Advance Journal of Food Science and Technology 6(5): 638-646, 2014
DOI:10.19026/ajfst.6.88
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: January 24, 2014 Accepted: February 10, 2014 Published: May 10, 2014

Research Article

Optimization of Combined Pulsed Electric Fields and Mild Temperature Processing Conditions for Red Apple Juice Polyphenol Oxidase and Peroxidase Inactivation

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Abstract: The effect on Polyphenol Oxidase (PPO) and Peroxidase (POD) enzyme activity in red apple juice was evaluated after combined Pulsed Electric Fields (PEF) and mild temperature processing using a response surface methodology. Changes in color were also analyzed and compared with thermally treated and unpasteurized juices. The studied factors were electric field strength (10-30 kV/cm), treatment time (200-1000 μs) and temperature (20-60°C). A significant second-order response function covering the whole range of experimental conditions was obtained for each enzyme. Treatments conducted at 30 kV/cm, 1000 μs and 60°C led to red apple juice with the lowest residual enzyme activity (0.04 and 0.16 for PPO and POD, respectively). Overall change in color was significantly lower ($p<0.05$), in comparison with severe thermal treatments. It was feasible to achieve comparable enzyme inactivation and better preserve natural juice color by this hurdle technique.

Keywords: Color, combined PEF and mild temperature, peroxidase, polyphenol oxidase, red apple juice, response surface methodology

INTRODUCTION

Apple juice clarification leads to drastic changes in the polyphenol profile compared to whole fruit. Consequently, clear apple juice is characterized by having low phenolic content, starch and pectic substances. However, some juices are not filtered and hence sold as naturally cloudy or opalescent. Cloudy apple juice has prominent sensory and nutritional quality. Nowadays, consumers prefer to obtain nutrients and health benefits naturally from food, thus the rise in cloudy apple juice market value. Research found that the total polyphenolic compounds in cloudy apple juice are 2.8 times higher than in clarified juice. Similarly, cloudy juice possessed notably higher (2.5 times) antioxidant activity (Candrawinata *et al*., 2012). Numerous studies have been carried out on the ability of cloudy apple juice to prevent changes that may produce cardiovascular diseases, cancer, elevated blood sugar and serious infections (Barth *et al*., 2007; Veeriah *et al*., 2008; Kujawska *et al*., 2011). Nevertheless, one of the major problems associated with cloudy apple juice processing is color deterioration. This essential quality parameter is strongly influenced by the activity of endogenous enzymes, namely Polyphenol Oxidase (PPO) and Peroxidase (POD). PPO catalyses the hydroxylation of monophenols and oxidation of colorless *o*-diphenols to *o*-quinones, followed by non-enzymatic polymerization of the quinones. This gives rise to melanins, pigments of high molecular mass and dark color (Espin *et al*., 1998). POD is a heme-containing enzyme that utilizes hydrogen peroxide to oxidize a large variety of hydrogen donors, such as phenols, aromatic amines, ascorbic acid and certain inorganic ions (Vernwal *et al*., 2006). Prevention of browning induced by PPO and POD is a critical subject in the juice industry. Reaction products of these enzymes not only affect color, flavor and texture, but when interacting with proteins, may also hinder digestibility thereby reducing the nutritional value of juices (Valvi *et al*., 2011). Reduction or complete inactivation of PPO and POD is an important goal. Conventionally, enzymatic effects have been controlled by thermal treatment, chemical inhibitors and pH variation. Heating is the most ideal because of its capacity to destroy spoilage and pathogenic microorganisms. It causes enzyme inactivation by denaturation, but also negatively affects thermo-sensitive nutrients, flavor, color and texture of cloudy juice. Chemical anti-browning effectors, such as sulfating agents, have adverse health effects (Ozoglu and Bayindirli, 2002).

The current trend in consumers' preference has led to increased interest by the food industry in new technologies with the ability to attain similar impact on microorganisms and enzymes as conventional

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treatment, yet with maximum preservation of sensory and nutritional quality. Pulsed Electric Fields (PEF) processing involves the application of short pulses (1-10 μs) of high intensity electric fields (15-40 kV/cm) to liquid foods placed between two electrodes (Jaeger *et al*., 2009). Inactivation of most microorganisms and enzymes has been demonstrated in fruit juices, with little or no impact on nutritional and sensorial properties (Martin-Belloso and Elez-Martinez, 2005a, b). Nonetheless, the combination of PEF and mild temperature was shown to be a more effective way of sufficiently reducing microbial populations to meet US Food and Drug Administration recommendations for fruit juice pasteurization and also enhance enzymatic damage (Martin-Belloso and Sobrino-Lopez, 2011). It is the balancing act of maintaining safety and preserving food quality that makes this hurdle concept very attractive.

