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Combined effect of temperature and pulsed electric fields on apple juice peroxidase and polyphenoloxidase inactivation

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Abstract

Pulsed electric fields (PEF) were applied to freshly prepared apple juice using a laboratory scale continuous PEF system to study the feasibility of inactivating peroxidase (POD) and polyphenoloxidase (PPO). Square wave PEF using different combinations of electric field strength, pre-treatment temperature and treatment time were evaluated in this study and compared to conventional pasteurisation (72 °C; 26 s). Inactivation curves for the enzyme were plotted for each parameter and inactivation kinetics were calculated. Results showed the highest level of decrease in the enzymatic activity of 71% and 68%, for PPO and POD, respectively, were obtained by using a combination of preheating to 50 °C, and a PEF treatment time of 100 μs at 40 kV/cm. This level of inactivation was significantly higher ($P < 0.05$) than that recorded in juice processed by conventional mild pasteurisation where the activity of PPO and POD decreased by 46% and 48%, respectively. The kinetic data for the inactivation of both enzymes could be described using a 1st-order model ($P < 0.001$).

Keywords: Pulsed electric field; Peroxidase; Polyphenoloxidase; Apple juice; Kinetics

1. Introduction

Unclarified apple juice is gaining an increased market share due to its enhanced sensory and nutritional qualities (Zenith International, 2001). This juice contains a high proportion of pulp in suspension and retains the flavour of freshly pressed apples (Okayasuand & Naito, 2001). However, cloudy apple juice is very sensitive under oxygen atmosphere to enzymatic browning since it contains considerable quantities of polyphenols and polyphenol oxidases (PPO) (E.C.1.14.18.1). These enzymes catalyze both the hydroxylation reaction of mono-phenols to di-phenols, and in a second step, the oxidation of colourless *ortho*-diphenols to form *o*-quinones, the condensation of which generates highly coloured melanines (Vamos-Vigyazo, 1981). These compounds negatively influence the organoleptic and nutritional qualities and subsequently the marketability of freshly squeezed apple juice. Another enzyme which has a considerable impact on the quality of apple juice is peroxidase (POD) (E.C.1.11.1.7). POD catalyzes the oxidation in the presence of H_2O_2 of a wide range of natural substances present in plant foods leading to deterioration of colour, flavour and nutritional quality. (Eskin & Robinson, 2001), all of which are a concern to food processors. Traditionally, prevention of enzymatic browning has been achieved by a combination of thermal treatment, chemical inhibitors and pH variation. However, thermal processing has an unfavourable impact on the quality of the final product (Chutintrasri & Noomhorm, 2006). A rising consumer demand for minimally processed and fresh-like food products has led to an increased interest by the food industry in innovative "non-thermal" processing technologies, which aim to achieve similar microbial and enzymatic inactivation with reduced or no application of heat. Among these technologies high hydrostatic pressure treatment has had the most commercial success in fruit

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juice processing to date but another technology showing promise is pulsed electric field (PEF) processing.

To date much of the PEF research has focused on microbial inactivation and its underlying mechanisms and kinetics. Only a small number of research groups have focused on the effects on chemical indicators of quality including enzyme activity (Fiala, Wouters, van den Bosch, & Creyghton, 2001; Wouters, Alvarez, & Raso, 2001; Wouters, Bos, & Ueckert, 2001).

In particular the literature available in relation to the effect of PEF on PPO and POD in food or in model systems is limited and the data available seems to indicate a certain resistance of these enzyme systems to PEF treatment. Ho, Mittal, and Cross (1997) observed a reduction of 30% in POD from soybean suspended in a buffer solution when it was treated at 75 kV/cm for a total of 60 μs. When 34.9 kV/cm and 126 μs were applied to soybean POD suspended in a buffer solution, an 18.1% POD inactivation was achieved (Yang, Li, & Zhang, 2004). By contrast a complete inactivation of orange juice POD was reported by Elez-Martinez, Aguilo-Aguayo, and Martin-Belloso (2005) when orange juice was processed at 35 kV/cm for 1500 μs. Zhong et al. (2007) achieved a reduction of POD activity of 14.3% at 25 kV/cm for 290 μs. In the same study Zhong et al. (2007) achieved a reduction of PPO activity of 16.9% after a treatment at 25 kV/cm for 124 μs. When 33.6 kV/cm and 126 μs were applied to PPO (Mushroom Tyronase) suspended in a buffer solution, a 38.2% PPO inactivation was achieved (Yang et al., 2004). When 24 kV/cm and 60 μs were applied to PPO suspended in a buffer solution, a 40% PPO inactivation was achieved (Ho et al., 1997). Although the effects of PEF on POD and PPO in apple juices has not been fully described in the scientific literature, PEF has tentatively emerged as worthwhile technique to inactivate POD and PPO in foods.

