Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 949–955
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Inactivation of tomato juice peroxidase by high-intensity pulsed electric fields as affected by process conditions

Ingrid Aguiló-Aguayo, Isabel Odriozola-Serrano, Luciano José Quintão-Teixeira, Olga Martín-Belloso *

Department of Food Technology UTPV-CeRTA, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain

Received 19 April 2007; received in revised form 20 August 2007; accepted 22 August 2007

Abstract

A response surface was used to establish the high-intensity pulsed electric fields (HIPEF) conditions in processing tomato juice to obtain the greatest peroxidase (POD) inactivation. Keeping constant the electric field strength at 35 kV/cm and the temperature below 35 °C, the treatments were set at pulse frequency from 50 to 250 Hz, pulse width from 1 to 7 μs and treatment time from 1000 to 2000 μs, using monopolar or bipolar mode. The effect of these parameters on POD inactivation was evaluated through a second order model that adequately fitted the experimental data ($p = 0.0001$), with a determination coefficient (R^2) of 0.85. HIPEF treatment resulted to be more effective in bipolar than monopolar mode to reduce POD activity and the longer the treatment time, the greater the reduction on the enzyme activity. A pulse frequency of 200 Hz was enough to reach a minimum value of residual POD activity. The significant interaction term pulse frequency and treatment time was included in the model, showing that different combinations of both variables can lead to the same level of residual POD activity. The effect of pulse width was enhanced by using a bipolar mode, being feasible to maximize POD inactivation selecting pulse width higher than 5.5 μs in bipolar mode.

Keywords: High-intensity pulsed electric field; Peroxidase; Response surface; Tomato juice

1. Introduction

The demand for fresh-like and lightly treated products has increased in the last few years, leading to a growing interest in new techniques for food processing. High-intensity pulsed electric field (HIPEF) is a non-thermal technology extensively studied as an alternative to the traditional thermal treatment (Dunn, 2001; Martín-Belloso & Elez-Martínez, 2005). High level of microbial destruction (Barsotti & Cheftel, 1999; Wouters & Smelt, 1997), and few losses of flavor, color, taste or nutrients (Yeom, Streaker, Zhang, & Min, 2000) have evinced the suitability of PEF technology to obtain high quality fresh-like foods. In comparison to the extensive research devoted to the destruction of microorganisms by HIPEF, the information available about the effect on enzymes is more limited. However, enzymes are less sensible than microorganisms to HIPEF and their inhibition depends on the enzyme itself, the media where are suspended and the processing parameter (Martín-Belloso, Bendicho, Elez-Martínez, & Barbosa-Cánovas, 2004).

Tomato derivatives such as tomato juice are highly consumed as important sources of minerals and vitamins in the diet (Hayes, Smith, & Morris, 1998; Thakur, Singh, & Nelson, 1996). In addition, a good retention of flavor and color are important attributes that influence the consumer's choice. Therefore, reducing peroxidase (POD) activity in tomato juice is an important goal to avoid color deterioration, off-flavor formation and loss of nutrients (Robinson, 1991). Nevertheless, the conventional heat treatment applied to tomato juice to inactivate the enzyme, can damaged other valuable properties. Some studies exist about the effects of HIPEF on enzymes suspended in milk,

* Corresponding author. Tel.: +34 973 702593; fax: +34 973 702596.
E-mail address: omartin@tecal.udl.es (O. Martín-Belloso).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.08.057

aqueous solutions and fruit juices (Bendicho, Barbosa-Cánovas, & Martín, 2002; Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003; Min, Evrendilek, & Zhang, 2007), but there is currently no published data on the HIPEF inactivation of tomato POD. Besides the scarce literature concerning POD inactivation by HIPEF, there are studies on the enzyme in aqueous solutions that might show controversial results about the effect of HIPEF which might be attributed to differences between the properties of the substratum to treat, the processing conditions and even the technical characteristics of the HIPEF equipment used. Elez-Martínez, Aguiló-Aguayo, and Martín Belloso (2006) completely inactivated the orange POD after processing the juice at 35 kV/cm for 1500 μs with 4 μs square pulses at 200 Hz. Grahl and Märkl (1996) achieved a 25% of POD inactivation in milk by the application of 20 pulses at 21.5 kV/cm whereas Van Loey, Verachtert, and Hendrickx (2002) reported less than 10% inactivation on horseradish POD suspended in a buffer solution after a treatment of 30 kV/cm for 40,000 μs and no inactivation of POD after processing milk at 19 kV/cm for 500 μs. The objective of this work was to study the effect of HIPEF parameters on the POD activity of tomato juice as well as to establish the treatment conditions to obtain the greatest POD inactivation in tomato juice.