Apples with white flesh and red peel are quite common. However, red-fleshed apples are triggering attention due to their remarkable anthocyanin profile and high antioxidant activity (Sadilova *et al*., 2006; Rupasinghe *et al*., 2010; Wang *et al*., 2013). A number of cultivars have been developed in New Zealand, Japan and Europe and more varieties are still being analyzed, with the primary intent of fruit consumption and juice processing (Volz *et al*., 2009; Umemura *et al*., 2011; Espley *et al*., 2013). A red cloudy apple juice could be beneficial for consumers as a novel functional beverage and as a result, has market potential for juice manufacturers. Generally, there is very little research on red-fleshed apple cultivars. Therefore, the aim of this study was to assess the effect of combined pulsed electric fields and mild temperature on PPO and POD activity in red cloudy apple juice (*Malus pumila* Niedzwetzkyana Dieck) using response surface methodology. Changes in overall juice color were also analyzed. Finally, a comparison was made with conventionally pasteurized juices.

MATERIALS AND METHODS

Materials and reagents: Ripe red-fleshed apples (*Malus pumila* Niedzwetzkyana Dieck) were obtained from Xinjiang Province, China, during the autumn season (2013). They were washed, packaged into polyethylene bags and frozen at -20°C until processing. Catechol and guaiacol were purchased from Sigma Chemical Company (Shanghai, China). All the other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents and chemicals were of analytical grade.

Red cloudy apple juice preparation: Red cloudy apple juice was prepared by defrosting the apples at 5°C and then juicing using a household juicer. Eight layers of cheese cloth were used for separating juice from apple pulp. The pH and conductivity of juice was 3.17±0.02 and 2.29±0.03 mS/cm, respectively. The PEF system requires materials of low electrical conductivity to prevent dielectric breakdown. The juice was immediately pasteurized by the different methods and assessed for residual enzyme activity and color.

Combined PEF and mild temperature treatment system: PEF treatment was carried out using a bench scale continuous system (OSU-4L, The Ohio State University, Columbus, Ohio, USA), as described and illustrated by Zhao and Yang (2008). A pulse generator provided bipolar square-wave pulses within six co-field flow chambers arranged in series. The chamber volume, diameter and distance between electrodes were 0.012 cm^3, 0.23 and 0.292 cm, respectively. Signals of voltage, current, frequency and waveform were monitored by a two channel digital real-time oscilloscope. Flow rate was adjusted by a micro-gear pump. An iced water bath was used, in which stainless steel coiled tubes connected to the treatment chambers were inserted for sample cooling. The inlet and outlet temperature for each pair of chambers was monitored by thermocouples. Temperature of the juice, for all treatments, was maintained at 20°C. Immediately after treatment, the juice was heated in a water bath for 60 sec and then cooled to room temperature before further analysis.

Conventional thermal treatment: An experimental set up was constructed consisting of a mineral oil bath, iced water for cooling and thermocouples for recording the temperature. Red apple juice was heated at 80, 90 and 115°C for 10 min, 5 min and 5 sec, respectively and then cooled to ambient temperature before further analysis.

Polyphenol oxidase assay: PPO enzyme activity was measured as described by Galli *et al*. (2009). A 3 mL reaction mixture consisting of 450 μL of 0.1 M catechol, 1650 μL of phosphate extraction buffer at pH 7.0 and 900 μL of diluted crude red apple juice was prepared. Catechol oxidation was followed for 10 min with absorbance being recorded at 30 sec intervals at 420 nm, against a blank consisting of catechol and buffer solution. An increase in absorbance was due to the formation of benzoquinone, the product of the reaction. Enzyme activity was calculated on the basis of the slope of the linear portion of the curve plotted of change in absorbance against time. PPO activity was expressed in units, as the change in absorbance at 420 nm/min/mL of juice or $0.001 \times A_{420}$ min/mL. Apple juice heated at 95°C for 2 min acted as control.

Peroxidase assay: POD activity was determined according to the method by Furumo and Furutani (2008). Guaiacol (4%) was freshly prepared and 0.15 mL thoroughly mixed with 2.70 mL of 0.1 M potassium phosphate buffer at pH 6.8 and 0.10 mL of 3% hydrogen peroxide. The mixture was incubated at room temperature for 3 min, and then 50 μL of diluted

Table 1: Face-centered central composite design for red apple juice PPO and POD residual activity after treatment by combined PEF and mild temperature