The objectives of this study were firstly to examine the effects of PEF (with or without additional preheating) on the inactivation of apple juice POD and PPO and to compare the inactivation with that achieved by conventional pasteurisation. A second objective was to evaluate the suitability of using a classic first order kinetic model for describing the inactivation of apple juice POD and PPO by PEF. The final aim of the study was to identify the impact of treatment time, electric field strength and preheating temperature on the inactivation of apple juice POD and PPO.

2. Materials and methods

2.1. Preparation of apple juice

Fresh apple juice was manufactured from apples (Jonagold Red; Ireland were purchased in a local grocery store. Apple juice was prepared immediately after purchase using a household juicer (Hinari, Model No. JEP 95 311 Domestic, Essex, UK) and then filtered through 100 mm mesh filters and poured into glass bottles prior to processing.

2.2. PEF Treatment system

The PEF equipment used was as described previously (Noci et al., 2008). The PEF process chamber consisted of two parallel stainless steel electrodes separated by a 2 mm gap, the length of the electrodes was 160 mm and the exposed electrode surface was 5 mm wide. The system was pulsed at a constant frequency of 15 Hz with a pulse duration of 1 μs.

2.3. Treatment conditions and experimental design

The following PEF conditions and pre-treatment temperatures were used to evaluate the effect of the main processing parameters on POD and PPO activity and their potential interactions. A 3×3 factorial arrangement was used with three different electric field strengths (20, 30 and 40 kV/cm), three treatment times (25, 50 and 100 μs) (number of pulses × pulse duration) and three pre-treatment temperatures (23, 35, 50 °C). Throughout these experiments the residual POD and PPO activity was calculated relative to activity to untreated apple juice. As a control apple juice was pasteurised in a pilot scale tubular heat exchanger (Armfield FT74, HTST/UHT Processing Unit, Hampshire, England) at 72 °C for a holding time of 26 s and then cooled to ambient temperature.

2.4. Enzyme analysis

2.4.1. Polyphenol oxidase (PPO) assay

The method of Dawson and Magee (1955) was used to measure PPO activity by measuring the increase in absorbance at 265 nm resulting from the reaction of PPO with L-3,4-dihydroxyphenylalanine. The assay was performed in a 10 mm glass cuvette the absorbance was monitored over a 10 min period at 25 °C with a spectrophotometer (Unicam; UV/VIS, MinUV1240 Cambridge, UK). Potassium phosphate buffer solution (50 mM) with the pH of 6.5 at 25 °C was used for the assay. One unit of POD activity was defined as a change in absorbance 265 nm of 0.001 per minute at pH 6.5 at 25 °C in a 3 ml reaction mix containing 50 mM potassium phosphate, 0.17 mM L-3,4-dihydroxyphenylalanine, 0.07 mM L-ascorbic acid and 50–100 units of polyphenol oxidase.

2.4.2. Peroxidase (POD) assay

The POD assay was carried out using the method of Chance and Maehly (1955), which measures the absorbance at 420 nm resulting from the oxidation of Pyrogallol to Purpurogallin catalyzed by POD in a 100 mM potassium phosphate buffer solution at a pH 6.0. The absorbance of both blank and juice sample was measured in a 10 mm glass cuvette over a 5 min period. The final concentrations of the reagents in the cuvette were 14 mM potassium phosphate, 0.027% (w/w) hydrogen peroxide, 0.5% (w/v) pyrogallol and 0.04–0.07 units of peroxidase. One unit of POD activity was defined as the change in absorbance (420 nm) over time

(min) per ml of enzymatic extract. One unit of POD was defined as the formation of 1.0 mg of purpurogallin from pyrogallol in 20 s at pH 6.0.

