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ORIGINAL PAPER

Conformation changes of polyphenol oxidase and lipoxygenase induced by PEF treatment

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Abstract Inactivation of polyphenol oxidase (PPO) and lipoxygenase (LOX) by pulsed electric fields (PEFs) has been investigated using a coaxial treatment chamber. Circular dichroism (CD) and fluorescence analysis have been used to study conformation changes in the protein. The experimental results show that PPO and LOX can be effectively deactivated by the PEF treatment and that the effect on PPO and LOX increases with the increase of the applied electric field and the number of pulses. The activity of PPO and LOX can be reduced by 69 and 88% when fields of 24 kV/cm were applied for 320 and 962 μs, respectively. The CD analysis showed that the PEF treatment caused a loss of α-helix and increase of β-sheet content, indicating that conformation changes occur in the secondary structure of the PPO and LOX enzymes. The fluorescence intensity of LOX increases after the PEF treatment while, at the same time, increases in the applied electric field increases the intensity of the fluorescence emitted. These results prove the occurrence of local tertiary structure changes in the LOX protein.

Keywords Pulsed electric field · Polyphenol oxidase · Lipoxygenase · CD analysis · Fluorescence spectroscopy analysis

1 Introduction

Nonthermal preservation of liquid food by pulsed electric fields (PEFs) has been extensively studied in recent years. The PEF treatment applies a high-intensity electric field generated between two electrodes in the liquid treated, which allows the passage of a large flux of electrical current through foods. Nonthermal treatment can be typically attained by use of a very short pulse of the order of microseconds or milliseconds. Contrary to conventional thermal processing, PEF treatment increases the shelf life of juice products while reducing the loss of their flavor, color, and nutrients [1, 2]. The potential of this technology has therefore drawn considerable attention from researchers and industry.

The inactivation effect of PEF on micro-organisms has been extensively studied over the past years [e.g., 1–5]. By contrast, there is relatively little information available concerning the effect of PEF on enzyme activity [6–11], most of it emphasizing the effect of the process parameters, product parameters, and microbial characteristics on the inactivation of selected enzymes. Far less information is available on the mechanisms and conformation changes of enzymes during the PEF treatment.

The browning of fruit and vegetables due to mechanical injury during post-harvest storage or processing is a well-known phenomenon. This browning is usually due to the catalytic action of the enzyme polyphenol oxidase (PPO), IUBMB code EC 1.14.18.1. The *o*-diphenols, which are present in the tissue of fruit and vegetables, are oxidized by PPO in the presence of oxygen. This oxidation reaction results in the production of *o*-quinones, which subsequently polymerize with other *o*-quinones, phenolic substances, proteins, amino acids, and so forth to produce brown pigments [12–14]. These pigments are sometimes referred to as melanins. This browning process causes a deterioration of sensory and nutritional quality and affects appearance and organoleptic properties [12–15], and hence, inactivation of PPO is desirable for preservation of foods. Several

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authors have reported that deactivation of PPO by thermal processing was the most effective method to control enzymatic browning [13, 14, 16]. However, this can also induce unacceptable texture and flavor changes [17, 18]. Furthermore, it has been reported that heat deactivation of PPO is unacceptable in anthocyanin-containing juice products, since the high temperatures required for PPO deactivation cause anthocyanin degradation [19].

Lipoxygenases (LOX), IUBMB code EC 1.13.11.12, are widely distributed in nature, having been found in almost all higher plants, fungi, and animals. Plant LOX are members of a class of nonheme iron-containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-l,4-pentadiene system to give an unsaturated fatty acid hydroperoxide [20]. The hydroperoxides generally decompose into acids, ketones, and aldehydes [21]. Also, the hydroperoxides and free radicals produced from the fatty acid by LOX can degrade vitamins and proteins in tomato juice during storage. Because many products of the LOX reaction (or derivatives thereof) are aromatic, the presence of LOX activity in many foodstuffs can affect their properties, particularly during long-term storage, in both desirable and undesirable ways [22, 23]. For example, tomato juice LOX initiates the formation of the fresh flavor compounds of tomato, but can destroy essential fatty acids and develop unpleasant flavors in tomato juice during storage [23]. Hence, the deactivation of LOX is an important processing procedure, especially for shelf-stable fruit juice.