Assay number[1]	E (kV/cm)	t (μs)	T (°C)	PPO_{RA}	POD_{RA}
1	30	200	60	0.24±0.090	0.53±0.08
2	20	600	40	0.71±0.020	0.75±0.06
3	30	1000	60	0.04±0.003	0.16±0.01
4	20	600	20	0.79±0.004	0.89±0.10
5	20	600	40	0.72±0.050	0.76±0.01
6	20	600	40	0.77±0.050	0.79±0.03
7	10	200	20	0.99±0.002	0.98±0.05
8	30	200	20	0.66±0.010	0.77±0.02
9	20	200	40	0.82±0.008	0.86±0.04
10	10	600	40	0.90±0.050	0.95±0.30
11	20	600	40	0.72±0.010	0.78±0.06
12	20	600	60	0.53±0.100	0.71±0.05
13	10	1000	60	0.87±0.100	0.91±0.20
14	10	200	60	0.95±0.300	0.96±0.10
15	20	600	40	0.70±0.010	0.78±0.04
16	10	1000	20	0.94±0.100	0.96±0.08
17	30	600	40	0.37±0.020	0.49±0.06
18	20	600	40	0.71±0.010	0.77±0.10
19	30	1000	20	0.39±0.090	0.42±0.07
20	20	1000	40	0.60±0.070	0.68±0.08

[1]: The order of experiments was randomized; Values are expressed as mean±standard deviation (n = 3)

sample was added. The reaction was monitored for 10 min at room temperature and absorbance recorded after every 30 sec at 470 nm, against a blank consisting of all reagents except sample. POD activity was expressed as described for PPO enzyme. POD was inactivated by heating juice at 95°C for 20 min. This was the control.

Color measurement: Changes in color were assessed at room temperature with a HunterLab UltraScan VIS spectrophotometer, monitored by EasyMatch QC software that calculated CIE-Lab color ordinates after calibration with a white standard ceramic tile. Total color difference (ΔE*) was considered as the parameter for overall color difference between a pasteurized and unpasteurized sample, denoted by '0'. It was calculated using Eq. (1):

$$\Delta E^* = [(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{1/2} \quad (1)$$

Hue (h°) and chroma (C*) values were calculated according to Eq. (2) and (3) (Palou *et al*., 1999):

$$h^\circ = \arctan(b^*/a^*) \quad (2)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

Experimental design: A face-centered central composite response surface design was used to determine the effect of Electric field strength (E), treatment time (t) and mild Temperature (T) on the residual activity of red cloudy apple juice PPO and POD. Residual activity of the enzymes was calculated with respect to unpasteurized red cloudy apple juice Eq. (4):

$$RA = \frac{A}{A_0} \quad (4)$$

where A_0 and A are the enzymatic activities of untreated and processed apple juice samples, respectively.

The independent variables were electric field strength (10-30 kV/cm), treatment time (200-1000 μs) and mild temperature (20-60°C) for 60 sec. Treatment time was calculated as the product of the pulse width and number of pulses delivered in all treatment chambers, taking into account pulse frequency and residence time of each unit volume of red apple juice. Samples were treated at a frequency and pulse width of 200 Hz and 2 μs, respectively, irrespective of the electric field strength, treatment time and mild temperature applied. This design generated a total of twenty experimental runs using Design-Expert 8.0.6 software (Stat-Ease, Inc. Minneapolis, USA). Three values (minimum, central and maximum) of each factor were considered (Table 1). The order of assays was randomized and performed in triplicate. Experimental data were fitted to a quadratic, second-order response surface model, predicted by Eq. (5):

$$RA = k_0 + k_1.E + k_2.t + k_3.T + k_4.E.t + k_5.E.T + k_6.t.T + k_7.E^2 + k_8.t^2 + k_9.T^2 + \varepsilon \quad (5)$$

where RA is the residual enzymatic activity of PPO (PPO_{RA}) or POD (POD_{RA}), E (kV/cm) is the electric field strength, t (μs) is the PEF treatment time, T (°C) is the mild temperature and ε the experimental error.

The significance of the second-order model was evaluated by Analysis of Variance (ANOVA). First or second order coefficients were generated by regression analysis with hierarchical backward elimination at 95% confidence level ($p<0.05$). The quadratic model was therefore reduced leaving only significant factors and interactions.

Statistical analysis: Results were given as mean and standard deviation of three independent determinations. Data for the impact on residual enzyme activity and color were compared with that of thermally treated juice by one-way ANOVA (Version 19.0 IBM SPSS Statistics Inc., New York, USA) and Duncan's post hoc test, at 95% confidence level.

RESULTS AND DISCUSSION

Effect of electric field strength, treatment time and mild temperature on red apple juice PPO residual activity: Table 1 shows the results for a face-centered central composite design applied to analyze the effect of combined PEF and mild temperature on red apple juice PPO enzyme. ANOVA for the response surface reduced quadratic model for PPO inactivation revealed that the model terms and overall model was significant

Table 2: Analysis of variance of the second-order response surface models for red apple juice PPO and POD residual activity

F-value		
Source[1]	PPO	POD
Quadratic model	369.3200	473.2300
E	1836.3800	1982.8400
t	153.0100	326.6100
T	306.0200	195.2600
E × t	45.9200	183.3300
E × T	154.0200	80.2300
E^2	23.3000	71.0900
T^2	13.1800	-
Lack of fit	0.8300	1.5400
Standard deviation	0.0200	0.0170
Mean	0.7200	0.7500
Coefficient of variation (%)	2.7900	2.2800
R^2	0.9954	0.9954
Adjusted R^2	0.9927	0.9933
Predicted R^2	0.9881	0.9891