2.5. Data analysis

The PEF treatments were carried out on two separate batches of juice. The residual activity (RA) of POD and PPO obtained after each PEF treatment was calculated as a percentage of the enzyme activity in untreated juice. Experimental data were fitted to a first order kinetic model (Eq. (1)) (Copeland, 2000; Eagerman & Rouse, 1976)

$$\ln(\mathrm{RA}) = -k_{\mathrm{E}}t \quad (1)$$

where t is the PEF treatment time (μs) and k_{E} is the inactivation rate constant.

Decimal reduction times (D) were then calculated using Eq. (2)

$$D = \frac{\ln 10}{k_{\mathrm{E}}} \quad (2)$$

2.6. Statistical analysis

An analysis of variance was performed, to investigate the effect of pre-treatment temperature, electric field strength and total treatment time as main factors and their interactions. All analyses of variance were performed using Genstat (Version 8.1, VSN International, Hemel Hempstead, United Kingdom) and a $P < 0.05$ was used to determine statistical significance. Sigmaplot (Version 8.2 SYSTAT, Point Richmond, CA, USA) was used for the calculation of the inactivation rate constant k_{E} and for graphical representation.

3. Results and discussion

3.1. Effect of pre-treatment temperature, electric field strength and treatment time on the inactivation of POD and PPO

The effects of pre-treatment temperature, electrical field strength and treatment time on POD and PPO are presented in Figs. 1 and 2, respectively. To further illustrate the effects of pre-treatment temperature and electrical field strength two additional figures (Figs. 3 and 4, respectively) have been prepared for selected processing conditions.

Increasing the pre-treatment temperature of the juice had a significant ($P < 0.01$) effect in the inactivation of POD and PPO. For example, the RA of POD decreased to 45.0% when the juice was preheated to 50 °C and followed by PEF treatment at 30 kV/cm for 100 μs (Fig. 3). Under the same PEF conditions with a pre-treatment temperature of 23 °C juice showed an RA of 63.6%. The inactivation of PPO showed a similar trend, as a 43.4% RA was achieved by PEF at 30 kV/cm for 100 μs at 50 °C, while there was 59.4% RA with a pre-treatment temperature of 23 °C. Fig. 3 also shows that the 30 kV/cm PEF treatment brought about a moderate rise in the temperature of the juice, within the PEF cell. This increase was constant at approximately 15 °C regardless of the inlet temperature. Under these specific PEF conditions the maximum temperature reached by the juice was 65 °C.

The impact of the PEF field strength on the RA for POD and PPO was highly significant ($P < 0.001$) and the relationship between the increase in field strength and the decrease in RA was found to be linear in both cases ($P < 0.05$). An example of the effects of electric field strength on POD and PPO activity in apple juice, under the most extreme processing conditions (i.e. 100 μs; 50 °C) is shown in Fig. 4. Under these conditions it was observed that before any PEF treatment was applied the RA was 88.5% and 85.1% for POD and PPO, respectively. Following PEF treatment POD activity decreased in a linear fashion ($P < 0.001$, $r^2 = 0.991$) as electrical field strength increased from 20 to 40 kV/cm. Conversely the outlet temperature increased linearly ($P < 0.001$, $r^2 = 0.9386$) with increasing electrical field strength. The highest electric field strength (40 kV/cm) reduced POD RA to 32.3%. Similarly, PPO RA also decreased in a linear manner in response to increasing electric field strength ($P < 0.05$, $r^2 = 0.933$), thus confirming the findings outlined by other researchers (Yeom, Zhang, & Chism, 2002; Yeom, Zhang, & Dunne, 1999).