In the investigation, under this study PPO and LOX were chosen study the deactivation effect of PEF treatment and of conformation changes to the protein.

2 Experimental setup

2.1 PEF treatment system

The deactivation of the enzymes was carried out using a laboratory scale pulse generator (Fig. 1), yielding exponentially decaying pulses of 0.5–15 μs duration at a frequency of 1 Hz. The applied voltage in the experiment ranged from 5 to 25 kV and the number of pulses varied from 207 to 1,449.

The treatment chamber (Fig. 2) had a coaxial cylinder electrode system. The total volume of the chamber was 60 mL and the effective volume directly exposed to the PEF was 30 mL. The radial gap between the electrodes was 5 mm. The electric field distribution of the electrode system is shown in Fig. 3 and was obtained using ANSYS 6.0.

A schematic diagram of the experimental system is given in Fig. 4. A peristaltic pump was used to pump the suspension fluid from the beaker to the treatment chamber at a

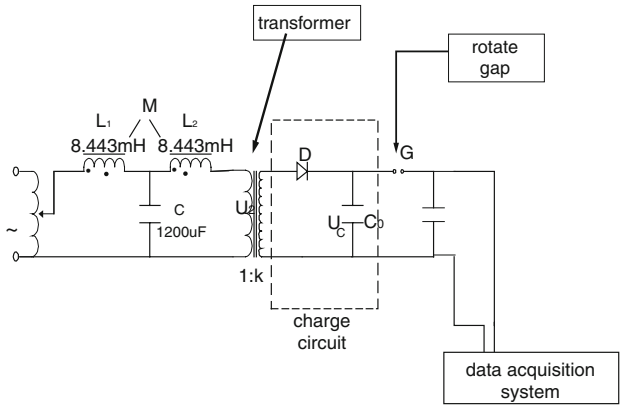

Fig. 1 Circuit of pulse power generator

Fig. 2 Photographs of the coaxial cylinder electrode treatment chamber

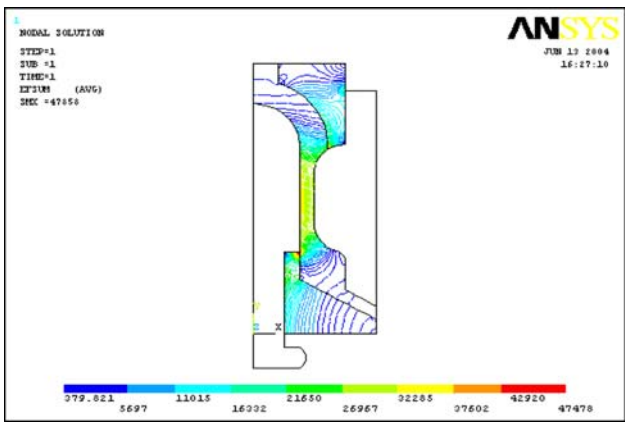

Fig. 3 Electric field distribution of parallel electrodes treatment chamber

velocity of 52.5 mL/min. A cooling coil submerged in an ice-water bath kept the temperature of the solution below 40 °C. The fluid temperature was monitored by thermocouples at the inlet and outlet of the treatment chamber.

The pulse waveform was recorded by a digital oscilloscope (Tektronix TDS 210) with a high-voltage probe (Tektronix P6015A).

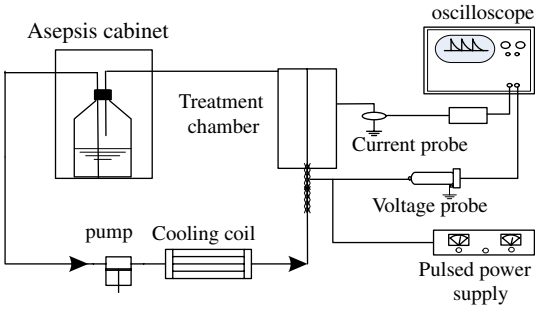

Fig. 4 The diagram of the experimental system

2.2 Materials and methods

The other reagents used in the study were Levodopa (L-DOPA, 3,4-dihydroxy-L-phenylalanine) and Linoleic acid (LA, EG/EC Number 2004709); both were of analytical grade. Both these two reagents and the enzymes used in the study (PPO and LOX) were produced by Sigma-Aldrich Co.Ltd.