[1]: E = electric field strength, t = treatment time, T = temperature

Table 3: Significant regression coefficient estimates and p-values of the quadratic models for PPO and POD residual activity in red apple juice pasteurized by combined PEF and mild temperature

	PPO		POD	
Source[1]	p-value	Coefficient	p-value	Coefficient
Intercept	-	0.770	-	0.780
E	<0.0001	-0.270	<0.0001	-0.240
t	<0.0001	-0.078	<0.0001	-0.097
T	<0.0001	-0.110	<0.0001	-0.075
E×t	<0.0001	-0.048	<0.0001	-0.081
E×T	<0.0001	-0.088	<0.0001	-0.054
E^2	0.0004	-0.054	<0.0001	-0.064
T^2	0.0034	-0.041	-	-

[1]: E = electric field strength, t = treatment time, T = temperature

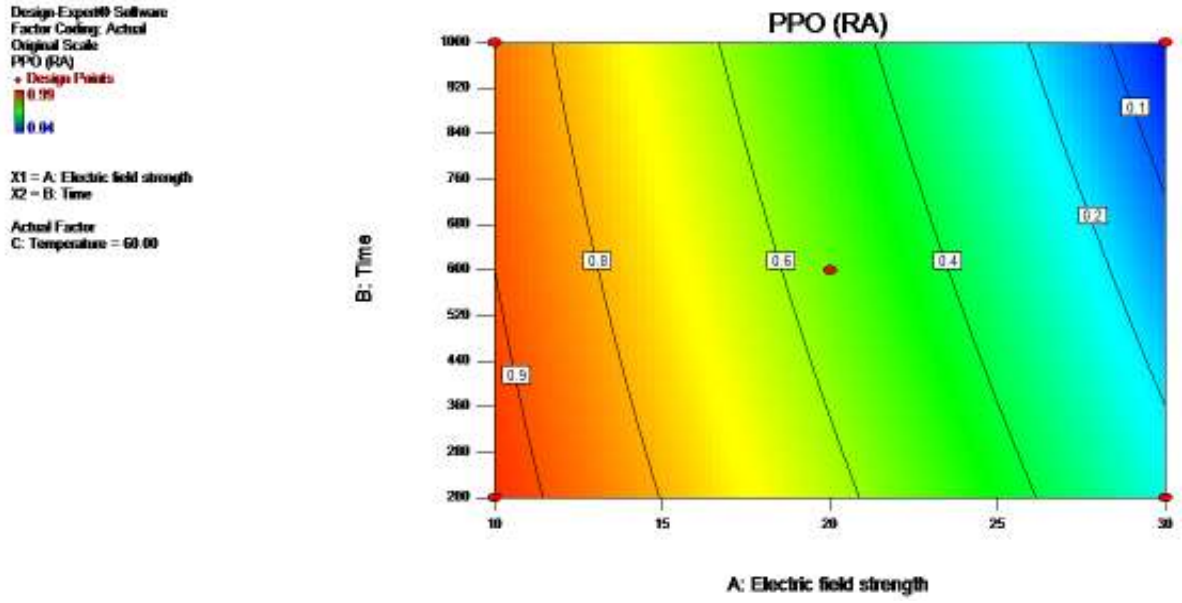
(*F*-value = 369.32), with the predicted R^2 (0.9881) in reasonable agreement with the adjusted R^2 (0.9927). No significant lack of fit was found ($F = 0.83$, $p = 0.6044$), indicating that the model was appropriate for prediction within the selected experimental factors (Table 2). Backward elimination showed that seven model terms (E, t, T, E×t, E×T, E^2 and T^2) were significant ($p < 0.05$) and only these were included in the final predictive equation (Table 3).

Figure 1a shows a two dimensional contour plot of the effect of electric field strength and time at a constant temperature of 60°C. It can be noted that at low electric field strength values (10-15 kV/cm), PPO residual activity in red apple juice was high regardless of increase in treatment time. At treatment times shorter than 360 μs, moderate enzyme inactivation was obtained only at electric field strengths above 20 kV/cm. However, at 27-30 kV/cm, there was substantial decrease in PPO residual activity (more than 80%), even at short treatment times. This suggests that treatment time had a pronounced effect on PPO inactivation only at high electric field strengths. The regression coefficients for both factors were negative, indicating their favorable impact on PPO inactivation. Nonetheless, the coefficient for electric field strength was higher, reflecting a stronger influence (Table 3). The effect of temperature and electric field strength on PPO residual activity at a constant treatment time of 1000 μs is shown in Fig. 1b. Similarly, increase in temperature after treatment at electric field strengths less than 15 kV/cm did not result in considerable enzyme inactivation. However, a combination of temperatures above ambient levels and high electric field strengths (25-30 kV/cm) resulted in pronounced effect on residual activity (less than 0.35). In this study, electric field strength and temperature were the most influential factors affecting PPO inactivation in red apple juice (Table 3).

