

Effect of pulsed electric field treatment on polyphenol oxidase, total phenolic compounds, and microbial growth of apple juice

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Abstract: A pulsed electric field (PEF) process was applied to unclarified apple juices at 30 and 40 kV cm⁻¹ for 50, 100, 150, and 200 pulses after preheating to 40 °C. Afterwards, polyphenol oxidase (PPO) enzyme levels, loss of total phenolic (TP) compounds, and alterations of the physiochemical parameters of the apple juice were determined. Total yeast/mold and mesophilic bacteria loads were periodically evaluated during storage at 25 °C for a 3-month period and the cloud quality of the apple juice was analyzed after storage. The results of the present study have indicated that total yeast/mold and mesophilic bacterial activity could be completely inactivated under all PEF processing conditions. Moreover, no microbial activity occurred in PEF-treated apple juices during the 3-month storage period, whereas the control had more than a 6 log cfu mL⁻¹ microbial load at the end of storage. PPO activity became completely inactive at 100 or more pulses at 40 kV cm⁻¹, while residual activity decreased by up to 32.1%, 7.0%, 2.9%, and 1.6% at 30 kV cm⁻¹ at 50, 100, 150, and 200 pulses, respectively. Kinetic data for PPO activity and TP content were described using a first-order kinetics model with a high R² value, ranging from 0.906 to 0.983. However, there was a negative correlation ranging from -0.907 to -0.938 between TP compounds and PPO inactivation. In addition, only minor changes were observed in physical appearance and color, pH, conductivity, and Brix levels of apple juices after the PEF processing.

Key words: Apple juice, microbial growth, phenolic, polyphenol oxidase, pulsed electric field

1. Introduction

Polyphenol oxidase (PPO) activity and yeast/mold growth are 2 of the most important quality parameters of apple juices. Thermal treatment is the traditional method used for production of apple juice. However, there are some disadvantages in controlling enzymes and microorganisms by thermal treatment. Studies have shown that thermal treatment may adversely affect the color, flavor, and nutrients in juices. Therefore, researchers have recently focused on nonthermal processing techniques (Barbosa-Canovas et al. 1999; Cemeroglu and Karadeniz 2009; Başlar and Ertugay 2013).

Nonthermal food processing techniques can resolve some heat-borne problems. These techniques have gained importance in recent years because of their usefulness in preserving the quality and safety of food systems. The main nonthermal processes in current use are ultrasound, pulsed electrical field (PEF), high hydrostatic pressure, and supercritical carbon dioxide systems. These all have the potential to inactivate microorganisms and enzymes in foods (Kuldiloke and Eshtiaghi 2008).

PEF involves the application of a high-voltage electric field for a very short time. While PEF is effective in inactivating enzymes and microorganisms, it has the added advantage of avoiding damage to the sensory and nutritional properties of foods. There have been several studies related to the effects of PEF on the enzyme inactivation processes. In one study, Giner et al. (2001) employed PEF treatment to inactivate PPO enzymes extracted from apples; they observed that the residual activity of apple PPO decreased by up to 3.15% at 24.6 kV cm⁻¹ for 6 ms of treatment time, whereas the maximum inactivation obtained for pear PPO was up to 38.0% at 22.3 kV cm⁻¹ for the same treatment time. Riener et al. (2008) observed the highest level of decrease in enzymatic activity (71%) for PPO in apple juice obtained by using a combination of preheating to 50 °C and a PEF treatment time of 100 µs at 40 kV cm⁻¹. Similarly, Sanchez-Vega et al. (2009) obtained a 70% reduction of residual PPO activity in apple juice using PEF treatment at 38.5 kV cm⁻¹ and 300 pulses per second after preheating to 50 °C. Schilling et al. (2008) achieved a maximum PPO deactivation of

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48% when apple juices were preheated to 40 °C and PEF-treated at 30 kV cm⁻¹. Mild preheating to 40 °C prior to the PEF treatment could potentially increase the antimicrobial effect of the PEF (Heinz et al. 2003) while avoiding detrimental effects on product quality (Walkling-Ribeiro et al. 2008). The effect of PEF on microbial inactivation in apple juice has been investigated by several researchers (Evrendilek et al. 2000; Charles-Rodriguez et al. 2007; Torkamani 2011).