2. Materials and methods

2.1. Sample preparation

Fresh ripened tomato fruits (*Lycopersicon esculentum* var. Flandia Prince) were washed and chopped. Then, they were crushed and the resulting product was filtered through a screen of 1.27 mm size to remove peel and seeds, obtaining the juice.

2.2. HIPEF equipment

Pulse treatments were carried out using a laboratory scale pulse generator (OSU-4F, The Ohio State University, Columbus) that provides square-wave pulses within eight cofield flow chambers in series whose treatment volume and gap distance are 0.012 cm^3 and 0.29 cm, respectively. The flow rate of the process was adjusted to 60 ml/min and controlled by a variable speed pump (model 75210-25, Cole Palmer, Vernon Hills, IL, USA). The treatment temperature was kept lower than 35 °C using a cooling coil connected before and after each pair of chambers and submerged in an ice-water shaking bath.

2.3. Experimental design

A response surface analysis was used to study the effect of the different HIPEF treatment variables on the inactivation of POD in tomato juice, keeping constant the electric field strength at 35 kV/cm. A central composite design with three factors and faced centered was the proposed experimental design. The independent numerical variables were pulse frequency (from 50 to 250 Hz), pulse width (from 1 to 7 μs) and treatment time (from 1000 to 2000 μs), which were coded as factor levels: −1, 0 and +1, where −1 corresponds to the lowest level of each factor, +1 the highest level, and 0 the middle level, whereas the polarity was a categorical variable in monopolar or bipolar mode. Variable levels were chosen according to previous studies. The experiment design was conducted in duplicate, resulting in two blocks of experiments. The order of assays within each block was randomized. Experimental data were fitted to a polynomial response surface. The second order response function was predicted by Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{i_i}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_{i_i} X_{j_i} \quad (1)$$

where Y is the dependent variable, β_0 is the center point of the system; β_i, β_{ii} and β_{ij} are the regression coefficients of the linear, quadratic and interactive effects of the independent variables, respectively; X_i, X_i^2 and X_iX_j represents the linear, quadratic and interactive effects of the independent variables, respectively. The nonsignificant terms ($P \geqslant 0.05$) were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to obtain the coefficients of the final equation for better accuracy. Design Expert 6.0.1 software (Stat Ease Inc., Minneapolis, Minn., USA) was used in all analyses and generated plots. A 95% confidence interval was used for all these procedures.

2.4. POD activity measurement

POD activity of tomato juice was measured using the method described by Elez-Martínez et al. (2006). All chemicals were purchased from Scharlab Chemie, SA (Barcelona, Spain). Enzyme extracts were obtained by homogenization of 10 ml tomato juice with 20 ml 0.2 mol/l sodium phosphate buffer (pH 6.5). Then, the homogenate was centrifuged (24,000*g*, 15 min) at 4 °C (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, USA) The supernatant was filtered through a Whatman No. 1 paper and the resulting liquid constituted the enzymatic extract, which was immediately used for the POD activity determination. POD activity was assayed spectrophotometrically by placing 2.7 ml 0.050 mol/l sodium phosphate buffer (pH 6.5), 0.2 ml *p*-phenylenediamine (10 g/kg) as H-donor, 0.1 ml hydrogen peroxidase (15 g/kg) as oxidant and 0.1 ml of enzymatic extract in a 1 cm path cuvette. The oxidation of *p*-phenylenediamine was measured at 509 nm and 25 °C using a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). POD activity was determined by measuring the initial rate of the reaction, which was computed from the linear portion of the plotted curve. One unit of POD activity was defined as a change in absorbance at

509 nm/min ml of enzymatic extract. Tomato juice was pumped through the HIPEF system without receiving any treatment to check that no differences in POD activity were observed before and after passing the tomato juice through the system. The percentage of residual POD activity (RA) was defined as indicated by Eq. (2):

$$RA = 100 \cdot \frac{A_t}{A_0} \quad (2)$$

where A_t and A_0 were the enzyme activities of treated and untreated samples, respectively. A_t and A_0 were determined immediately after processing to avoid the effects of storage time.

3. Results and discussion

Table 1 shows the POD-RA after applying different combinations of HIPEF variables. No POD-RA was observed at 250 Hz for 2000 μs using a 4 μs-pulse width in bipolar mode. In contrast, POD kept all its initial activity when tomato juice was treated in monopolar mode at 1 μs pulse width and 50 Hz of pulse frequency for 1000 μs treatment time.