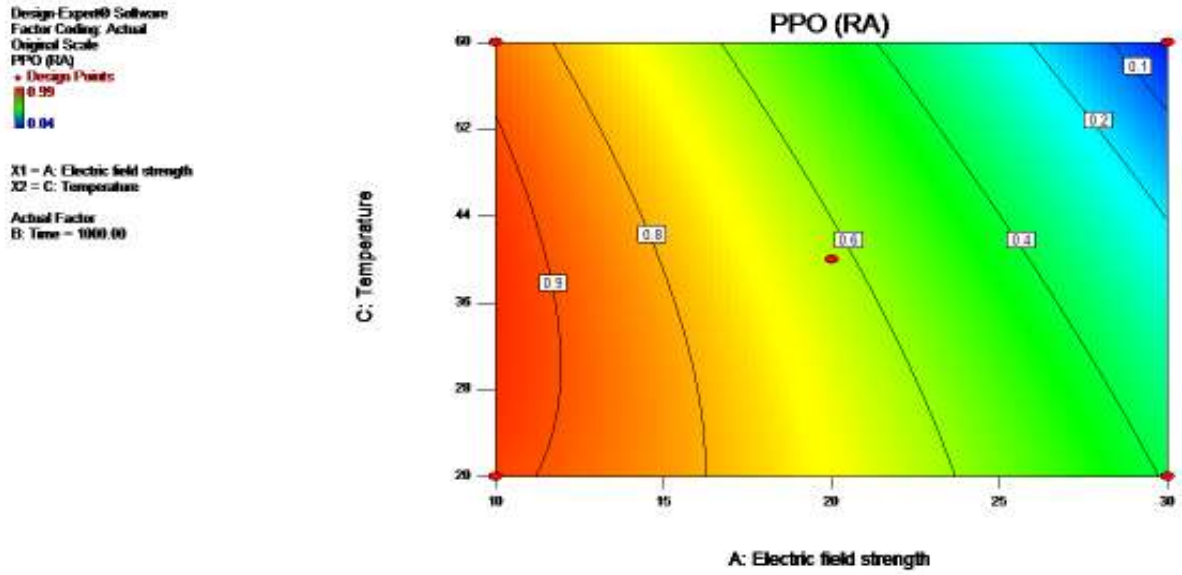
Most studies report enzyme inactivation in juices after heating prior to PEF treatment. In this study, red apple juice was treated by PEF at 20°C and then subjected to heat for 60 sec. Generally, comparison of data on products treated by PEF in various studies is challenging due to the application of different processing conditions and devices. Nevertheless, the present results are in agreement with other findings showing that increase in temperature and electric field strength effectively promotes PPO enzyme inactivation. The residual activity of mushroom PPO decreased from 0.98 to 0.83 after an increase in electric field strength from 10-25 kV/cm. Treatment time, pulse frequency and temperature were kept constant at 290 μs, 10 Hz and 4°C, respectively (Zhong *et al*., 2007). Riener *et al*. (2008) also observed a similar trend as apple juice relative activity decreased from 65 to 44% when treated at 20 and 40 kV/cm, respectively. Increase in temperature from 23 to 50°C, further reduced the activity to 24%. The mechanism of enzyme inactivation by PEF processing is still unclear. However, some observations have led to the suggestion that conformational changes in enzyme native structure maybe responsible for loss of activity (Martin-Belloso and Elez-Martinez, 2005a).

Effect of electric field strength, treatment time and mild temperature on red apple juice POD residual activity: The same criteria were used for analyzing POD inactivation results (Table 1). Only six model terms (E, t, T, E×t, E×T and E^2) were significantly affecting ($p<0.05$) POD residual activity (Table 3). The predictive model was significant (*F*-value = 473.23) with predicted and adjusted R^2 values of 0.9891 and 0.933, respectively. In addition, model lack of fit was insignificant ($F = 1.54$, $p = 0.3305$) indicating that it was also acceptable (Table 2).

