

# Electrochemical Reaction and Oxidation of Lecithin under Pulsed Electric Fields (PEF) Processing

Wei Zhao,\* Ruijin Yang,\* Qi Liang, Wenbin Zhang, Xiao Hua, and Yali Tang

State Key Laboratory of Food Science and Technology and School of Food Science and Technology, Jiangnan University, No. 1800 Lihu Road, Wuxi 214122, China

**ABSTRACT:** Pulsed electric fields (PEF) processing is a promising nonthermal food preservation technology, which is ongoing from laboratory and pilot plant scale levels to the industrial level. Currently, greater attention has been paid to side effects occurring during PEF treatment and the influences on food qualities and food components. The present study investigated the electrochemical reaction and oxidation of lecithin under PEF processing. Results showed that electrochemical reaction of NaCl solutions at different pH values occurred during PEF processing. Active chlorine, reactive oxygen, and free radicals were detected, which were related to the PEF parameters and pH values of the solution. Lecithin extracted from yolk was further selected to investigate the oxidation of food lipids under PEF processing, confirming the occurrence of oxidation of lecithin under PEF treatment. The oxidative agents induced by PEF might be responsible for the oxidation of extracted yolk lecithin. Moreover, this study found that vitamin C as a natural antioxidant could effectively quench free radicals and inhibit the oxidation of lipid in NaCl and lecithin solutions as model systems under PEF processing, representing a way to minimize the impact of PEF treatment on food qualities.

**KEYWORDS:** *pulsed electric fields, lecithin, oxidation, electrochemical reaction, free radicals*

## ■ INTRODUCTION

Pulsed electric fields (PEF) processing, which has gotten more attention in the past few years, is a nonthermal food processing technology mainly used in liquid food to inactivate microorganisms and enzymes.<sup>1–3</sup> As compared with thermal preservation methods, the most important advantages of PEF are effectively preserving food freshness and reducing energy consumption.<sup>5,6</sup> PEF is a potential complement to or replacement of thermal preservation methods and has undergone rapid development close to commercial application.<sup>7,8</sup>

The mechanism of microbial inactivation by PEF is attributed to the permeabilization and damage on the cell membrane when the applied electric field exceeds a certain threshold value.<sup>9</sup> As compared with the extensive studies on the inactivation of microorganisms and enzymes by PEF, there are few reports about the effects of PEF on food components, especially food lipids.<sup>9–11</sup> To our best knowledge, only a few studies mainly discussed the possible occurrence of an electrochemical reaction and electrode corrosion during PEF processing.<sup>4,5</sup> However, there are few experimental reports on the electrochemical reaction during PEF processing and the influence on food components.<sup>4</sup>

PEF has been successfully applied to different kinds of foods rich in lipids such as milk, soymilk, fruit juice–soymilk beverage, and liquid egg.<sup>12–18</sup> However, the effects of PEF, especially the electrochemical reaction under PEF processing on food lipids, have not been studied so far. Knowledge on electrochemical reaction and food lipids oxidation in PEF processing would be useful for the application and development of PEF technology in the food industry. For example, egg is rich in lecithin, which is an important functional lipid. The research on the effects of PEF on egg yolk lecithin is particularly necessary and significant. Therefore, yolk lecithin was selected

to investigate the effect of PEF on the physical and chemical properties of food lipids in the present study. The aim of this study was to investigate the lipid oxidation and related electrochemical reaction during PEF processing.

## ■ MATERIALS AND METHODS

**Materials.** Fresh eggs were purchased from the market. Standard lecithin (L- $\alpha$ -PC, lyophilized powder) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were from Sigma & Aldrich. All other chemicals were analytical grade.

**PEF Processing.** A bench scale continuous system (OSU-4L, The Ohio State University, Columbus, OH) with square-wave pulses was used in this study. After going through each pair of chambers, the treated sample was cooled by passing through a coiled tube with a 2.3 mm inner diameter, which was submerged in a heat exchange bath (Fisher Scientific Inc., Pittsburgh, PA) with cold water (5 °C). The pre- and post-PEF exposure temperatures ( $T_{\text{inlet}} - T_{\text{outlet}}$ ) at the inlet and outlet of the treatment chamber were measured using a K-type thermocouple (OMEGA, Stamford, CT). The highest temperature achieved in all tests was lower than 30 °C. The flow rate, pulse repetition rate, and pulse width were set 0.187 mL/s, 600 Hz, and 2  $\mu$ s, respectively. Sterilized dark brown glass bottles of 20 mL were directly filled from PEF treatment system. After that, the containers were tightly closed, leaving as less amount of headspace as possible, and stored away from light at 4 °C.