The 2nd order model fit properly the experimental data ($p = 0.0001$) (Table 2). The determination coefficient (R^2) was 0.85 and the lack of fit was no significant, indicating that the model was adequate for predicting the response

Table 1
Experimental central composite design and POD relative residual activity in tomato juice treated by different combinations of high-intensity pulsed electric field (HIPEF) variables[a]

Assay number[b]	Coded variables[c]			Uncoded variables				Relative residual POD activity (%)	
	X_1	X_2	X_3	Pulse frequency (Hz)	Pulse width (μs)	Treatment time (μs)	Polarity	Block 1	Block 2
1	0	0	0	150	4	1500	Monopolar	24.3	25.1
2	1	−1	−1	250	1	1000	Monopolar	51.6	51.8
3	0	0	0	150	4	1500	Monopolar	32.6	33.4
4	−1	1	1	50	7	2000	Monopolar	23.5	23.2
5	−1	1	−1	50	7	1000	Monopolar	74.7	75.1
6	0	0	0	150	4	1500	Monopolar	31.7	32.0
7	−1	−1	1	50	1	2000	Monopolar	88.2	89.5
8	0	0	0	150	4	1500	Monopolar	25.7	26.1
9	0	−1	0	150	1	1500	Monopolar	95.1	96.2
10	0	1	0	150	7	1500	Monopolar	19.6	19.1
11	1	1	−1	250	7	1000	Monopolar	9.6	9.2
12	0	0	0	150	4	1500	Monopolar	55.3	57.2
13	1	−1	1	250	1	2000	Monopolar	51.6	49.7
14	−1	−1	−1	50	1	1000	Monopolar	100	100
15	0	0	−1	150	4	1000	Monopolar	75.5	75.2
16	1	0	0	250	4	1500	Monopolar	52.4	52.8
17	−1	0	0	50	4	1500	Monopolar	77.9	78.6
18	1	1	1	250	7	2000	Monopolar	1.3	1.1
19	0	0	0	150	4	1500	Monopolar	61.2	60.7
20	0	0	1	150	4	2000	Monopolar	15.7	16.5
21	−1	1	−1	50	7	1000	Bipolar	55.2	54.9
22	0	0	0	150	4	1500	Bipolar	12.9	14.5
23	1	−1	−1	250	1	1000	Bipolar	35.3	36.8
24	1	0	0	250	4	1500	Bipolar	2.7	3.1
25	−1	0	0	50	4	1500	Bipolar	45.9	46.8
26	0	0	0	150	4	1500	Bipolar	14.1	13.8
27	−1	−1	−1	50	1	1000	Bipolar	90.2	92.5
28	1	−1	1	250	1	2000	Bipolar	31.9	32.5
29	−1	−1	1	50	1	2000	Bipolar	43.2	44.8
30	0	0	1	150	4	2000	Bipolar	7.3	5.8
31	0	0	0	150	4	1500	Bipolar	18.5	19.2
32	1	1	1	250	7	2000	Bipolar	0	0
33	0	0	0	150	4	1500	Bipolar	17.2	15.2
34	0	−1	0	150	1	1500	Bipolar	35.3	36.2
35	0	0	0	150	4	1500	Bipolar	21.3	22.1
36	0	0	−1	150	4	1000	Bipolar	25.7	27.2
37	1	1	−1	250	7	1000	Bipolar	2.6	3.4
38	0	1	0	150	7	1500	Bipolar	8.4	8.0
39	−1	1	1	50	7	2000	Bipolar	52.6	57.2
40	0	0	0	150	4	1500	Bipolar	12.2	16.4

[a] HIPEF treatment was set at 35 kV/cm of electric field strength and treatment temperature kept below 35 °C.
[b] Assay number does not correspond to the order of processing.
[c] X_1, frequency; X_2 pulse width; X_3 treatment time.

Table 2
Significance of the effect of processing parameters in the residual POD activity on HIPEF-treated tomato juice by a response surface quadratic model

Source[b]	Mean square	F-value	Prob > F
Quadratic model	2033.75	11.04	<0.0001[a]
f	8483.08	46.10	<0.0001[a]
τ	7027.50	38.15	<0.0001[a]
t	2103.30	11.42	0.0023[a]
P	4730.63	25.68	<0.0001[a]
f^2	728.53	3.95	0.0456[a]
t^2	224.16	1.22	0.2832
t^2	25.80	0.14	0.7113
$f\times\tau$	106.61	0.58	0.4537
$f\times t$	603.93	3.28	0.0316[a]
$f\times P$	14.11	0.072	0.7841
$\tau\times t$	0.39	0.002	0.9636
$\tau\times P$	989.82	5.37	0.0286[a]
$t\times P$	163.02	0.88	0.3555
Lack of fit	217.66	1.66	0.2085
Standard deviation		13.57	
Mean		37.50	
R^2		0.85	
Adjusted R^2		0.77	

[a] Significant at 95% confidence interval.
[b] f = frequency; τ = pulse width; t = treatment time; P = polarity.