Figure 2a shows the effect of electric field strength and treatment time on POD residual activity when temperature was kept at 60°C. Low electric field strengths were not very effective at enzyme inactivation. At 20 kV/cm, prolonging treatment time resulted in residual activity higher than 0.65. To obtain residual activities less than 0.40, treatment times longer than 500 μs and electric field strengths higher than



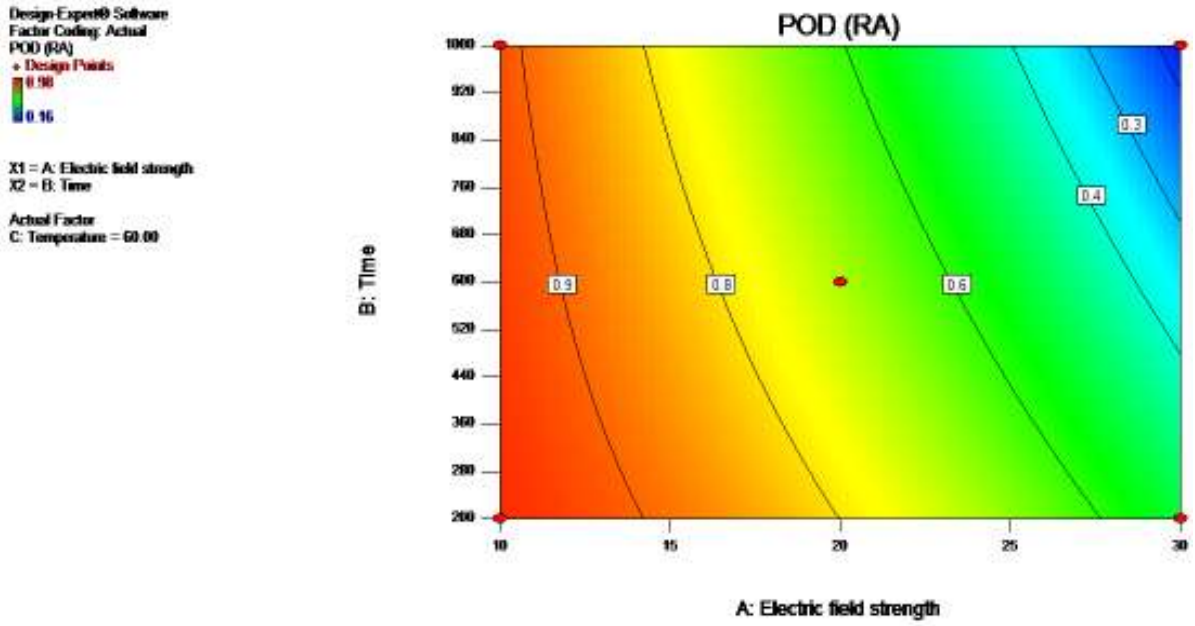
(a)



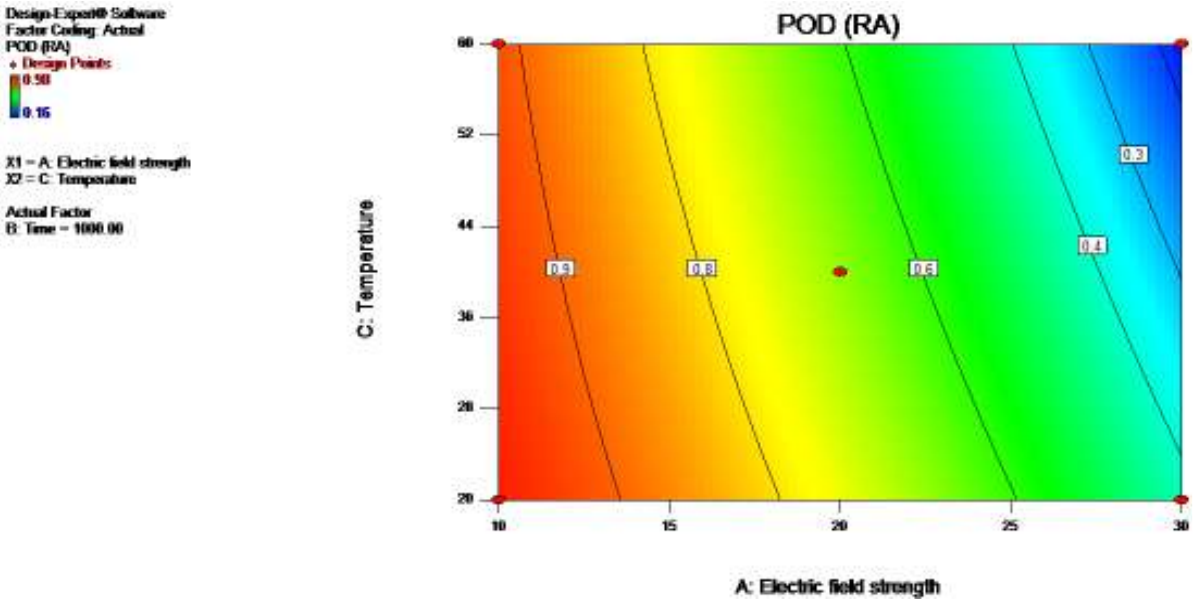
(b)

Fig. 1: Effect of electric field strength, treatment time and temperature on red apple juice PPO_{RA} when temperature was set at 60°C, (a) and treatment time 1000 μs, (b) the t×T interaction was insignificant