The highest electric field strength (40 kV/cm) produced an RA of 28.9%, but as stated above this field strength also increased juice temperature from 50 °C to approximately 72 °C. This temperature is close to that used in mild pasteurisation treatments for juice and could therefore contribute to some thermal inactivation of both POD and PPO. Since the residence time of the juice in the PEF cell is only 6.6 s the magnitude of the thermal inactivation would probably be fairly modest as indicated by the findings of Espachs-Barroso, Van Loey, Hendrickx, and Martin-Belloso (2006). However, if potentially undesirable effects of heat on juice quality are to be avoided, a limitation of the electric field strength to 30 kV/cm is advisable, as under these conditions the outlet temperature of the juice would not exceed 65 °C.

Overall, the present results are in agreement with the findings for other products showing that increasing the intensity of the electric field strength also increased the enzyme inactivation effectiveness of PEF treatments (Sepulveda, Gongora-Nieto, San-Martin, & Barbosa-Canovas, 2005; Yeom, Streaker, Zhang, & Min, 2000).

While the precise nature of enzyme deactivation by PEF is not fully understood some workers suggested that enzyme inactivation may be caused by PEF induced conformational changes in the tertiary structure of the enzyme (Ho et al., 1997; Vega-Mercado, Powers, Barbosa-Canovas, & Swanson, 1995; Yeom et al., 1999). A recent study by Zhong et al. (2007) showed that the secondary structure of the enzymes are also affected by PEF.

The impact of PEF treatment time on the RA can be observed in Figs. 1 and 2. Prolonging the treatment time from 25 to 100 μs linearly decreased ($P < 0.001$) the RA

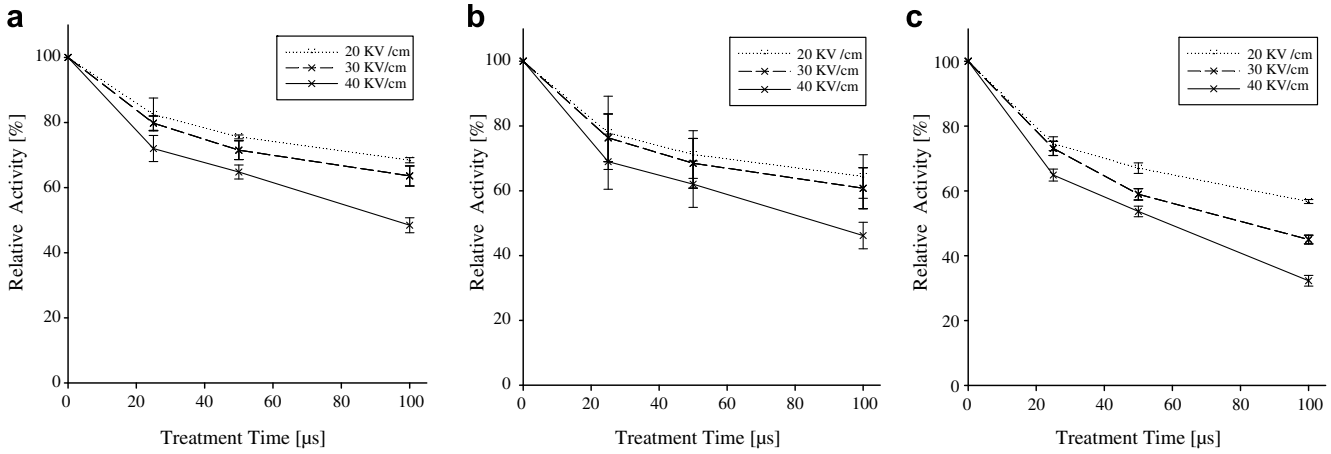

Fig. 1. Relationship between peroxidase (POD) residual activity and pulsed electric field (PEF) treatment time. PEF treatment at (a) 23 °C, (b) 35 °C and (c) 50 °C at 20, 30 or 40 kV/cm.