**Extraction of Yolk Lecithin.** Yolk lecithin was extracted by the organic solvent precipitation method.<sup>19</sup> Egg yolk was separated from the whole egg and resolved in acetone. The suspension was filtered after stirring at 40 °C for 2 h. The filter residue was dissolved in the solution of ethanol and petroleum ether (3:1; v/v), then stirred, and extracted for 2 h. After suspension by air pump filtration, a filter liquor

**Received:** October 2, 2012

**Revised:** November 27, 2012

**Accepted:** November 27, 2012

**Published:** November 27, 2012

was obtained. Rough yolk lecithin was quantified and dissolved in NaCl solution (pH 7.0) with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  after rotary evaporation. Lecithin was re-extracted from yolk lecithin solution samples according to the method of Bligh and Dyer.<sup>19</sup> The re-extracted lecithin was redissolved in chloroform and stored in the dark at  $-50\text{ }^{\circ}\text{C}$  for further analysis.

For HPLC analysis, both rough and standard samples were directly dissolved in chilled chloroform to achieve a concentration of 5 mg/mL. The HPLC analysis were performed on the Agilent 1100 (Agilent 1100, United States) with a Waters 2996 ultraviolet detector (Waters, United States) at 210 nm. Chromatography was carried out at 30  $^{\circ}\text{C}$ , using a Lichrosorb Si60 column (4.6 mm  $\times$  250 mm; 5  $\mu\text{m}$  particles) (Merck, Germany). The injection volume was 5  $\mu\text{L}$ . The mobile phases consisted of *n*-hexane, isopropanol, and deionized water (6:8:1, v/v/v) containing 1% acetate solution. The premixed mobile phase first passed through solvent filtration, followed by column washing for 0.5 h. It was sufficient to achieve a background current in the range 0.4–1.0 nA. According to the chromatogram and calculation, the content of rough yolk lecithin was 90.9% (Figure 1). Yolk lecithin (5 mg/mL) was dissolved into NaCl solution (pH 7.0) with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  for PEF processing.

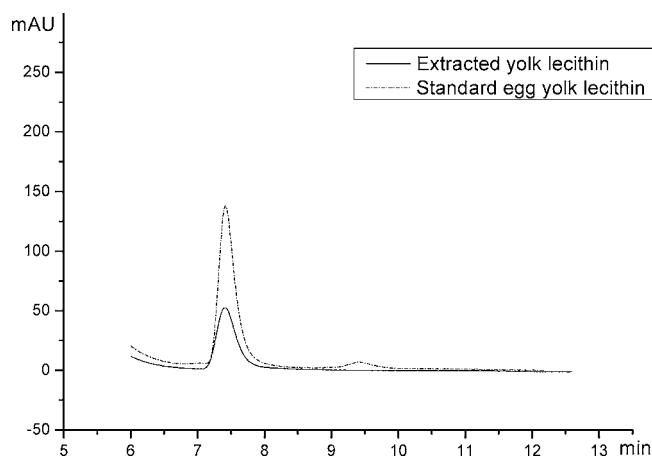


Figure 1. Chromatogram of yolk lecithin with HPLC-UV detection.

**Determination of Oxidation of Yolk Lecithin.** *Acid Value (AV) and Peroxide Value (POV).* The POV was determined according to AOAC.<sup>20</sup> The results were expressed as mg KOH/g lipid and mequiv/kg, respectively.

**Carbonyl Value.** The carbonyl value was determined according to the method described by Azad Shah et al.<sup>21</sup> with some modification. In brief, 2,4-dinitrophenylhydrazine (2, 4-DNPH) solution was prepared by dissolving 50 mg of 2,4-DNPH in 100 mL of 1-BuOH containing 3.5 mL of HCl. The lecithin sample (20–200 mg) was weighted into a 10 mL volumetric flask and filled with 1-BuOH. One milliliter of lecithin solution was transferred to a 15 mL test tube and then mixed with 1 mL of 2,4-DNPH solution. The test tube was incubated at 40  $^{\circ}\text{C}$  for 20 min and mixed with 8 mL of 8% KOH in 1-BuOH. The mixture was separated centrifugally (2000g, 5 min) to obtain the upper solution and then measured at 420 nm with ultraviolet spectrophotometer (723N, Shanghai Precise Scientific Instrument Co., Shanghai, China) and compared with standard series.