25 kV/cm were required. Likewise, Fig. 2b shows that increase in post treatment temperature caused substantial POD inactivation (more than 60%) at 25 kV/cm or higher. However, the larger regression coefficient indicates that increase in electric field strength was more influential than treatment time and temperature (Table 3). Published works on the impact of PEF on enzyme residual activity agree that POD is highly thermostable, but more susceptible to intense electric fields (Yang *et al*., 2004; Elez-Martinez *et al*., 2006; Aguilo-Aguayo *et al*., 2010). Overall, it is clear that POD was more resistant to the applied processing conditions than PPO. In a similar study (Riener *et al*., 2008), higher levels of PPO (71%) than POD (68%) were inactivated after being subjected to a combination of preheating at 50°C and PEF treatment time of 100 μs at 40 kV/cm. Comparably, grape juice PPO is also more liable to combined PEF and mild heat than POD (Marselles-Fontanet and Martin-Belloso, 2007). The difference between inactivation of PPO and POD in



(a)



(b)

Fig. 2: Effect of electric field strength, treatment time and temperature on red apple juice POD_{RA} when temperature was set at 60°C, (a) and treatment time 1000 μs, (b) the t×T interaction was insignificant

fruit juices maybe due to the huge distinction in molecular structure and size displayed among enzymes (Martin-Belloso and Elez-Martinez, 2005a).

Optimization of combined PEF and mild temperature processing conditions: A combination of processing factors leading to red apple juice with the lowest residual enzyme activity was determined. Overall, low residual enzyme activity was achieved at high electric field strength, treatment time and temperature (Fig. 1 and 2). A high desirability value of 0.998 was obtained when PEF treatment at 30 kV/cm for 1000 μs was combined with post treatment temperature of 60°C. The predicted residual activity for PPO and POD at the optimum processing conditions was 0.038 and 0.167, respectively. Data comparison shows that the proposed predictive models were accurate to fit experimental results. PPO and POD

Table 4: Comparison between combined PEF with mild temperature (optimized conditions) and conventional pasteurization on red apple juice PPO and POD residual activity

Pasteurization method	PPO_{RA}	POD_{RA}
Unpasteurized juice	1.00 ± 0.020^{a}	1.00 ± 0.04^{a}
PEF and mild temperature	0.04 ± 0.003^{bc}	0.16 ± 0.01^{bc}
80°C, 10 min	0.02 ± 0.004^{b}	0.12 ± 0.03^{c}
90°C, 5 min	n.d[1]	0.10 ± 0.02^{d}
115°C, 5 sec	0.06 ± 0.010^{c}	0.20 ± 0.02^{b}

[a-d]: Means with different letters within a column were significantly different ($p<0.05$); Values are expressed as mean±standard deviation ($n = 3$); [1]: n.d = not detected

residual activity at the same conditions was 0.04 and 0.16 (Table 1), respectively, hence validating the model equations.

Comparison between PEF and conventional pasteurization on red apple juice residual enzyme activity and color: The inactivation of red apple juice PPO and POD by combined PEF and mild temperature (optimized conditions) was compared to conventional thermal pasteurization. There is wide research on comparison between PEF and less severe thermal conditions, generally ranging from 70-90°C for 20-100 sec. Riener *et al*. (2008) reported that thermal pasteurization of apple juice at 72°C for 26 sec resulted in PPO and POD residual activities of 0.54 and 0.52, respectively. This activity is considerably higher than that attained in this study after combined PEF and mild temperature optimization (0.04 and 0.16 for PPO and POD, respectively). Consequently, to enable an objective comparison, more severe thermal conditions were selected. Table 4 shows that pasteurization by the four treatments had substantial impact ($p>0.05$) on residual activity of both enzymes. For PPO enzyme, there was no significant difference between all the treatments ($p<0.05$). However, complete inactivation was achieved at 90°C for 5 min. For POD enzyme, thermal treatment at 90°C for 5 min resulted in significantly lower ($p<0.05$) residual activity than PEF treated juice. All the applied treatments could not completely inactivate POD. It is worth noting that combined PEF and mild temperature conditions were more effective in enzyme inactivation than ultra-high temperature (115°C, 5 sec). Overall, POD was more thermos table than PPO. The larger an enzyme and more complex its structure, the more susceptible it is to high temperatures (Yang *et al*., 2004).