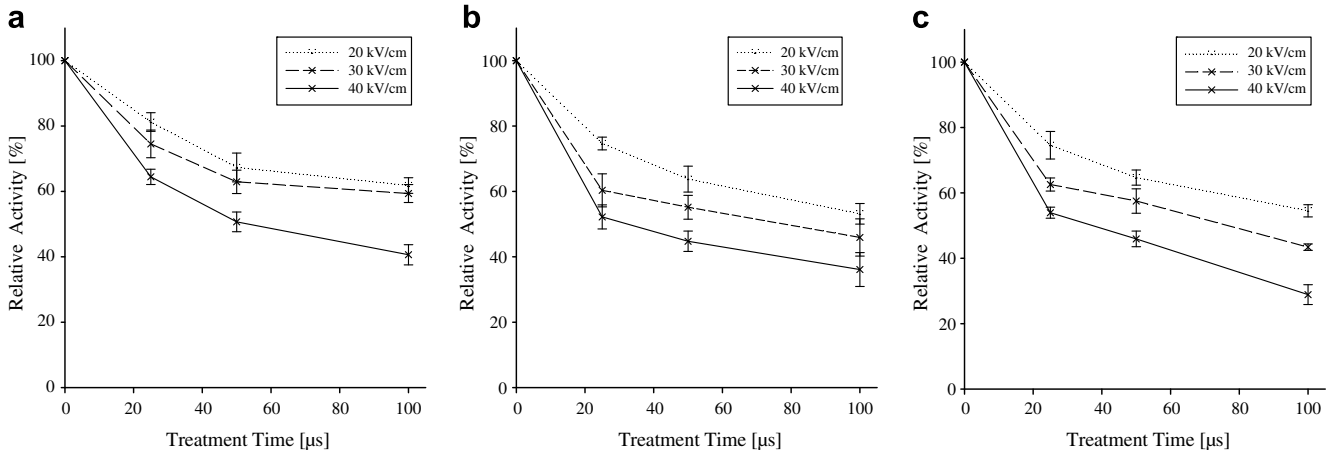

Fig. 2. Relationship between polyphenoloxidase (PPO) residual activity and pulsed electric field (PEF) treatment time. PEF treatment at (a) 23 °C, (b) 35 °C and (c) 50 °C at 20, 30 or 40 kV/cm.

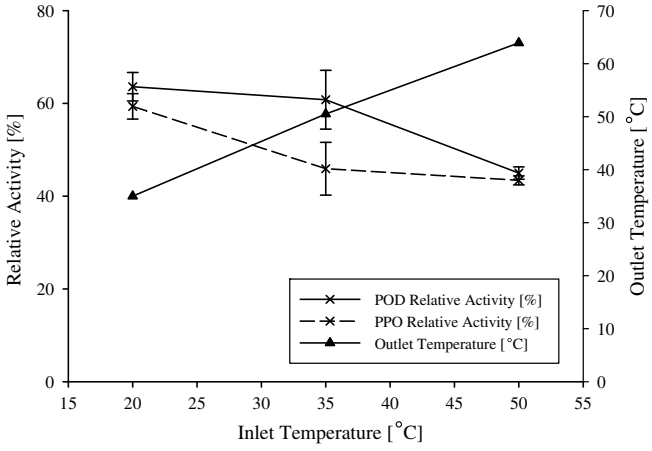

Fig. 3. Effect of preheating temperature on the inactivation of peroxidase (POD) and polyphenoloxidase (PPO) using pulse electric field Protocol 2 (30 kV/cm, 100 μs).

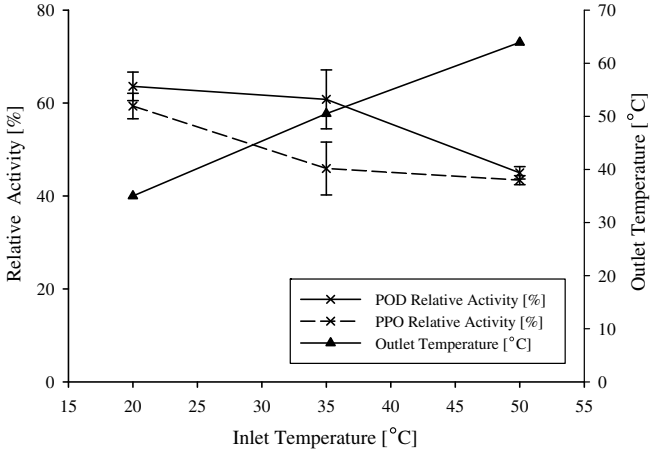

Fig. 4. The effect of electric field strength on peroxidase (POD) and polyphenoloxidase (PPO) activity and outlet temperature on freshly squeezed apple juice preheated to 50 °C prior to pulsed electric field treatment.

for both POD and PPO, from 75% to 54%, and from 67% to 48%, respectively. In a similar study (Yang et al., 2004) showed a decrease of soybean POD and PPO by 72% and 63%, respectively, when using similar PEF conditions in the absence of any pre-treatment heating.