The variable *k* represents enzyme stability. The higher the *k* value, the more sensitive the enzyme is. Giner et al. (2001) modeled the inactivation of PPO activity in apple and pear extracts using PEF as an exponential decay model with a high determination coefficient (*R*²) of between 0.90 and 0.98 and reported that the higher the electric field intensities were, the higher the *k* value was. Similarly, Riener et al. (2008) reported that higher electric field intensities or treatment temperatures resulted in higher *k* values.

The PEF technology, as a nonthermal process, has good potential for use in the production of liquid foods. PEF is a suitable technology in particular for fruit juices and has been widely studied. Some nonthermal processing techniques such as ultrasound (Başlar and Ertugay 2013), high-pressure treatment (Baron et al. 2005), supercritical carbon dioxide (Gui et al. 2007), and high-pressure carbon dioxide treatment (Xu et al. 2011) were applied to cloudy apple juice; however, the number of studies in the literature regarding PEF-treated cloudy apple juice is limited. Thus, our study makes a meaningful contribution to the literature via determining the effect of PEF treatment on PPO inactivation, total phenolic (TP) compounds, microbial growth, and the physiochemical parameters of unclarified apple juice, which are all important parameters in the production of cloudy apple juice.

2. Materials and methods

2.1. Materials

Apples (Golden Delicious) were collected from Dissmore's in Pullman, WA, USA and stored at 4 °C until the PEF processing was done. The juices of the washed apples were extracted using a home juice extractor (GE-169201, USA). The apple juices were then filtered using cheesecloth and PEF treatment was immediately applied to the unclarified apple juice samples.

2.2. PEF treatment

A PowerMod 25 kW PEF treatment system from Diversified Technologies Inc. (Bedford, MA, USA) that had been installed at Washington State University's pilot plant was used in this study. The PEF treatment system has 2 treatment chambers with 2 electrodes. The electrode diameter was 0.5 cm and the gap distance was 0.65 cm (Bermúdez-Aguirre et al. 2011). A schematic diagram of

the PEF system is shown in Figure 1. The experiment was carried out using 2 different electric field intensities (30 and 40 kV cm⁻¹) and 4 different pulses (50, 100, 150, and 200) at a constant initial temperature (40 °C), pulse width (2.0 μs), frequency (0.2 kHz), and flow rate (500 mL min⁻¹). These conditions were designed based on the results obtained in preliminary experiments. In some preliminary experiments, the equipment could not be run above 40 kV cm⁻¹ because cloudy components in the unclarified apple juice caused an arcing problem and the equipment stopped numerous times. According to the preliminary studies that we have done, a 40 kV cm⁻¹ PEF treatment resulted in an increase of 15 °C in the apple juices; therefore, the samples were warmed up to 40 °C in order to avoid any negative effects of higher temperatures on taste, flavor, and nutritional value of the apple juices. Two thermocouples (k-type, Omega Engineering Inc., Stamford, CT, USA) were used to measure the temperature before entering the input chamber and immediately after exiting the output chamber. After PEF treatment, the apple juices were cooled down to 20 °C using a cooling coil. Untreated samples of the same apple juice served as control samples.

2.3. PPO activity

A PPO activity test was performed according to a modified method of Özoğlu and Bayındırlı (2002). According to the test procedure, 0.5 mL of apple juice was mixed with 0.5 mL of catechol solution (0.2 M) and 1.5 mL of McIlvane buffer solution (pH 6.5). Immediately afterwards, absorbance values were recorded at 420 nm every 10 s. The PPO activity curve plotted *A*₄₂₀ against time and then the enzyme activity was calculated on the basis of the slope of the linear portion of the curve. One unit of PPO enzyme activity (*E*) was defined as the amount of enzyme that caused an increase in 0.001 Δ*A*₄₂₀ min⁻¹ under assay conditions, and residual PPO enzyme activity was calculated as follows:

$$\% \text{ Residual PPO Enzyme Activity} = \frac{A}{A_0} \times 100 \quad (1)$$

where *A* is the PPO activity after PEF treatment and *A*₀ is the PPO activity before PEF treatment.