2.3 PEF treatment

In order to explore the deactivation effects of pulsed electric fields on PPO and LOX enzymes, pulses having a range of peak electric fields and pulse numbers were used.

The PEF in this article is produced by directly discharging a capacitor though the treatment chamber with the purely resistive load of the solution between the electrodes and no other associated loads. The pulse shape is thus an exponentially decaying pulse with a decay time τ (= RC), corresponding to the time required for a given pulse to decay from its peak voltage to 37% of the peak voltage. For PPO and LOX solutions, the decay time was set at 12.5 and 37 μs, respectively. The peak electric fields were set at 8, 12, 16, 20, and 24 kV/cm. The treatment pulse numbers for each applied electric field were 6, 9, 12, 19 and 26, respectively.

In order to investigate the conformation change of the protein by means of circular dichroism (CD) and fluorescence analysis, the PPO and LOX samples were treated at 8, 12, 16, and 20 kV/cm for 300 μs.

2.4 PPO and LOX activity analysis

The activity of PPO and LOX was determined spectrophotometrically using a spectrophotometer (UV-762, Lingguang, Shanghai).

The PPO activity was assayed with catechol as a substrate. The reaction mixture included 2 mL of 0.1 M catechol and 1 mL of 0.1 M phosphate buffer at pH 6.8 which were incubated at 37 °C. Then 2.0 mL of 0.0133 lM (3.4 U/mL) enzyme solution was added to the reaction mixture to initiate the enzyme reaction. The mixed solution was allowed to stand for 4 min at 37 °C. Absorbance at 420 nm was monitored at room temperature (25 ± 1) °C.

$$\text{Specific activity} = \text{A420 nm (PPO)}/\text{min}/0.1\ \text{mL}$$

Lipoxygenases activity was measured, with linoleic acid as the substrate, by monitoring the increase in absorbance at 234 nm (ΔA234) due to the formation of the conjugated diene ($\varepsilon = 25{,}000/\text{M cm}$) [24]. 1 U corresponds to the amount of enzyme which forms 1 μmol linoleic acid hydroperoxide per minute at pH 9.0 and 25 °C with linoleic acid (Fluka No. 62240) as substrate.

The relative activities of PPO and LOX as percentages were calculated using the following formula:

$$\text{RA} = \Big[(\text{Activity of PPO (LOX) after PEF treatment})/(\text{Activity of PPO (LOX) before PEF treatment})\Big] \times 100\%$$

Three trials were made for each experimental condition and a 5% confidence level was adopted.

2.5 Circular dichroism analysis of PPO and LOX

Circular dichroism is a valuable spectroscopic technique for studying protein conformation in solution because many common conformational motifs containing α-helices, β-pleated sheets, poly-L-proline ‖-like helices and turns, have characteristic far UV (178–250 nm) CD spectra [25], and directly characterize the change of protein secondary conformation [26].

Circular dichroism spectra were recorded with a JASCO J-720 CD spectropolarimeter (Japan Spectroscopic Company, Tokyo), using a quartz cuvette of 1-mm optical path length at room temperature (25 ± 1 °C). CD spectra were scanned at the far UV range (250–200 nm) with four replicates at 50 nm/min and a bandwidth of 1 nm. The CD data were expressed in terms of the mean residue ellipticity, $[\theta]$, in deg cm^2 dmol^{-1}. The PPO and LOX concentrations for CD analysis were 1.560 and 1.852 μmol/L, respectively. The relative content of α-helix (or β-sheet) was calculated using the following expression:

$$\alpha\text{-helix (or } \beta\text{-sheet) relative content} = \frac{\text{content of } \alpha\text{-helix (}\beta\text{-sheet) after treatments}}{\text{content of } \alpha\text{-helix (}\beta\text{-sheet) before treatments}} \times 100\%$$

2.6 Fluorescence spectroscopy analysis

Fluorescence spectra were measured with a spectrofluorometer (HITACHI F-4500, Japan), using a quartz cuvette of 10-mm optical path length at room temperature (25 ± 1 °C). The emission spectra of the enzymes were recorded

after excitation at 285 nm in the region 300–600 nm before and after the PEF treatment. The LOX concentrations for the fluorescence spectroscopy analysis was 0.617 μmol/L. The relative fluorescence intensity was expressed as:

$$\text{Relative fluorescence intensity} = \frac{\text{fluorescence intensity after PEF}}{\text{fluorescence intensity before PEF}} \times 100\%$$