Table 1
Kinetic constants (k_E) of the first order inactivation model, correlation coefficients (r^2) and decimal reduction time (D) for peroxidase (POD) and polyphenoloxidase (PPO)

T (°C)		POD Electric field strength (kV/cm)			PPO Electric field strength (kV/cm)		
		20	30	40	20	30	40
23	k_E (1/μs)	0.004	0.004	0.007	0.005	0.005	0.009
	r^2	0.8974	0.8935	0.9485	0.8768	0.7954	0.8926
	D (μs)	615.4	539.9	337.5	491.4	471.3	269.8
35	k_E (1/μs)	0.004	0.005	0.007	0.006	0.007	0.009
	r^2	0.8668	0.8644	0.9329	0.9189	0.7945	0.7954
	D (μs)	559.2	497.8	319.0	383.2	331.2	251.2
50	k_E (1/μs)	0.005	0.008	0.011	0.006	0.008	0.012
	r^2	0.8769	0.9577	0.9789	0.9081	0.8779	0.923
	D (μs)	449.9	297.3	212.2	401.9	302.8	199.5

3.2. D-values

In order to establish the link between treatment time and enzyme activity the D-values (decimal reduction times) were calculated. Kinetic data from the POD and PPO inactivation studies measured as a function of preheating temperature and electric field strength are summarised in Table 1. The D-value in the present context denotes the PEF treatment time (μs) required at a given PEF temperature and electric field strength to reduce the enzyme activity by 90%. At a pre-treatment temperature of 23 °C the D-values obtained using the rate constant data (k_E) and Eq. (2) decreased in a linear manner ($r^2 = 0.935$) from 615.4 to 337.5 μs, when the electric field strength was increased from 20 to 40 kV/cm for POD. Similarly, the D-values for PPO also decreased in a linear fashion ($r^2 = 0.8174$) from 491.4 μs to 269.8 μs. These data represent further evidence that higher electric field strengths result in higher inactivation level of POD and PPO. It is also worth noting from the data in Table 1 that a large decrease in D-values for both enzymes was observed, when PEF field strength or preheating temperature was increased ($P < 0.001$). Furthermore, there were substantially lower D-values at all PEF field strengths for PPO compared to POD at treatment temperatures of 23 and 35 °C; these data highlight the greater susceptibility of PPO to inactivation by PEF. However, when a pre-treatment temperature of 50 °C was used, D-values for both enzymes at electric field strengths of 30 and 40 kV/cm were very similar suggesting a proportionally greater contribution to enzyme deactivation by heat compared to the PEF effect. In general, the larger an enzyme and the more complex its structure, the more susceptible it is to high temperature (Yang et al., 2004).

3.3. Comparison between conventional pasteurisation and PEF

The inactivation of POD and PPO by PEF was compared to a conventional thermal pasteurisation, which is one of the common treatments used by the juice industry (72 °C for 26 s). The residual activity of POD was 52% and that of PPO 54% which was considerably higher than that achieved by PEF under less severe thermal conditions (32.3% for POD, 28.9% for PPO).

4. Conclusions

PEF in conjunction with moderate preheating was applied to fresh apple juice in order to evaluate its impact on POD and PPO inactivation. The combined treatment was found to cause greater inactivation, than that obtained by heat treatment alone at a comparable temperature. Additionally, the degree of inactivation of POD and PPO was significantly higher than that obtained from a mild conventional pasteurisation. The residual activity of POD and PPO decreased with an increase in electric field strength and treatment time. PPO appeared to be more susceptible than POD to a combination of temperature increase and PEF treatment. The proposed processing strategy may lead to higher quality apple juice, as the latter is exposed to milder temperatures and for shorter times, than by conventional heat processing methods.

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