Table 3
Significant regression coefficients of the quadratic model for the residual POD activity in HIPEF-treated tomato juice

Factor[a]	Coefficient estimate[b]	Low	High	Standard error
Monopolar pulses				
Intercept	183.496	169.003	195.200	5.458
f	−0.693	−23.490	−11.765	2.443
τ	−12.821	−2.200	3.325	1.135
t	−0.018	−0.029	2.160	0.456
f^2	1.151×10^{-3}	−3.970	0.012	0.830
$f\times t$	1.229×10^{-4}	−4.350	0.500	1.010
Bipolar pulses				
Intercept	128.376	122.479	155.200	6.817
f	−0.709	−21.297	−6.324	3.119
τ	−8.131	−2.100	3.600	1.188
t	-6.824×10^{-3}	−18.157	1.158	4.024
f^2	1.151×10^{-3}	−3.970	0.012	0.830
$f\times t$	1.229×10^{-4}	−4.350	0.500	1.010

[a] f = frequency; τ = pulse width; t = treatment time.
[b] 95% confidence interval.

across the design space. The variables pulse frequency, pulse width and treatment time affected linearly the POD inactivation and only the quadratic term of pulse frequency was significant. There were also differences in the effect of the treatment achieved applying monopolar and bipolar pulses. The mutual influence between the effects of pulse frequency and treatment time as well as treatment time and polarity on the POD-RA were observed.

3.1. Effect of pulse polarity

Among HIPEF processing parameters, the polarity, P, in the application of pulses is one of the most important factors that influenced the POD inactivation ($p = 0.001$) (Table 2).

Owing to the polarity is a categorical variable and significant differences between mono- and bipolar pulses were observed, the POD-RA in HIPEF-treated tomato juice was modeled through two different polynomial Eqs. (3) and (4) when applying mono- or bipolar pulses, respectively.

$$Y = 183.496 - 0.693\cdot f - 12.821\cdot\tau - 0.018\cdot t + 1.151\cdot10^{-3}\cdot f^2 + 1.229\cdot10^{-4}\cdot f\cdot t \quad (3)$$

$$Y = 128.376 - 0.709\cdot f - 8.131\cdot\tau - 6.824\cdot10^{-3}\cdot t + 1.151\cdot10^{-3}\cdot f^2 + 1.229\cdot10^{-4}\cdot f\cdot t \quad (4)$$

where Y is the residual POD activity (%), f the pulse frequency (Hz), τ the pulse width (μs) and t the treatment time (μs). Coefficients of the fitted model are shown in Table 3.

Bipolar mode improved the effectiveness of the HIPEF treatments on the tomato juice POD-RA reduction as the intercept terms of Eqs. (3) and (4) show. When tomato juice was submitted to a bipolar HIPEF treatment of 35 kV/cm for 1000 μs using 250 Hz and 4 μs pulse width (Fig. 1), the POD-RA was 7.9%, whereas applying monopolar pulses was 37.1%. In spite of the limited literature about the effect of pulse polarity on POD activity, Elez-Martínez et al. (2006) reported better effect on POD inactivation in monopolar than bipolar mode, obtaining approximately 30% and 65% of residual activity, respectively, when orange juice was HIPEF-processed at 25 kV/cm for 300 μs using 4 μs-pulse width and 200 Hz of pulse frequency. However, when the electric field strength increased from 25 kV/cm to 30 or 35 kV/cm, the inactivation levels of orange POD were greater in bipolar than monopolar mode, being the POD completely inactivated.

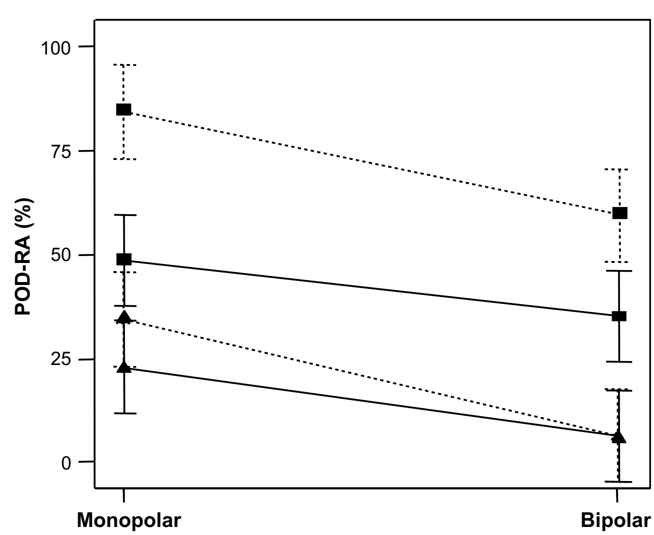

Fig. 1. Effect of the polarity on the residual POD activity when high-intensity pulsed electric field (HIPEF) treatment was set at 35 kV/cm, 4 μs of pulse width and 50 (■) or 250 (▲) Hz of pulse frequency for 1000 μs (···) or 2000 μs (—) of treatment time.