3 Results and discussion

3.1 Deactivation of PPO and LOX

Figure 5 shows that the relative activity of PPO decreases with the increase of the electric field and treatment time. A reduction of some 69% in the activity of PPO can be obtained at 24 kV/cm applied for 320 μs. For short treatment times (<50 μs), the relative activity of PPO differs little whatever be the applied electric field. However, as the treatment time increases, the effect of the electric field becomes much more obvious. Thus, the relative activity of PPO treated at 8 and 24 kV/cm for 75 μs was 94 and 80%, respectively. However, increasing the treatment time to 325 μs would greatly decrease the PPO relative activity, to 68 and 31%, respectively.

The effect of electric field and treatment time on LOX follows the same trends (Fig. 6). However, comparing the data in Figs. 5 and 6, it is clear that LOX tends to be less sensitive to the PEF treatment. Thus, the time required to deactivate 88% of the LOX at 24 kV/cm 962 μs.

Summarizing, increasing the field strength and the treatment time increases the degree of deactivation. The same trend was found by Giner and coworkers for tomato, apple, pear, and peach PPO [9, 27, 28]. The patterns of the influence of electric field and pulse number is similar to that observed in the survival fraction of microorganisms where the higher the electric field or number of pulses, the higher the microbial inactivation [29–31]. Ho [8] reported that the PPO from mushroom, dissolved in 50 mM potassium phosphate buffer (pH 6.5) and subjected to 30 exponential decay pulses with instant charge reversal at 80 kV/cm produced only a moderate 40% reduction in enzyme activity. Giner et al. [27, 28] investigated the effect of HELP treatments on PPO extracted from apples, peaches, and pears. Apple PPO was reduced by 97% after 300 bipolar pulses of 20 μs at 24 kV/cm. Increasing the field strength or the number of pulses, again increased the degree of inactivation. For peach PPO, a 70% reduction in activity was achieved at 24 kV/cm in bipolar mode. For pear PPO, a maximal reduction in activity of 62% was achieved after 300 pulses at 22.3 kV/cm. Min et al. [32] reported that the LOX activity was not changed significantly after PEF treatment at 10 kV/cm at 10, 20, or 30 °C ($p > 0.05$), but decreased significantly at 15, 20, 30, and 35 kV/cm for any level of PEF treatment temperature ($p < 0.05$). A maximum 88% deactivation of tomato juice LOX was observed with the PEF treatment at 30 kV/cm for 60 μs at 50 °C. Van Loey reported that PPO and LOX could not be deactivated by more than 10% by high voltage pulses [33], except that, after a high number of pulses at a low pulse frequency (e.g., after 1,000 pulses at 1 Hz), deactivation of 64 and 21% of LOX and PPO, respectively was noticed, but they attributed these significant deactivations of LOX and PPO to a small off-state current through the IGBT switch used.

3.2 Conformational change of PPO and LOX by PEF

3.2.1 CD spectra of PPO and LOX

Circular dichroism spectroscopy is used to study the secondary and tertiary structure of proteins. The specific