The most distinct feature of red apple juice is its appealing color. The color is due to the substantial amount of anthocyanins present. Several factors affect the color stability of juices, including pH, non-enzymatic reactions, enzyme activity, pasteurization conditions and so on. In this study, the effect of pasteurization conditions is reported. Figure 3 shows the L*, a*, b*, C*, h° and ΔE* values for red apple juice before and after pasteurization. All the juices were affected significantly by treatment ($p<0.05$). Unpasteurized juice was darker, brighter and redder. Nevertheless, lower L* (lightness) and higher a* (redness) values indicate that PEF treated juice was significantly darker and more red ($p<0.05$) than thermally pasteurized juices. The b* parameter reflects the degree of yellowness or blueness in the sample. Yellowness of PEF treated juice was similar to that treated at 115°C for 5 sec. The overall color difference (ΔE*) from the unpasteurized juice is calculated by taking into account changes in L*, a* and b* parameters. The overall difference in color was great (>9) and clearly visible for thermally treated juices. The C* (chroma) parameter indicates vividness or dullness of the juice. In this case, C*, L* and a*

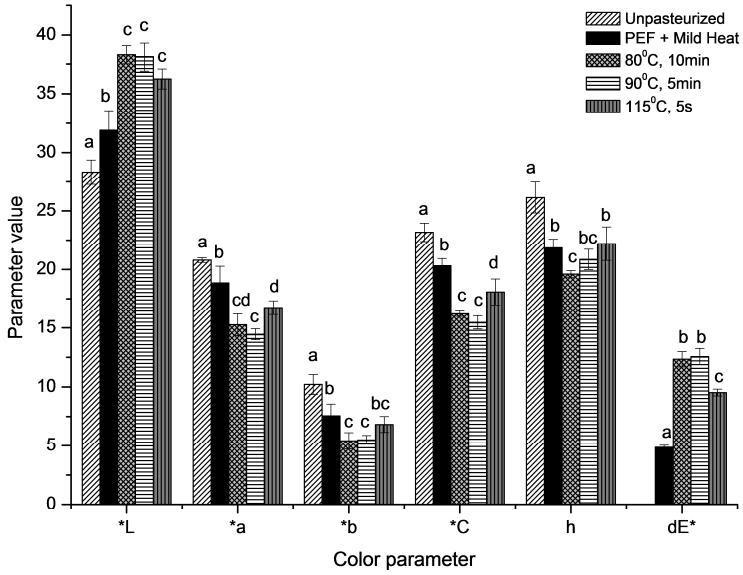


Fig. 3: Comparison between combined PEF with mild temperature (optimized conditions) and conventional pasteurization on red apple juice color parameters. Means with different letters were significantly different ($p<0.05$)

parameter values are in agreement, demonstrating that PEF juice was brighter, more vivid and redder than thermally treated juices. Description of color by the hue index (h°) revealed a similar trend. However, PEF treated juice was different significantly ($p<0.05$) only with juice treated at 80°C for 10 min. High temperatures and long treatment conditions are detrimental to juice color.

The results observed in this study somewhat concur and contradict with other literature. Aguilo-Aguayo *et al*. (2009) reported color parameters of three red juices (strawberry, watermelon and tomato) after PEF and heat treatments. L* values for the three juices increased, a* and b* values also increased for strawberry and watermelon juices, but decreased for tomato juice. Overall change (ΔE*) was greater for strawberry and watermelon juices. Cserhalmi *et al*. (2006) also observed irregularities with a variety of citrus fruit juices (pink grapefruit, lemon, orange and tangerine). Overall change was greater for tangerine juice. Min *et al*. (2003) found that PEF treated tomato juice was even more red than the control samples, thereby enhancing the color. These findings seem to indicate that the impact of PEF or thermal treatments on color parameters of juices from various fruits and vegetables is inconsistent, maybe due to the different intrinsic characteristics.

Overall, in comparison with severe heat treatments, combined PEF and mild temperature optimized conditions had comparable adverse effect on PPO and POD residual activity and better retained the natural color of cloudy red apple juice.

CONCLUSION

The optimization of red apple juice pasteurization was achieved by response surface methodology. Application of response surface models not only gave optimum operating conditions, but also revealed the interaction between factors under concern and their level of influence towards the required output. Electric field strength, treatment time and post treatment temperature had significant influence on the inactivation of red apple juice PPO and POD enzymes. However, POD was more resistant to this hurdle technique than PPO. High electric field strength, treatment time and temperature were crucial for substantial enzyme inactivation. The optimum combined PEF and mild temperature processing conditions were 30 kV/cm, 1000 μs and 60°C. Under these conditions, enzyme inactivation was comparable with severe conventional thermal treatments. Moreover, the initial color of red apple juice was better preserved. This study demonstrates the feasibility of ensuring significant residual enzyme activity reduction in red apple juice and avoiding considerable color changes by the application of combined PEF and mild temperature pasteurization as an alternative to heat. There is potential for red cloudy apple juice to meet consumer demand for natural, nutritious and functional foods. This study will provide a basis for new product development as more red-fleshed apple cultivars are being developed and PEF technology is progressing towards industrial application.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support provided by National “863” Project (Project 2011AA100801-02, 31000829 and 31101376 of the National Natural Science Foundation of China) and Project BK2010148 of Jiangsu Provincial Natural Science Foundation.

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