2.4. Total phenolic content

Colorimetric determination of TP content in apple juice was based on the procedure of Singleton and Rossi (1965). Apple juice (1 mL) was mixed with 75 mL of distilled water and 5 mL of Folin-Ciocalteu reagent. After 3 min, 5 mL of saturated sodium carbonate (75 g L⁻¹) was added to the mixture and then filled to 100 mL with water. The absorbance of the solution was measured at 720 nm after 60 min. Results were expressed as milligrams of gallic acid equivalent in milliliters (Spanos and Wrolstad 1990; Özkan et al. 2010).

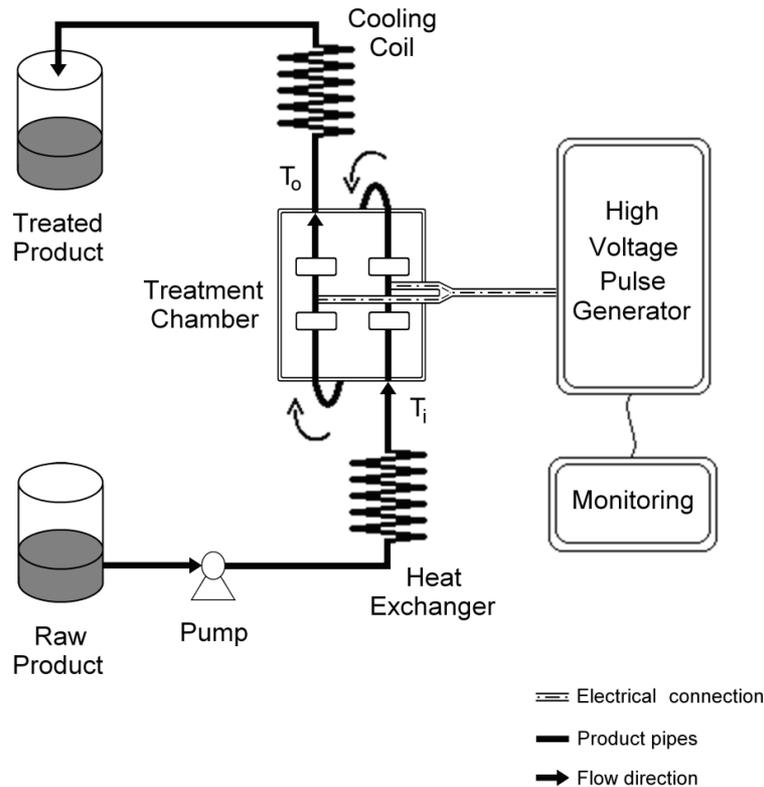


Figure 1. Schematic diagram of the pulsed electrical field system. T_i = temperature before entering the input chamber, T_o = temperature after exiting the output chamber.

2.5. Microbiological enumerations

After the PEF treatment, the apple juices were put in test tubes and stored in an incubator set at 25 °C for 3 months. Each sample was serially diluted with 0.1% sterile peptone water, and 0.1 mL of each diluted (or nondiluted) sample was distributed into identical plates of appropriate agar using the spread plate method. A plate count agar (Merck) was used for detecting the viable cells of total aerobic mesophilic bacteria (TAMB). The plates were incubated at 32 °C for 48 h. Potato dextrose agar (Merck) was acidified to a pH of 3.5 ± 0.01 with 10% lactic acid and used to detect viable cell counts of yeast and molds. The plates were incubated at 25 °C for 5 days. The detection limit of yeast and mold was $1 \log_{10}$ cfu mL⁻¹.

2.6. Cloud value and cloud stability

Apple juice samples were centrifuged at $760 \times g$ for 10 min in order to determine the cloud values. Cloud value was measured as supernatant absorbance at 660 nm using a spectrophotometer with distilled water serving as a blank (Versteeg et al. 1980; Tiwari et al. 2008). In order to measure cloud stability, the samples were centrifuged at $4200 \times g$ for 15 min, the supernatant was taken, and

the absorbance at 625 nm was measured. Cloud stability (CS) was calculated according to the following equation (Stahle-Hamatschek 1989; Genovese et al. 1997):

$$CS = \frac{C}{C_0} \times 100 \quad (2)$$

where C is the absorbance after centrifuging and C_0 is the absorbance before centrifuging.