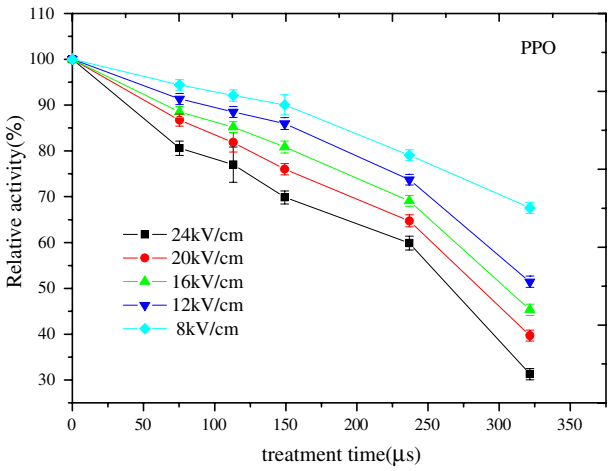

Fig. 5 Effect of electric field and treatment time on PPO activity

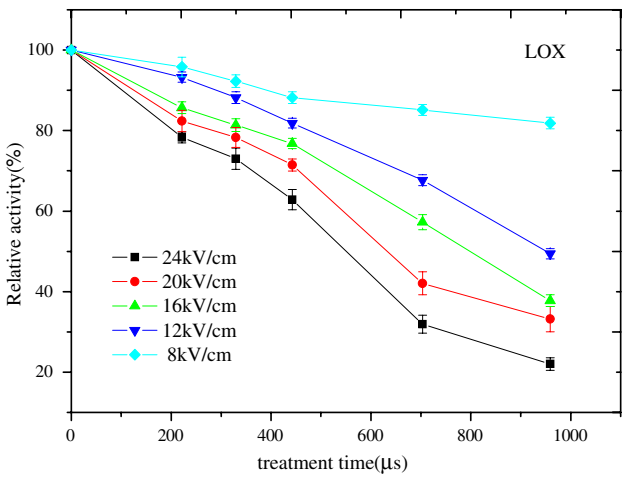

Fig. 6 Effect of electric field and treatment time on LOX activity

conformation of amide bonds in secondary structure elements results in a particular patterns of the far-UV CD spectra [34]. The amount of α-helix and β-sheet can be estimated after fitting the far-UV CD spectra to the corresponding spectra of proteins with known secondary structures [35]. This method is used to study protein folding as it allows the easy detection of changes in the secondary structures of proteins [36]. The characteristic features of the α-helix and β-sheet conformation of proteins are reported to be at 209, 222, and 215 nm, respectively [37, 38].

The CD spectra of PPO in the control and after PEF treatment are shown in Fig. 7. PPO has double negative peaks in the far-UV CD spectra at around 209 and 215 nm. Those two peaks are features characteristic of the α-helix secondary conformation and β-sheet of protein [37], and their intensity reflects the amount of helical structure and β-sheet in protein. CD spectra PPO indicate that it has β-sheets in its secondary structure besides α-helix. CD spectra of PPO decrease after PEF treatment. In addition, the negative peak at 215 nm shifted bathochromially to 218 nm after treatment at 12 kV/cm for 52 μs (see curve b in Fig. 7). However, significant changes occur in the spectra profile at an applied electric field of 20 kV/cm (see curve c in Fig. 7), indicating that great changes had occurred in the local structure of the PPO protein.

Similarly, the CD spectrum of LOX has two negative peaks at around 208 and 218 nm (Fig. 8), indicating that α-helical and β-sheet were simultaneously present in the second conformation of LOX. The effect of the electric field on the LOX profile follows similar trends to that of PPO.

Figure 9 shows the variation of the α-helical and β-sheet content of the PPO and LOX conformation during PEF

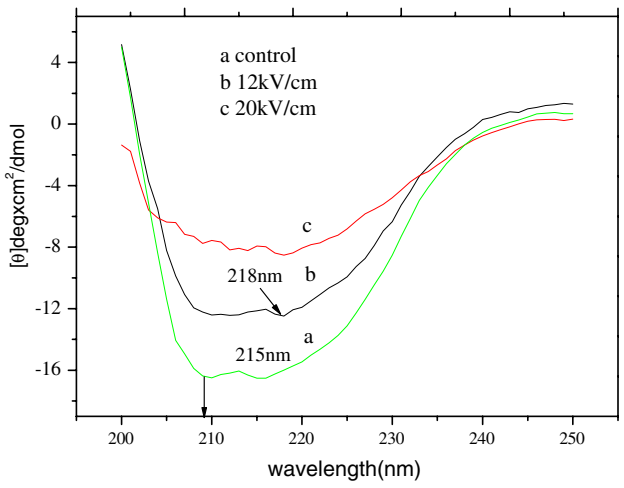

Fig. 7 Far-UV CD spectra of control PPO, PEF-treated PPO. PPO concentration was 1.560 μM. (*a*) control; (*b*) PEF treatment at 20 kV/cm for 52 μs; (*c*) PEF treatment at 12 kV/cm for 52 μs