These results contrast with those observed in a HIPEF-treated cold Mediterranean vegetable soup (Aguiló-Aguayo, Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006), which has tomato as the main ingredient. In the latter product, bipolar pulses enhanced the POD reduction at 30 or 35 kV/cm for 1500 μs using 4 μs-pulse width and 200 Hz of pulse frequency. Elez-Martínez et al. (2006) suggested the existence of a critical value of electric field strength depending on the polarity of the pulses applied, when treated orange juice by HIPEF. However, this parameter did not significantly affect the PME of tomato. The differences on the reported results could be attributed to the type of enzyme and the food where it is present (Giner, Gimeno, Espachs, Elez, Barbosa-Cánovas, & Martin, 2000).

3.2. Effect of treatment time and pulse frequency

The linear coefficient of the variable treatment time, t, was negative, meaning that the higher the HIPEF treatment time, the lower the POD-RA was. When the HIPEF parameters were set at 35 kV/cm, 4 μs bipolar pulses and 150 Hz, the tomato juice POD-RA decreased from 24% to 9.2% with a rise of t from 1000 μs to 2000 μs, keeping the temperature below 35 °C. These results agreed with those reported by Zhong et al. (2007) who studied POD from horseradish in a buffer solution using HIPEF equipment with exponentially-decaying pulse-wave. They observed a gradual depletion in POD-RA between 32% and 10%, when the electric field strength was maintained at 25 kV/cm and the treatment time increased from 290 μs to 1740 μs.

Equations (3) and (4) show a higher absolute value of coefficient for monopolar than bipolar mode. As can be seen in Fig. 2a, when a HIPEF treatment at 150 Hz for 7 μs is applied in bipolar mode, it was obtained 18.8% and 3.7% of POD-RA after 1000 μs and 2000 μs, respectively. However, the POD-RA increased on monopolar mode (Fig. 2b), being 32.2% for 1000 μs and 5.7% for 2000 μs of treatment time. The difference of RA after applying different treatment times suggested that, at the same level of t, bipolar mode led to lower values of POD-RA than monopolar pulses. The same behaviour was observed by Elez-Martínez et al. (2006) in orange juice. In addition, greater enzymatic reduction levels were obtained at similar t values, using monopolar than bipolar HIPEF pulses. Orange POD was totally inactivated when orange juice was treated at 35 kV/cm for 1500 μs in bipolar mode (Elez-Martínez et al., 2006). Changes in the conformational α-helix of POD were attributed as the main reason of the loss of activity in the HIPEF-treated products, demonstrating that α-helix relative content decrease after the treatment (Zhong, Hu, Zhao, Chen, & Liao, 2005; Zhong et al., 2007).

The POD inactivation also depended on the pulse frequency, f. Therefore an increase in its value, keeping constant the rest of variables, induced a decrease in the