2.7. pH, conductivity, Brix, and color measurement

The pH and electrical conductivity of samples were determined through direct immersion of an electrode using a pH/conductivity Orion R 4-Star Meter (Beverly, MA, USA). The total soluble solids (Brix) of the apple juices were measured using an Abbe refractometer. L , a , and b values of the apple juice samples were analyzed using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in reflection mode (Bermúdez-Aguirre et al. 2011). Each sample was analyzed in triplicate for all analyses.

2.8. Mathematical modeling

The kinetics of apple juice PPO activity and TP content were described by fitting a first-order kinetic model, Eq. (3), to the experimental data:

$$A = A_0 \text{Exp}(-k P) \quad (3)$$

where A is PPO activity and TP content of the samples, A_0 is the PPO activity and TP content of the samples before PEF processing, P is the number of pulses, and $-k$ is the first-order rate constant.

Eq. (4) was also applied to predict PPO activity and TP content as a function of electric field intensity (E) and number of pulses (P) at fixed conditions following Giner et al. (2001):

$$A = A_0 (-K \text{Exp}(-\omega E) P), \quad (4)$$

where $-K$ and $-\omega$ (cm kV^{-1}) are constants for the number of pulses and electric field intensity.

Both models were evaluated using statistical parameters such as root mean square error (RMSE), modeling efficiency (EF), and chi-square (χ^2) in addition to the determination coefficient (R^2), following Toğrul and Arslan (2004).

2.9. Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Data represent the means of experiments performed in duplicate and replicated at least twice. Significance was defined at $P < 0.05$.

3. Results

3.1. Polyphenol oxidase and total phenolic content

PPO activity of control apple juice was determined as $227 \Delta A_{420} \text{ min}^{-1}$. PPO was not completely inactivated at 30 kV cm^{-1} ; however, activity decreased up to 32.1%, 7.0%, 2.9%, and 1.6% at 50, 100, 150, and 200 pulses, respectively. Figure 2 shows the residual inactivation of the PPO

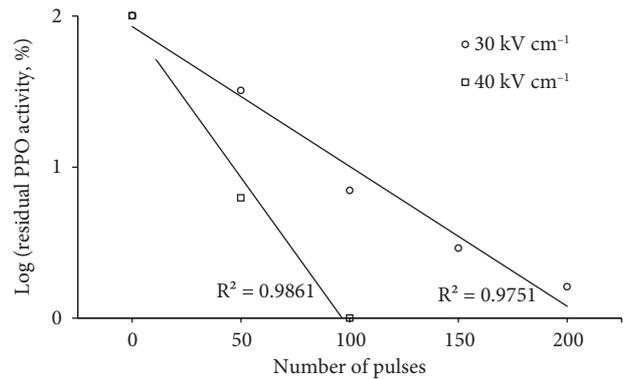


Figure 2. PPO residual activity during PEF treatment.

enzymes on a logarithmic scale. However, residual activity decreased up to 6.2% for 50 pulses at 40 kV cm^{-1} and was completely inactivated after 100 pulses. Thus, the rate of inactivation obtained at 30 kV cm^{-1} for 100 pulses was reached at 50 pulses with 40 kV cm^{-1} . The research results indicated that the PPO activity of apple juices significantly decreased as the electrical field intensity and the number of pulses increased ($P < 0.05$). The inactivation kinetics of PPO were adjusted according to the first-order kinetic model of Eq. (3) as a function of the number of pulses. The model fitted the experimental data at 30 and 40 kV cm^{-1} with high determination coefficients ($R^2 = 0.968$ and 0.983 , respectively). Table 1 shows R^2 , RMSE, EF, and χ^2 used as statistical measures for comparison of the experimental and model-simulated values. The statistical test parameters indicated that the assayed model explains the relationship between PPO activity and number of pulses well enough.

The TP content of the untreated apple juice was 263 mg L^{-1} . Figure 3 shows the degradation of the TP component on a logarithmic scale. The maximum loss of TP content of the apple juice was measured as 26% at 30 kV cm^{-1} and 57% at 40 kV cm^{-1} for 200 pulses. Degradation of

Table 1. Kinetic constant (k) of the first-order model, determination coefficient, and statistical test parameters for PPO activity and TP content.

