2$ and standard error $S$ are listed in Tables 1–3.

#### 3.2.1. First-order fractional conversion model

The first-order fractional conversion model of Eq. (3) describes successfully the inactivation of soybean LOX in the evaluated range of treatment times with higher regression parameters ($R^2 = 0.999$–$0.993$) and lower standard error ($S = 1.083$–$3.749$) (Table 1). The value of residual enzyme activity stabilization $RA_\infty$ ranged from 16.844% to 27.458% and PEF strength $E$ did not affect significantly the $RA_\infty$ values ($p < 0.05$). The $k$ values increased from $1.85 \times 10^{-3}$ to $6.08 \times 10^{-3}$ μs$^{-1}$ with $E$ from 20 to 40 kV/cm. A plot of $k$ as a function of $E$ showed a positive linear relationship in Eq. (6) ($R^2 = 0.949$). This indicates $E$ played an important role on the mechanism of soybean LOX inactivation

$$k = (0.000157 \pm 0.00004)E + (-0.00098 \pm 0.00001) \quad (6)$$

Espachs-Barroso et al. (2002) reported the inactivation of a commercial pectic enzyme by the first-order fractional conversion model, which $k$ (from 2.04 to 5.26 ms$^{-1}$) were higher than that of soybean LOX (from $1.85 \times 10^{-3}$ to $6.08 \times 10^{-3}$ μs$^{-1}$). This indicates soybean LOX was more resistant to PEF than a commercial pectic enzyme. The first-order fractional conversion model could predict well the inactivation of orange juice pectin methyl esterase by PEF with high $R^2$ (0.955–0.996). Kinetic rates $k$ values were no statistically influenced by $E$ (the mean value of $k$ was $2.3 \pm 1.3 \times 10^{-3}$ μs$^{-1}$) ($p < 0.05$). The $RA_\infty$ values decreased from 89% to 23% with $E$ (Elez-Martínez, Suárez-Recio, & Martín-Belloso, 2007). A plot of $RA_\infty$ as a function of $E$ showed a negative linear relationship ($R^2 = 0.948$). This indicated the inactivation mechanism of orange juice pectin methyl esterase was different from that of soybean LOX because there were different conformations of different enzymes.

Table 1
Kinetic constants of first-order fractional conversion model for inactivation of soybean LOX activity in soymilk by PEF

| $E$ (kV/cm) | First-order fractional conversion model | | | |
|---|---|---|---|---|
| | $RA_\infty$ | $k_t \times 10^3$ (μs$^{-1}$) | $R^2$ | $S$ |
| 20 | 21.993 ± 1.2 | 1.85 ± 0.23 | 0.999 | 1.083 |
| 30 | 27.458 ± 3.3 | 3.43 ± 0.56 | 0.999 | 1.516 |
| 35 | 16.844 ± 2.9 | 5.57 ± 0.17 | 0.995 | 3.402 |
| 40 | 20.533 ± 0.9 | 6.08 ± 0.89 | 0.993 | 3.749 |

Table 2
Kinetic constants of Weibull distribution function for inactivation of soybean LOX activity in soymilk by PEF

| $E$ (kV/cm) | Weibull distribution function | | | |
|---|---|---|---|---|
| | $a$ (μs) | $\gamma$ | $R^2$ | $S$ |
| 20 | 904.94 ± 5.6 | 0.794 ± 0.69 | 0.996 | 0.0383 |
| 30 | 664.27 ± 4.3 | 0.583 ± 0.78 | 0.999 | 0.0259 |
| 35 | 240.67 ± 7.0 | 0.478 ± 0.22 | 0.998 | 0.0456 |
| 40 | 322.16 ± 1.8 | 0.492 ± 0.54 | 0.999 | 0.0338 |