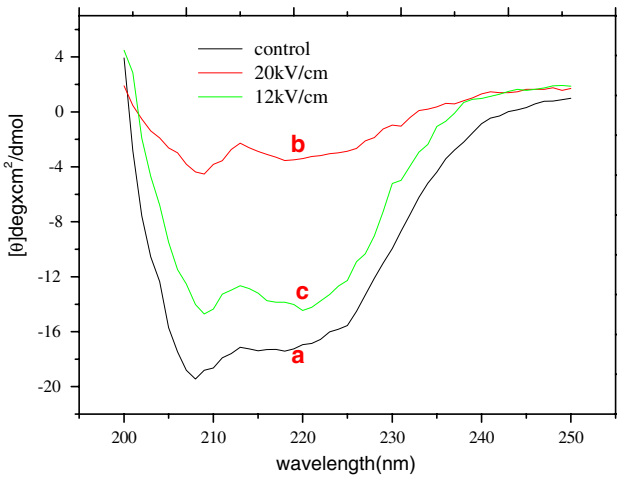

Fig. 8 Far-UV CD spectra of control, PEF-treated LOX. LOX concentration was 1.852 μM. (*a*) control; (*b*) PEF treatment at 20 kV/cm; (*c*) PEF treatment at 12 kV/cm, 173 pulse

treatment. As can be seen from the figure, the α-helical content of the PPO and LOX decreases after the PEF treatment while the β-sheet content increases. This effect was strengthened as the applied electric field increased. For example, the α-helical content of the PPO and LOX was 56 and 29% after being treated at 8 kV/cm, however, when the electric field was increased up to 20 kV/cm, the α-helical content of PPO and LOX decreased to 21 and 16%, respectively. By contrast, the β-sheet content of the PPO and LOX increased from 11 and 43 to 41 and 67%, respectively. In addition, the decrease of α-helix and increase of β-sheet in the PPO are higher than in the LOX, indicating that the second conformation of PPO is less resistant to PEF treatment, which may be because of the higher molecular weight of PPO.

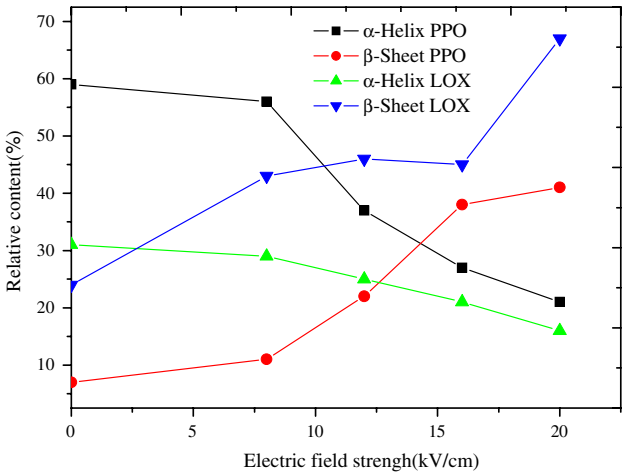

Fig. 9 Variation of α-helical and β-sheet content in the PEF treated PPO and LOX at different electric field strength (52 μs)

In addition, it was noted that after storage for 24 and 48 h at 4 °C, the relative activity of the PPO and LOX did not recover. The relative residual activity of the PPO and the LOX showed a slight change, but no significant difference was exhibited between 0 and 48 h storage at 4 °C ($p > 0.05$). These findings are similar to those in [10]. The enzyme deactivation throughout storage was not significantly ($p < 0.05$) dependent on the storage temperature. Regarding thermal and PEF processing, both technologies yielded low stable enzyme activity during the storage time, indicating that the changes induced in the enzyme structure were irreversible for both treatments.

3.2.2 Fluorescence spectrum analysis of LOX

The intrinsic fluorescence of protein molecules is very sensitive to changes in their tertiary structure. Thus, fluorescence spectroscopy is an effective method for studying the local tertiary structure of proteins.

The fluorescence spectra of proteins gives information about the local environment and the dynamics of the fluorescent amino acid residues Trp, Tyr, and Phe and of the fluorescent cofactors such as flavin, if present. LOX protein has many Phe residues, and so fluorescence spectroscopy was used to inspect possible local tertiary structure changes after the PEF treatment. By contrast, local tertiary structure changes in the PPO protein cannot be investigated through fluorescence spectra because of the lack of aromatic amino acids, which absorb ultraviolet light.