Parameters	E (kV cm^{-1})	A = $A_0 \text{Exp}(-kP)$		Statistical test parameters		
		k	R^2	RMSE	χ^2	EF
PPO activity	30	0.024 ± 0.001	0.968	5.096	28.332	0.968
	40	0.056 ± 0.001	0.983	0.800	0.709	0.982
TP content	30	0.001 ± 0.000	0.906	6.431	45.12	0.906
	40	0.004 ± 0.000	0.981	5.61	35.38	0.981

E = electric field intensity, P = number of pulses, RMSE = root mean square error, χ^2 = chi-square, EF = modeling efficiency.

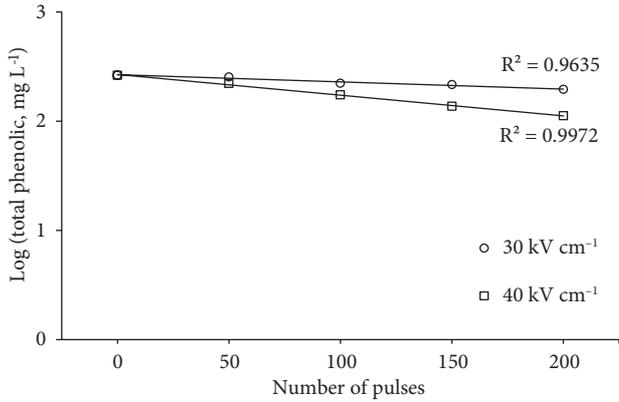


Figure 3. Change of total phenolic content during PEF treatment.

the TP content was also modeled using the first-order kinetics models of Eqs. (3) and (4). A first-order kinetics model fitted the experimental data with high R^2 values of 0.968–0.983 for electric field intensities of 30 and 40 kV cm⁻¹, respectively. The rate constants (k) were found to be 0.024 and 0.056 for the inactivation of PPO activity and

0.001 and 0.004 for the degradation of TP content at the electric field intensities of 30 and 40 kV cm⁻¹, respectively (Table 1). K and ω values for PPO activity and TP content as a function of electric field intensity (E) and number of pulses (P) at a fixed condition are shown in Table 2. The parameters show the combined effects of electric field intensity and number of pulses on PPO inactivation and TP content. K is a constant of number of pulses, while ω is a constant of electric field intensity.

3.2. Microbial growth

The microbial growth of mesophilic bacteria and yeast/mold in apple juice stored for 13 weeks is shown in Table 3. The numbers of TAMB and yeast/mold were 5.03 and 5.05 log cfu mL⁻¹ in the control apple juices, respectively; however, the TAMB and yeast/mold numbers were below detectable levels (<10 cfu mL⁻¹) in the PEF-treated samples during the first week of storage. The microorganisms in the apple juice samples were inactivated (<1 log cfu mL⁻¹) under selected PEF conditions and did not proliferate during a 3-month storage period. However, microorganisms in untreated apple juice multiplied to 6.87 log and 6.46 log for mesophilic bacteria and yeast/mold, respectively.

Table 2. Kinetic constants (K and ω) of PPO activity and TP content as a function of electric field intensity and number of pulses according to first-order kinetics models.

	$A = A_0 \text{ Exp} [-K \text{ Exp} (-\omega E) P]$			Statistical test parameters		
	K	Ω (cm kV ⁻¹)	R^2	RMSE	EF	χ^2
PPO activity	0.002 ± 0.000	-0.086 ± 0.006	0.975	1.76	0.970	4.149
TP content	5.63E-5 ± 0.000	-0.108 ± 0.005	0.981	2.310	0.981	7.11

E = electric field intensity, P = number of pulses, RMSE = root mean square error, χ^2 = chi-square, EF = modeling efficiency.

Table 3. Microbial growth (log mL⁻¹) of PEF-treated apple juice during storage.

	Storage (weeks)	30 kV cm ⁻¹				40 kV cm ⁻¹				Control
		50	100	150	200	50	100	150	200	
Mold and yeast	1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.05 ± 0.07
	4	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.91 ± 0.13
	9	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.90 ± 0.07
	13	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	6.46 ± 0.13
Total mesophilic bacteria	1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.03 ± 0.01
	4	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.10 ± 0.02
	9	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	5.81 ± 0.01
	13	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	6.87 ± 0.12

3.3. Physicochemical properties

The L , a , and b values of the control apple juice were found to be 26.02, 1.00, and 11.99, respectively. The L value decreased significantly in the apple juice processed at 30 kV cm⁻¹, but the value increased significantly after PEF processing at 40 kV cm⁻¹. Regarding the a and b values of apple juice given in Table 4, it was found that the parameters gradually increased at 30 kV cm⁻¹ and they decreased at 40 kV cm⁻¹. However, when we consider the enzymatic browning that might occur during processing, this difference does not result in a noticeable change.