**Determination of Active Chlorine and Active Oxygen of NaCl Solutions with Different pH Values.** NaCl was dissolved in deionized water to achieve an electrical conductivity of 830  $\mu\text{s}/\text{cm}$ . The pH of NaCl solution was adjusted to 4.0, 7.0, and 10.0 using 0.1 mol/mL HCl and NaOH solution. The active chlorine was determined by using iodometric titration.

For the detection of active oxygen species,  $\text{O}_3$  was determined by ultraviolet spectrophotometer method. KI (15 mL), fresh sample (30 mL), and 3 mol/mL HAC (5 mL) were added into 50 mL colorimetric tube in sequence. The solution was determined on an ultraviolet spectrophotometer (UV1201, Ruili Instrument Co., Beijing,

China) at 352 nm. The  $\text{O}_3$  concentration was calculated according to  $\text{KIO}_3$  standard curve using the equation:

$$\text{O}_3 \text{ (mg/L)} = \frac{\text{O}_3 \text{ (mg) corresponding to absorption value}}{\text{volume of the sample}}$$

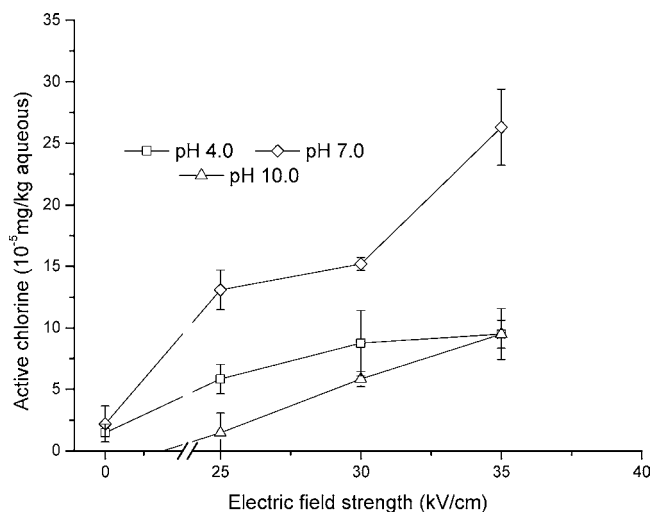
$\text{H}_2\text{O}_2$  was determined by fluorescence spectrophotometry immediately after PEF treatment. A 0.47 mL amount of 1.6 mmol/L  $\text{H}_2\text{SO}_4$ , 0.2 mL of 1.5 mmol/L  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ , 0.15 mL of 5.32 mmol/L benzoic acid, and 3 mL of different NaCl solutions were added to 10 mL test tubes, mixed, and allowed to stand for 20 min followed by diluting to 5 mL with redistilled water, and the pH was adjusted to 12. The excitation wavelength, emission wavelength, and slit width were set at 300, 401, and 500 nm, respectively, on a fluorospectrophotometer (RP-530IPC, Shimadzu manufacturing institute, Japan). The concentration of  $\text{H}_2\text{O}_2$  was calculated according to the standard curve, and the value was expressed as  $\mu\text{mol/L}$  solution.

**Electron Spin Resonance Spectroscopy (ESR) Detection of Free Radicals.** The NaCl solutions (pH 4.0, 7.0, and 10.0) with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  were anaerobic (flushed with nitrogen for several minutes). The spin trap (DMPO, 220 mM) dissolved in NaCl solutions, containing 1.5% acetonitrile. The liquid samples were enclosed into glass capillary (100 mm  $\times$  1 mm i.d.) and then were stored in nitrogen canister for the further ESR measurements. ESR measurements were conducted with a Bruker EMX 12/10 computer-controller spectrometer. The spectra of spin adducts were recorded at the following conditions: microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 2 G; and scan time, 84 s. Every sample was swept five times to acquire a suitable signal-to-noise ratio. The *g* factor and hyperfine coupling constants of DMPO spin adducts were determined from the measured spectra.

**Statistical Analysis.** All measurements were carried out in triplicate, and the results are expressed as means  $\pm$  standard deviations. Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to identify differences among the means at  $P < 0.05$  using a SPSS software package (version 11.03, SPSS, United States).