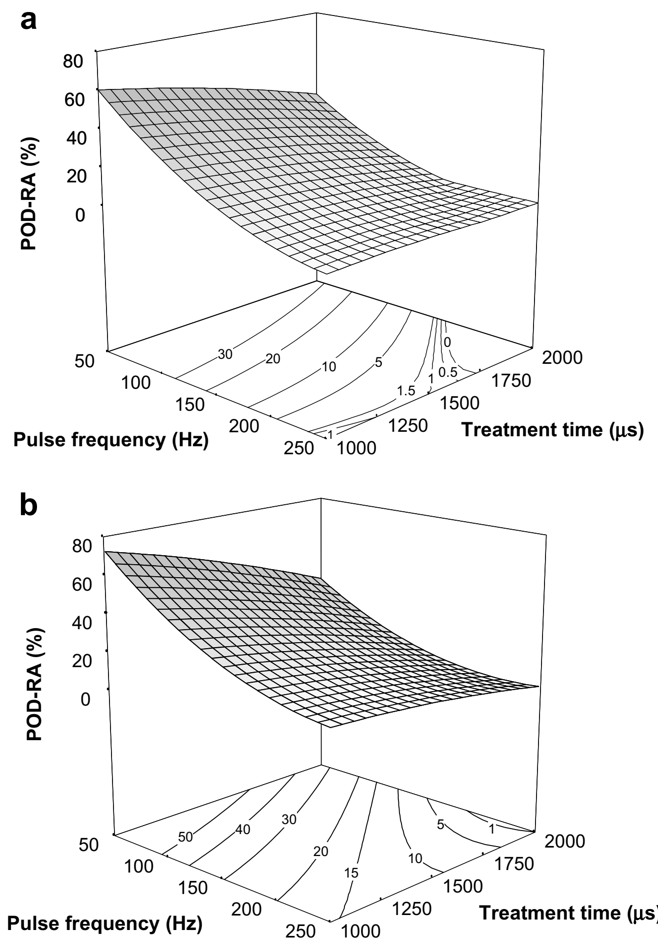

Fig. 2. Effect of the pulse frequency and total treatment time on residual POD activity when high-intensity pulsed electric field (HIPEF) treatment was set at 35 kV/cm, 7 μs of pulse width in bipolar (a) or monopolar (b) mode.

POD-RA. When f increased from 50 to 200 Hz, applying bipolar 7 μs-pulses for 2000 μs at 35 kV/cm (Fig. 2a), the POD-RA decreased from 32.1% to 0%. Some studies on orange POD and PME, described a depletion in these enzyme activities when the pulse frequency increased (Elez-Martínez et al., 2006; Elez-Martínez, Suárez-Recio, & Martín-Belloso, 2007). A lipase and a protease in simulated milk ultrafiltrate also showed the same trend when the pulse frequency raised and the remaining treatment conditions were kept constant (Bendicho, Barbosa-Cánovas, & Martín, 2003).

On the other hand, the positive value of the quadratic term f^2 ($p = 0.0456$) indicated that POD-RA of tomato juice reached a minimum within the studied range of f. Fig. 2a showed a region of pulse frequency between 50 and 250 Hz, where the tomato juice POD could be totally inactivated. These results suggested that pulse frequency of 200 Hz was enough to reach the highest POD inactivation rates.

The effect of pulse frequency, f, on the reduction of POD activity from tomato juice was affected by the treatment time, t, which was included in the response model as the interaction $f \cdot t$ (Eqs. (3) and (4)). The positive value of the interaction term suggests that greater RA reduction can be achieved by a rise in both variables. However, the effect of the mutual influence of f and t on POD-RA followed a nonlinear curve (Fig. 2). Thus, different combinations of the variables f and t may lead to the same level of RA. In this way, 8% of POD-RA was observed with either 1911 μs at 138 Hz or 1030 μs at 192 Hz, when the HIPEF treatments were carried out at 35 kV/cm using 7 μs-bipolar pulses (Fig. 2a). Bendicho, Marsellés-Fontanet, Barbosa-Cánovas, and Martín-Belloso (2005) observed that the combination of these parameters did not have a clear impact on the effectiveness of protease inactivation. Nevertheless, an interaction of these factors might lead to differences in the effect on the enzyme RA. As far as we know, little information is available about the effect of the simultaneous influence of pulse frequency and treatment time on POD of HIPEF-treated juices. In addition, the enzymatic inactivation process induced by HIPEF is more complex than the microbial reduction. The possible presence of a number of isoperoxidases that have different resistance to HIPEF could be the reason of why different percentages of enzymatic inactivation have been reported in the literature. The presence of a number of isoperoxidases with different thermostability was also attributed to the nonlinearity heat inactivation on POD orange juice (Bruemmer, Roe, & Bowen, 1976; Clemente, 1998).

3.3. Effect of pulse width

The pulse width, τ, influenced significantly ($p = 0.0001$) the POD-RA. Eqs. (3) and (4) reflected the negative coefficient of the variable pulse with, indicating a linear decrease of POD-RA with τ in both mono and bipolar mode (Fig. 3). Moreover pulse width, τ, and pulse polarity, P, show a reciprocal influence, as revealed by the significance of the interaction term $\tau \cdot P$ ($p = 0.0286$) (Table 2). Considering the application of pulses in bipolar mode, the effect of pulse width on POD-RA was enhanced irrespective of the treatment time and pulse frequency used (Fig. 3). In this way, greater POD inactivation values could be reached selecting pulse widths higher than 5.5 μs in bipolar mode. Moreover it was observed that a complete POD inactivation could be reached if the HIPEF treatment was applied at 35 kV/cm for 2000 μs and pulse frequency 200 Hz, using 5.5 μs of pulse width applying bipolar pulses. On the other hand, when monopolar mode was used, it was needed to applied higher values of pulse width than in bipolar mode, in order to be effective on the POD inactivation. Pulse width, τ, should be taken into account in order to elucidate the mechanism of enzyme inactivation by HIPEF because it is a processing factor that induces significant changes in enzyme activity during HIPEF treatments (Elez-Martínez et al., 2007). Elez-Martínez et al. (2006) and Elez-Martínez et al. (2007) reported that a decrease in POD and PME activities from orange juice were observed when the pulse width changed from 1 to 10 μs, for a constant electrical energy density input (2336 MJ/m^3). Giner, Ortega, Mesegué, Gimeno, Barbosa-Cánovas and Martin (2002) also observed the same trend in the PPO inactivation of peach.