PEF-treated apple juice showed a slight variability in pH and Brix for different electric field strengths and pulses. PEF treatment at 30 kV cm⁻¹ slightly changed the conductivity of apple juices, while PEF treatment at 40 kV cm⁻¹ caused an increase.

Even though we did not obtain a noticeable effect on the cloudy quality of PEF-processed apple juice, the cloud stability of the PEF-treated apple juice samples increased

up to 50% compared to the control (Figure 4). After 3 months of storage, the 40 kV cm⁻¹ PEF-treated apple juices had visible levels of sediments. In fact, even the PEF processing at 30 kV cm⁻¹ for 200 pulses draws attention to the lack of sediment. These alterations could be very important in terms of the cloud quality of apple juices during production.

4. Discussion

This study indicates that PPO activity could be completely inactivated by the selected conditions of PEF. However, Giner et al. (2001) reported that the maximum residual activity of apple PPO decreased up to 3.15% at 24.6 kV cm⁻¹ for 6 ms of treatment time. Researchers also reported that tomato pectin methyl esterase (PME) activity was not completely inactivated by PEF treatment at 24 kV cm⁻¹ for 400 pulses, which is the maximum condition, and PME and PPO activity decreased as the electrical field intensity and the number of pulses increased. Riener et al. (2008)

Table 4. Physicochemical parameters of apple juice treated under selected PEF processing conditions.

PEF (kV cm ⁻¹)	Number of pulses	pH	Conductivity (μs cm ⁻¹)	Brix (°B)	Color parameters			T _{input} (°C)	T _{output} (°C)	ΔT (°C)
					L	a	b			
30	50	3.56 ^b	1864 ^b	12.6 ^a	25.76 ^c	1.08 ^d	13.39 ^e	40.0	43.4	3.4
		(±0.02)	(±15)	(±0)	(±0.05)	(±0.02)	(±0.25)	(±0.9)	(±0.6)	(±0.4)
	100	3.65 ^c	1920 ^c	12.6 ^a	24.50 ^b	2.57 ^f	12.65 ^d	40.3	43.8	3.5
		(±0.03)	(±20)	(±0)	(±0.18)	(±0.07)	(±0.07)	(±0.7)	(±0.6)	(±0.3)
	150	3.59 ^b	1866 ^b	12.6 ^a	24.07 ^a	2.48 ^f	13.40 ^e	40.5	44.3	3.8
		(±0.03)	(±11)	(±0)	(±0.09)	(±0.04)	(±0.33)	(±0.6)	(±0.9)	(±0.5)
	200	3.52 ^a	1970 ^d	12.7 ^b	23.84 ^a	2.17 ^e	15.18 ^f	40.6	44.5	3.9
		(±0.02)	(±10)	(±0)	(±0.13)	(±0.21)	(±0.32)	(±0.6)	(±0.8)	(±0.5)
40	50	3.69 ^c	2162 ^e	12.6 ^a	28.35 ^d	0.64 ^c	12.80 ^d	40.4	54.2	13.8
		(±0.02)	(±23)	(±0)	(±0.14)	(±0.07)	(±0.27)	(±1.4)	(±1.0)	(±0.5)
	100	3.57 ^b	2181 ^e	12.6 ^a	28.11 ^d	0.05 ^b	11.22 ^b	40.6	54.6	14.0
		(±0.02)	(±20)	(±0)	(±0.05)	(±0.06)	(±0.47)	(±1.9)	(±1.7)	(±0.9)
	150	3.54 ^{a,b}	2174 ^e	12.7 ^b	28.26 ^d	-0.15 ^a	9.43 ^a	40.9	54.9	14.0
		(±0.03)	(±9)	(±0)	(±0.37)	(±0.13)	(±0.51)	(±1.7)	(±1.5)	(±0.8)
	200	3.57 ^b	2172 ^e	12.7 ^b	28.33 ^d	-0.07 ^{a,b}	9.61 ^a	40.9	54.9	14.0
		(±0.02)	(±9)	(±0)	(±0.37)	(±0.08)	(±0.19)	(±1.5)	(±1.4)	(±0.7)
Control (±0.03)		3.58 ^b	1834 ^a	12.6 ^a	26.02 ^c	1.00 ^d	11.99 ^c	NA	NA	NA
		(±5)	(±0)	(±0.03)	(±0.07)	(±0.20)				

NA = not available. Values with different superscript lowercase letters are significantly different as per the Duncan test ($P < 0.05$).