The spectrum of LOX in solution shows fluorescence emission maxima at 337 and 583 nm once excited at 285 nm (Fig. 10). The fluorescence intensity increases quickly as the applied electric strength increases (Fig. 11). The emission peaks at 337 and 583 nm [39, 40] are reported to be due to the emission of tryptophane and phosphorescence, respectively. These results indicate that conformation changes occur in the tertiary structure of LOX protein.

Srinivasulu [40] reported that lipoxygenase-1 (LOX1) from soybean was irreversibly deactivated at 54 °C at pH 9.0, in the presence of two nonionic surfactants, Brij 35 and Tween 20. The fluorescence emission maximum did not change after thermal inactivation, but fluorescence intensity decreased slightly.

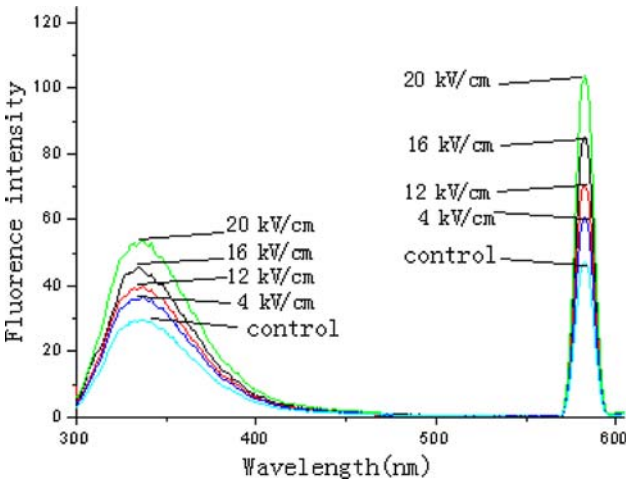

Fig. 10 Intrinsic relative fluorescence intensity (RFI) of LOX at 351 nm before and after PEF treatment, LOX concentration was 0.617 μM

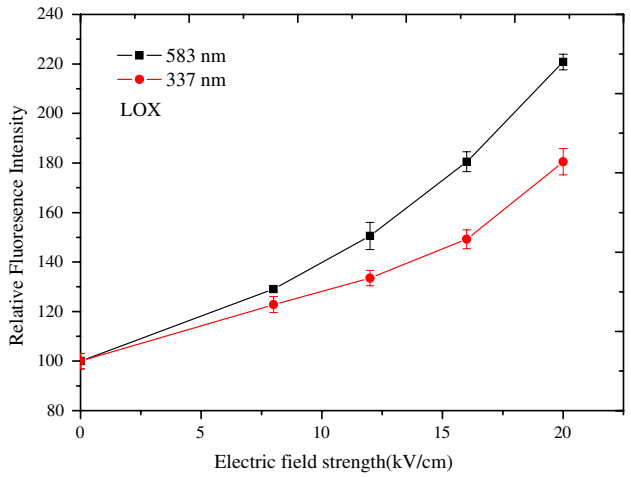

Fig. 11 Intrinsic relative fluorescence intensity (RFI) of LOX at 337 and 583 nm after PEF treatment

4 Conclusion

Deactivation of PPO and LOX has been investigated and the structural changes associated with PEF induced inactivation of LOX were probed by CD and fluorescence. It was found that PPO and LOX can be effectively deactivated during the PEF treatment. A 69% reduction in PPO activity can be attained after being treated at 24 kV/cm for 320 μs. LOX activity decreases 88% after being treated for 962 μs at 24 kV/cm. The decrease of the negative peaks (208 and 215 nm in PPO spectra, 208 and 218 nm in the LOX spectra) in the CD spectra of PPO and LOX proves conformation changes occur in the secondary structure of the enzymes. α-Helical content in PPO and LOX decreases after PEF treatment, while β-sheet content increases. Fluorescence measurements confirmed that tertiary conformation changes occur in the local structural of LOX. However, the possible mechanisms for the conformation changes induced by the PEF treatment is beyond the scope of the present investigation.