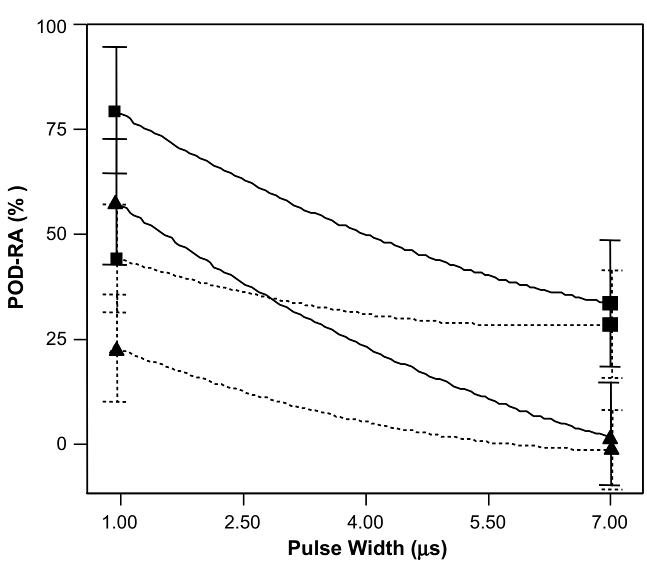

Fig. 3. Effect of the pulse width on the residual POD activity when high-intensity pulsed electric field (HIPEF) treatment was set at 35 kV/cm for 2000 μs of treatment time, at 50 (■) or 250 Hz (▲) for 2000 μs in bipolar (···) or monopolar (—) mode.

4. Conclusions

High-intensity pulsed electric fields were effective to inactivate POD in tomato juice. A complete POD inactivation was observed when HIPEF treatment was performed at 35 kV/cm for 2000 μs using 7 μs-bipolar pulses at 200 Hz. Among the studied variables; treatment time, pulse frequency, pulse width and the simultaneous influence of treatment time and pulse frequency could be modeled by a second-order equation. Treatment time, pulse frequency and pulse width affected linearly ($p < 0.05$) the POD inactivation. Only the quadratic term of pulse frequency was significant, showing at 200 Hz a minimum value in the residual POD activity. Furthermore the use of bipolar pulses raised the POD inactivation within the defined ranges of the HIPEF variables more than those applied in monopolar mode. The effect of the mutual influence between pulse frequency and treatment time showed the same level of POD-RA at different combinations of both parameters. In this way, only a 8% of residual POD activity remained with either 1911 μs at 138 Hz or 1030 μs at 192 Hz, when the HIPEF treatments were carried out at 35 kV/cm in bipolar mode and using a pulse width of 7 μs. Moreover, a reciprocal influence of pulse width and polarity was revealed, being feasible to maximize POD inactivation selecting pulse widths higher than 5.5 μs in bipolar mode.

Hence, this study demonstrated the effectiveness of HIPEF treatment on tomato juice POD inactivation and described the behaviour of its activity at different HIPEF processing conditions.

Acknowledgements

This work was supported by the Ministerio de Ciencia y Tecnología (Spain) through the Project ALI 2005-05768. This study has also been carried out with financial support from the Commission of the European Communities, Framework 6, Priority 5'Food Quality and Safety', Integrated Project NovelQ FP6-CT-2006-015710.

References

Aguiló-Aguayo, I., Elez-Martínez, P., Soliva-Fortuny, R., & Martín-Belloso, O. (2006). Effects of high-intensity pulsed electric fields on the inactivation of peroxidase in mediterranean vegetable soup. In *Workshop on applications of novel technologies in food and biotechnology* (Abstract book, pp. 90), Cork, Ireland.

Barsotti, L., & Cheftel, J. C. (1999). Food processing by pulsed electric fields II. Biological aspects. *Food Reviews International, 15*, 181–213.

Bendicho, S., Barbosa-Cánovas, G. V., & Martín, O. (2002). Milk processing by high intensity pulsed electric fields. *Trends in Food Science and Technology, 13*, 195–204.