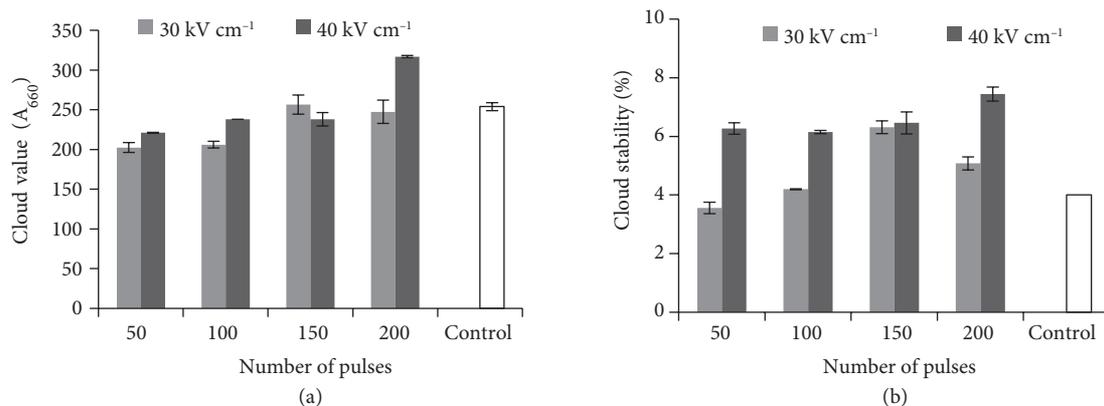


Figure 4. The effect of PEF treatment on cloud value (a) and cloud stability (b) of apple juice.

subjected apple juices to temperatures of 2, 35, and 50 °C; electrical field intensities of 20, 30, and 40 kV cm⁻¹; and treatment times of 25, 50, and 100 μ s. The authors reported that an increasing inactivation rate of PPO was obtained by using a combination of preheating to 50 °C and a PEF treatment time of 100 μ s at 40 kV cm⁻¹. Similarly, our results indicated that PPO enzyme inactivation increased with electrical field intensity and pulses. However, a combination of preheating to 40 °C and a pulse count of 100 at 40 kV cm⁻¹ resulted in a complete inactivation of PPO. The inactivation rate of PPO (approximately 70%) obtained at 30 kV cm⁻¹ for 50 pulses (Riener et al. 2008) and 38.5 kV cm⁻¹ at 300 pulses per second (Sanchez-Vega et al. 2009) was reached at 30 kV cm⁻¹ for 50 pulses in the present study.

Based on the R^2 and statistical test parameters of the kinetic models, the PPO activity and TP content as a function of electric field intensity (E) and number of pulses (P) fit the model with good agreement. Giner et al. (1999, 2000, 2001) and Riener et al. (2008) reported that this model can be appropriate for this kind of prediction, and the kinetic behavior of apple PPO during PEF treatment had a similar behavior. As in previous studies on clear apple juice, the PPO and TP content in unclarified apple juice that has cloudy particles decreased logarithmically and a first-order kinetic model fit the data well.

The mechanism of enzyme inactivation by PEF treatment was not clearly illuminated. However, it is assumed that the main reason for the inactivation mechanism may be the changing of the natural structure of the enzyme. Enzyme molecules are not hydrolyzed; rather, the loss of enzyme activity is a result of protein denaturation (Yeom and Zhang 2001; Picart and Cheftef 2003). We also think that the major cause of the PPO activity loss might be protein denaturation by the PEF treatment.