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References

1. MacGregor SJ, Farish O, Fouracre R, Rowan NJ, Anderson JG (2000) IEEE Trans Plasma Sci 28:144–149
2. Castro AJ, Barbosa-Canovas GV, Swanson BG (1993) J Food Process Preserv 17:47–73
3. Timoshkin IV, MacGregor SJ, Fouracre RA, Crichton BH, Anderson JG (2006) J Phys D 39:596–603
4. Beveridge JR, MacGregor SJ, Marsili L, Anderson JG, Rowan NJ, Farish O (2002) IEEE Trans Plasma Sci 30:1525–1531
5. Garcia D, Gomez N, Manas P et al (2005) J Appl Microbiol 99:94–104
6. Espachs-Barroso A, Van Loey A, Hendrickx M, Martin-Belloso O (2006) Innov Food Sci Emerg Technol 7:40–48
7. Vega-Mercado H, Powers JR, Barbosa-Canovas GV, Swanson BG, Luedecke L (1995) In: Processings IFT annual meeting, California, USA, p 267
8. Ho SY, Mittal GS, Cross JD (1997) J Food Eng 31:69–84
9. Giner J, Gimeno V, Espachs A, Elez P, Barbosa-Cánovas GV, Martin O (2000) Innov Food Sci Emerg Technol: 57–67
10. Yeom HW, Streaker CB, Zhang QH, Min DB (2000) J Agric Food Chem 48:4597–4605
11. Bendicho S, Estela C, Giner J, Barbosa-Cánovas GV, Martin Belloso O (2002) J Dairy Sci 85:19–27
12. Vámos-Vigyázó L (1981) CRC Crit Rev Food Sci Nutr 15(5): 49–127
13. Mcevily AJ, Iyengar R, Otwell WS (1992) CRC Crit Rev Food Sci Nutr 32:253–273
14. Sapers GM (1993) Food Technol 47(10):75–84
15. Golan-Goldhirsh A, Whitaker JR (1984) J Agric Food Chem 32:1003–1009
16. Jankov SI (1962) Fruchtsaft-Industrie 7:13–32
17. Labuza TP, Lillemo JH, Taoukis PS (1992) Flussiges Obst 59: 15–20
18. Eskin M (1990) In: Eskin M (ed) Biochemistry of foods. Academic Press, California, pp 401–427
19. Siddiq M, Sinha NK, Cash JN (1992) J Food Sci 57:1177–1179
20. James N (1991) Annu Rev Plant Physiol Plant Mol Biol 42:145–148
21. Leoni O, Iori R, Palmieri S (1985) J Food Sci 50:88–92
22. Axelrod B (1974) ACS Adv Chem Ser 136:324–348
23. Eskin NAM, Grossman S, Pinsky A (1977) Crit Rev Food Sci Nutr 9:1–41
24. Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwich A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells B, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) Theor Appl Genet 86:705–712
25. Greenfield NJ (1999) Trends Anal Chem 18:236–244
26. Venyaminov SY, Yang JT (1996) Circular dichroism and the conformational analysis of biomolecules. Plenum Press, New York, pp 69–107
27. Giner J, Gimeno V, Barbosa-Cánovas GV, Martín O (2001) Food Sci Technol Int 7:339–345
28. Giner J, Gimeno V, Ortega M, Mesegué M, Barbosa-Cánovas GV, Martín O (2002) J Food Sci 67:1467–1472
29. Evrendilek G, Zhang QH, Richter E (1999) J Food Protect 7:793–796
30. Wang LM, Shi ZN, Guan ZC, Cheng L, Liao XJ, Zhang K (2005) High Volt Eng 31:64–66
31. Zhang RB, Wang LM, Wu Y, Guan ZC, Jia ZD (2006) IEEE Trans Plasma Sci 34:1370–1374
32. Min S, Min SK, Zhang QH (2003) J Food Sci 68(6):1995–2001
33. Van Loey A, Verachtert B, Hendrickx M (2002) Trends Food Sci Technol 12:94–102
34. Johnson WC Jr (1990) Proteins 7:205–214
35. Greenfield NJ (1996) Anal Biochem 235:1–10
36. Kelly SM, Price NC (1997) Biochim Biophys Acta 1338:161–185
37. Chang CT, Wu CSC, Yang JT (1978) Anal Biochem 91:13–31
38. Myer YP (1968) Biol Chem 243:2115–2122
39. Maccarrone M, Salucci MS, Zadelhoff GV, Malatesta F, Veldink G, Vliegenthart JFG, Finazzi-Agro A (2001) Biochemistry 40:6819–6827
40. Srinivasulu S, Rao AGA (1996) J Agric Food Chem 44(11): 3489–3493