Table 3
Parameters of the Fermi model relating to the electric field strength, for the inactivation of soybean LOX activity in soymilk by PEF

| $t$ (μs) | Fermi's models | | | |
|---|---|---|---|---|
| | $E_h$ (kV/cm) | $k_F$ (kV/cm) | $R^2$ | $S$ |
| 172 | 35.83 ± 4.7 | 12.58 ± 0.98 | 0.982 | 0.146 |
| 345 | 27.93 ± 2.1 | 13.32 ± 1.32 | 0.987 | 0.116 |
| 517 | 23.25 ± 4.2 | 14.73 ± 2.00 | 0.991 | 0.090 |
| 690 | 17.67 ± 3.6 | 15.49 ± 3.12 | 0.977 | 0.134 |
| 1036 | 12.09 ± 2.1 | 15.19 ± 0.98 | 0.973 | 0.150 |

#### 3.2.2. Weibull distribution function

The inactivation of soybean LOX as a function of PEF time could also be described by the Weibull distribution

function of Eq. (4) with higher regression parameters ($R^2 = 0.996$–$0.999$) and lower standard error ($S = 0.0259$–$0.0383$). The scale factor $\alpha$ and the shape parameter $\gamma$ decreased from 904.94 to 322.16 μs and from 0.794 to 0.492 with the increase of $E$ (Table 2). Moreover $\alpha$ and $\gamma$ were affected significantly by $E$. A plot of $\alpha$ as a function of $E$ showed a negative linear relationship ($R^2 = 0.974$) in Eq. (7)

$$\alpha = (-35.186 \pm 5.789)E + (1632.57 \pm 185.87) \quad (7)$$

A plot of $\gamma$ as a function of $E$ and showed a negative and exponential relationship ($R^2 = 0.988$) in Eq. (8):

$$\gamma = (1.374 \pm 0.125)e^{[(-0.0279 \pm 0.00313)E]} \quad (8)$$

Rodrigo, Barbosa-Canovas, Martınez, and Rodrigo (2003) applied Weibull distribution function to describe the inactivation of pectin methyl esterase in orange-carrot juice by PEF and found scale factor $\alpha$ (120.57–288.48 μs) were lower than those of soybean LOX (322.16–904.94 μs), which indicated soybean LOX was more resistant to PEF than the pectin methyl esterase. The shape parameter $\gamma$ of the pectin methyl esterase were higher than 1, which implies a convex inactivation curve (Rodrigo et al., 2003). But in our study, the shape parameter $\gamma$ values were lower than 1, which implies a concave inactivation curve. This indicated the shape curve of enzyme inactivation relates to the enzyme source. Elez-Martínez, Aguiló-Aguayo, and Martín-Belloso (2006) reported that the Weibull distribution function could also predict well the inactivation of orange juice peroxidase by PEF with high $R^2$ values ($R^2 > 0.866$). The shape parameter $\gamma$ ranged from 0.22 to 0.70, indicating concave inactivation curve, and were affected significantly by $E$ ($p < 0.05$). The scale factor $\alpha$ decreased from 20,000 to 18 μs with the increase of $E$. This indicated further the inactivation of enzyme by PEF follows a different pattern depending on the enzyme source.

### 3.2.3. Fermi's model

The parameters of the Fermi's model of Eq. (5) describing the inactivation of soybean LOX by PEF at different treatment times are shown in Table 3. Fermi's model could explains well the experimental data with good agreement ($R^2 > 0.973$) and predicted the inactivation of soybean LOX with enough low standard error ($S < 0.150$). The steepness parameters of the curve ($k_F$) were 12.58–15.16 kV/cm (the mean value 14.62 kV/cm) with the increase of treatment time $t$ from 172 to 1036 μs, indicating treatment time $t$ did not affected significantly the $k_F$ values ($p < 0.05$). However, the critical level of pulse strength $E_h$ decreased from 35.83 to 12.09 kV/cm with the increase of $t$. A plot of $E_h$ as a function of $t$ showed a negative and exponential relationship ($R^2 = 0.997$), as described in Eq. (9):