Interestingly, microorganisms did not grow in the PEF-treated apple juice during the 3-month storage period. Min et al. (2003) processed orange juice by PEF at 40 kV cm⁻¹ for 97 ms and reported that TAMB and yeast/mold were initially completely inactivated (<1 log cfu mL⁻¹), but bacteria, yeast, and mold started growing slightly after 133 days of storage of the orange juices. In another study, PEF treatment at 37 kV cm⁻¹ for 166 μ s caused a 2 log reduction of total mesophilic bacteria and a 1.5 log reduction of yeast and mold in clear apple juice (Evrendilek et al. 2000). In our study, however, the microorganisms were completely inactivated by PEF and we did not observe any growth of microbes in the PEF-treated samples until 13 weeks of storage. Based on these results, PEF treatment has the potential for promoting microbial safety for cloudy apple juice during 3-month storage periods.

PEF treatment is usually not adequate to inhibit enzyme activity (Sanchez-Vega et al. 2009; Riener et al. 2008) and microbial growth in clear apple juice. In a similar study, Barbosa-Canovas et al. (1999) suggested that microorganisms were inactivated because of the electroporation and electrofusion during the PEF treatment. On the other hand, it is more efficient in orange juice, which contains turbidity elements (Min et al. 2003). In the present study, PPO enzymes along with mesophilic bacteria, mold, and yeast were effectively inactivated. We believe the reason for this may have something to do with elements of turbidity in apple juices.

When PPO, yeast/mold, and mesophilic bacteria were completely inactivated by PEF conditions of 40 kV cm⁻¹ for 100 pulses, the loss of TP content was 34%. Aguilar-Rosas et al. (2007) reported that heat treatment performed on apple juices at 90 °C for 30 s caused a 32.2% loss of phenols, while PEF treatment caused only a 14.49% reduction. However, a negative correlation ranging from -0.907 to -0.938 between TP compounds and PPO inactivation was calculated.

In work done by Charles-Rodriguez et al. (2007), it was reported that PEF had no effect on the value of L of apple juices and it increased the value of the a and b parameters. Bermúdez-Aguirre et al. (2011) showed that color changes of PEF-processed milk are associated with the wearing down of the electrodes, which are made of stainless steel with platinum inserts, during arching. They think that the changes could also be due to the disintegration of enzymatic browning compounds as a result of arching during the PEF processing. Falguera et al. (2011) also reported that some pigment impairment could be a cause of color change. Thus, color changes in our study could have been due to arching or impairment of some pigments.

Evrendilek et al. (2000) applied the PEF process to apple juice; they did not report any changes in pH and Brix values but the conductivity increased, just as in our study. In several studies, it has been reported that PEF does not have a significant impact on pH (Charles-Rodriguez et al. 2007) and Brix (Min et al. 2003; Charles-Rodriguez et al. 2007). This observation on unclarified apple juice in our study was similar to earlier reports on clear apple juice. Sanchez-Vega et al. (2009), who applied the PEF treatments at ranges between 33 and 42 kV cm⁻¹ with frequencies of 150, 200, 250, and 300 pulses per second, reported that there was no variability in pH at different electric field strengths and frequencies, which is similar to the findings of our study. The PEF process increased the temperature of the apple juice as shown in Table 4; however, the temperature was reduced immediately after the processes by means of a heat exchanger. This increase was small at 30 kV cm⁻¹ while it was greater at 40 kV cm⁻¹.

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- Control of treatment temperature is necessary to avoid the negative effects of heat during PEF treatment of apple juice. The present study showed the level of temperature rising during PEF treatment.
- Rivasa et al. (2006) reported that both PEF treatment (25 kV cm⁻¹ for 280 ms at a maximum temperature of 68 °C) and heat processes (98 °C, 21 s) on mixed orange and carrot juice led to a significant increase in the level of turbidity (absorbance at 660 nm). In addition, it has been stressed that the properties of cloud quality can be modified by the PEF process. In contrast, some of the processing conditions of our study had a negative influence on the cloud quality parameters of apple juice. This could have been due to the variation in the electrical load of the particles in the juice during the PEF treatment.
- In conclusion, this study has shown that PEF is very effective for inactivation of mesophilic bacteria, yeast/mold, and PPO enzymes. PEF technology can be more effective in the processing of cloudy apple juice than of clear apple juice. However, the effect of PEF process on the cloudiness should not be forgotten, since results indicated that some of the PEF conditions could decrease the cloud quality of apple juices.

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