Bendicho, S., Barbosa-Cánovas, G. V., & Martín, O. (2003). Reduction of protease activity in simulated milk ultrafiltrate by continuous flow high intensity pulsed electric field treatments. *Journal of Food Science, 68*, 952–957.

Bendicho, S., Marsellés-Fontanet, R., Barbosa-Cánovas, G. V., & Martín-Belloso, O. (2005). High intensity pulsed electric fields and heat treatments applied to a protease from *Bacillus subtilis*. A comparison study of multiple systems. *Journal of Food Engineering, 69*, 317–323.

Bruemmer, J. H., Roe, B., & Bowen, E. R. (1976). Peroxidase reactions and orange juice quality. *Journal of Food Science, 41*, 186–189.

Clemente, E. (1998). Purification and thermostability of isoperoxidase from oranges. *Phytochemistry, 49*, 26–29.

Dunn, J. E. (2001). Pulsed electric field processing: An overview. In G. V. Barbosa-Canovas & Q. H. Zhang (Eds.), *Pulsed electric fields in food processing: Fundamental aspects and applications* (pp. 1–30). England: Lancaster, PA: Technomic Publishing Company.

Elez-Martínez, P., Aguiló-Aguayo, I., & Martín Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture, 86*, 71–81.

Elez-Martínez, P., Suárez-Recio, M., & Martín-Belloso, O. (2007). Modeling the reduction of pectin methyl esterase activity in orange juice by high intensity pulsed electric fields. *Journal of Food Engineering, 78*, 184–193.

Espachs-Barroso, A., Barbosa-Cánovas, G. V., & Martín-Belloso, O. (2003). Microbial and enzymatic changes in fruit juice induced by high-intensity pulsed electric fields. *Foods Reviews International, 19*, 253–273.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G. V., & Martín, O. (2000). Inhibition of tomato (*Licopersicon esculentum* Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technologies, 1*, 57–67.

Giner, J., Ortega, M., Mesegué, M., Gimeno, V., Barbosa-Cánovas, G. V., & Martín, O. (2002). Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. *Journal of Food Science, 67*, 1467–1472.

Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology, 45*, 148–157.

Hayes, W. A., Smith, P. G., & Morris, A. E. J. (1998). The production and quality of tomato concentrates. *Critical Reviews in Food Science and Nutrition, 38*, 537–564.

Martín-Belloso, O., Bendicho, S., Elez-Martínez, P., & Barbosa-Cánovas, G. V. (2004). Do high intensity pulsed electric fields induce changes in enzymatic activity, protein conformation and vitamin and flavor stability? In G. V. Barbosa-Cánovas, M. S. Tapia, & M. P. Cano (Eds.), *Novel food processing technologies* (pp. 87–104). New York: Marcel Dekker Inc.

Martín-Belloso, O., & Elez-Martínez, P. (2005). Food Safety aspects of pulsed electric fields. In D. W. Sun (Ed.), *Emerging technologies for food processing* (pp. 183–217). London: Elsevier.

Min, S., Evrendilek, G. A., & Zhang, H. Q. (2007). Pulsed electric fields: Processing system, microbial and enzyme inhibition, and shelf life extension of foods. *IEEE Transactions on Plasma Science, 35*, 59–73.

Robinson, D. S. (1991). Peroxidases and catalases in foods. In D. S. Robinson & N. A. M. Eskin (Eds.), *Oxidative enzymes in foods* (pp. 1–47). London: Elsevier.

Thakur, B. R., Singh, R. K., & Nelson, P. E. (1996). Quality attributes of processed tomato products: A review. *Food Reviews International, 12*, 375–401.

Van Loey, A., Verachtert, B., & Hendrickx, M. (2002). Effects of high electric field pulse on enzymes. *Trends in Food Science & Technology, 12*, 94–102.

Wouters, P. C., & Smelt, J. P. P. M. (1997). Inactivation of microorganisms with pulsed electric fields: Potential for food preservation. *Food Biotechnology, 11*, 193–229.

Yeom, H. W., Streaker, C. B., Zhang, Q. H., & Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry, 48*, 4597–4605.

Zhong, K., Hu, X., Zhao, G., Chen, F., & Liao, X. (2005). Inactivation and conformational change of horseradish peroxidase induced by pulsed electric field. *Food Chemistry, 92*, 473–479.

Zhong, K., Wu, J., Wang, Z., Chen, F., Liao, X., Hu, X., et al. (2007). Inactivation kinetics and secondary structural change of PEF-treated POD and PPO. *Food Chemistry, 100*, 115–123.