$$E_h = (44.391 \pm 0.912)e^{(-0.00129 \pm 0.00005)t} \quad (9)$$

Giner et al. (2000), Giner et al. (2005) applied Fermi's model to fit the inactivation of tomato pectin methylesterase and commercial pectinesterase by PEF and found a plot of $E_h$ as a function of pulse number $N$ showed a negative and exponential relationship. This model was also applied to the inactivation of orange juice pectin methyl esterase by PEF and concluded the exponential decay relationship between $E_h$ and $t$. Moreover, the steepness parameters of the curve ($k_F$) were significantly ($p < 0.05$) non-depending on treatment time $t$ (Elez-Martínez et al., 2007). Min et al. (2003) reported the $E_h$ values of tomato juice lipoxygenase decreased with the $t$. Results of our study were consistent with their data.

### 3.2.4. Kinetic inactivation model comparison

The capability of these models to predict the inactivation of soybean LOX by PEF was evaluated through the analysis of $R^2$ (regression parameters) and $S$ (standard error) (Tables 1–3).

There were very high regression parameters ($R^2$) of Weibull distribution function, the first-order fractional conversion model and Fermi's model. But the standard errors (S) of Weibull distribution function were the lowest, and those of the first-order fractional conversion model and Fermi's model were second. So Weibull distribution function was most suitable model describing the inactivation of soybean LOX as a function of the treatment time and strength, and was reflected by a higher shape parameter $\gamma$ if the curves of inactivation of enzyme exhibited some lag time at low PEF strength. The first-order fractional conversion model provided the residual enzyme activity stabilization value after a prolonged treatment time. Fermi's model provided the critical level of pulse strength where RA is 50%. The first-order exponential decay model was the simplification of them. The data provided the important reference for the application of PEF in soymilk and soybean products.

## 3.3. Effects of pulse frequency on soybean LOX activity

To evaluate the influence of pulse frequency on inactivation of soybean LOX, different pulse frequencies $f$ were tested at constant $t$ 345 μs, $E$ 30 kV/cm. Fig. 2 shows that

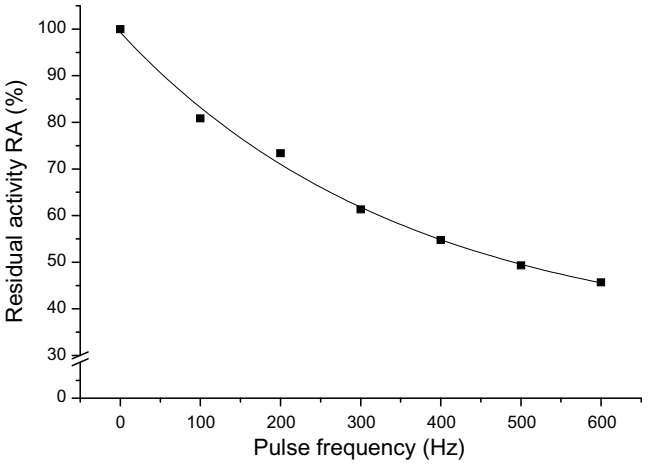


Fig. 2. Effect of pulse frequency $f$ on the residual activity (RA) of soybean LOX by PEF (mean ± SD, $n = 3$).

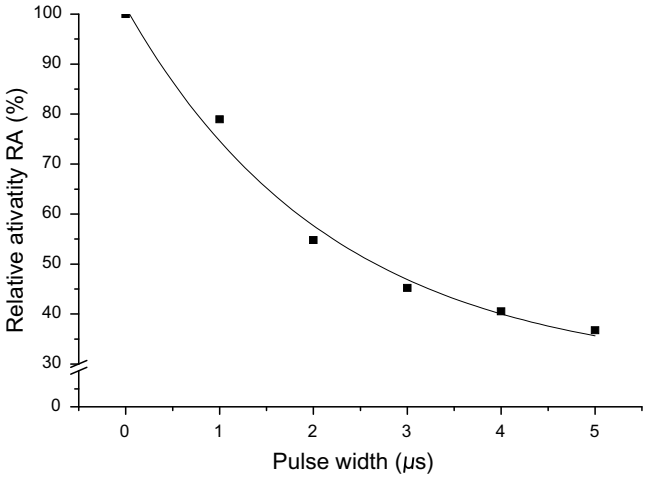


Fig. 3. Effect of pulse width $w$ on the residual activity (RA) of soybean LOX by PEF (mean ± SD, $n = 3$).

the higher the pulse frequency was, the greater the enzyme inactivation was achieved ($p < 0.05$). The RA of soybean LOX was reduced to 45.67% when pulse frequency reached the maximum of 600 Hz. A plot of RA as a function of pulse frequency $f$ followed Weibull distribution function ($R^2 = 0.991$, $S = 2.901$), as described in Eq. (10):

$$\mathrm{RA} = 100\mathrm{e}^{-(f/802.82)^{0.764}} \tag{10}$$

Bendicho et al. (2003a), Bendicho et al. (2003b) found more inactivation of a protease from *Bacillus subtilis* with higher pulse frequency. Elez-Martínez et al. (2006), Elez-Martínez et al. (2007) also found the same phenomenon when they studied the inactivation of orange juice peroxidase and pectin methyl esterase.

### 3.4. Effects of pulse width on soybean LOX activity

Pulse width is another factor to influence inactivation of soybean LOX in PEF processing. Maintaining constant $t$ 345 μs, $E$ 30 kV/cm and $f$ 400 Hz, the RA of soybean LOX was tested at different pulse widths $w$. Fig. 3 shows the RA of soybean LOX decreased with the increase of $w$, reaching the minimum 36.73% when $w$ was 5 μs. This result indicated that $w$ significantly influenced inactivation of soybean LOX ($p < 0.05$). A wider pulse width $w$ had not been applied due to sparking problem during PEF treatment. A plot of RA as a function $w$ followed Weibull distribution function ($R^2 = 0.994$, $S = 3.945$), as described in Eq. (11):

$$\mathrm{RA} = 100\mathrm{e}^{-(w/4.67)^{0.711}} \tag{11}$$

Giner et al. (2000) and Ho, Mittal, and Cross (1997) pointed out that pulse width has an important role in the activity reduction of tomato pectin methyl esterase and alkaline phosphatase. More inactivation due to wider pulse width was found for tomato pectin methyl esterase (Giner et al., 2000) and alkaline phosphatase (Ho et al., 1997) subjected to exponential decay pulse. Giner, Gimeno, Palomes, Barbosa-Cánovas, and Martín (2003) found 72% RA of polygalacturonase was achieved after 200 pulses of 40 ms width at 5.18 kV/cm electric field intensity, whereas 160 ms width reached 41% RA at the same other PEF parameters. Bendicho et al. (2003a), Bendicho et al. (2003b) observed that pulse width has significant effect on changes in protease inactivation if total treatment time was considered ($p < 0.05$). But the 4 μs pulse width process requires a higher number of pulses than the 7 μs pulse width process to achieve similar treatment times. So, the latter might be considered more effective to achieve similar inactivation with a lower number of pulses.

## 4. Conclusion

The present results indicate that PEF parameters (treatment time, strength, pulse frequency and width) affected significant activity of soybean LOX in soymilk. A stronger parameters caused a higher degree inactivation of soybean LOX. About 88% of maximum inactivation was achieved when soybean LOX was exposed to PEF with 42 kV/cm for 1036 μs using 400 Hz pulse frequency and 2 μs pulse width in square wave pulse of bipolar mode. In comparison with different kinetic inactivation models, Weibull distribution function was most suitable model describing the inactivation of soybean LOX as a function of PEF process parameters. PEF may cause denaturation of enzymes by changing their conformations. We shall investigate further to determine how PEF affects internal conformations and find the mechanism of inactivation enzymes by PEF.

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