## RESULTS AND DISCUSSION

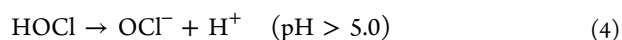
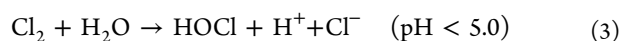
**Effects of PEF Treatment on the Concentration of Active Chlorine in NaCl Solution.** To investigate the electrochemical reaction of foods with different acidic and alkaline properties during PEF treatment, NaCl solutions with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  and different pH values were used in this study, because sodium ion ( $\text{Na}^+$ ) and chloride ion ( $\text{Cl}^-$ ) are the most common ions in foods. During PEF treatment, electrochemical reactions may result in partial electrolysis of the solutions, leading to corrosion of the electrode and introduction of small particles of electrode material into the liquid.<sup>4,22,23</sup> Electrolysis of NaCl solution with direct current (DC) is known to generate different types of effective bactericidal components. The primary bactericidal components are active chlorine species (ACS) with high redox potentials, including dissolved chlorine gas ( $\text{Cl}_2$ ) produced by electrolysis at the anode, hypochlorous acid ( $\text{HOCl}$ ) formed by the hydrolysis of  $\text{Cl}_2$ , and hypochlorite anions ( $\text{OCl}^-$ ).<sup>24–26</sup> Effects of PEF treatment on the active chlorine of NaCl solutions with different pH values are shown in Figure 2. The results showed that electric field strength had significant effects ( $p < 0.05$ ) on the concentration of active chlorine generated during PEF treatment. The concentration of ACS steadily increased as the electric field strength increased at a fixed pH, indicating that the stronger the PEF strength was, the greater the increment of ACS concentration was. The pH value of NaCl solution also had significant effects ( $p < 0.05$ ) on the ACS



**Figure 2.** Effects of PEF treatment on the active chlorine of NaCl solutions with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  and different pH values.

concentration. There are more ACS generated in neutral aqueous than that in both acidic and alkaline aqueous.

During PEF treatment, it could be thought to produce ACS according to the following reactions:<sup>27,28</sup>



From the eqs 3 and 4, it can be inferred that pH was an important factor on the type and the quantity of ACS generated by PEF treatment. Similar results were found in the studies of Chen et al.<sup>29</sup> and Czarnetzki et al.,<sup>30</sup> where more ACS generated when the pH of NaCl solution under electrolysis was between acidic and alkaline pH. The possible reason was deduced that the solubility of chlorine dramatically decreased at low pH, and high pH would induce disproportionation of ACS, which is also known as dismutation and is a specific type of redox reaction in which a species is simultaneously reduced and oxidized to form two different products. Although electrolysis is very different from PEF treatment, these studies might be helpful to explain the phenomenon in the present study. The knowledge on the electrochemical reaction under PEF treatment is still very limited. Further experimental research is very worthwhile to elucidate how electrochemical reactions occur in PEF treatment.

**Effects of PEF Treatment on the Reactive Oxygen in NaCl Solution.** The oxidation of lipids is often related to reactive oxygen species (ROS). ROS are very strong oxidants including  $\text{O}_3$  (ozone),  $^1\text{O}_2$  (single oxygen),  $\text{O}_2^{\bullet-}$  (superoxide radical),  $\text{HO}_2^\bullet$  (hydroperoxide radical),  $\cdot\text{OH}$  (hydroxyl radical),  $\text{H}_2\text{O}_2$ , etc. Table 1 illustrates the effects of PEF treatment on the  $\text{H}_2\text{O}_2$  of NaCl solutions with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  and different pH values. The results showed that PEF treatment had significant effects on the  $\text{H}_2\text{O}_2$  concentration. The increment of  $\text{H}_2\text{O}_2$  concentration was a function of the applied electric field strength and pH of NaCl solution. At each pH of NaCl solution,  $\text{H}_2\text{O}_2$  was detected until the applied electric field strength exceeded a critical value. The pH of NaCl

**Table 1.** Effects of PEF Treatment on the  $\text{H}_2\text{O}_2$  of NaCl Solutions with an Electrical Conductivity of 830  $\mu\text{s}/\text{cm}$  and Different pH Values

PEF treatment for 400 $\mu\text{s}$ at each strength (kV/cm)	$\text{H}_2\text{O}_2$ ( $\mu\text{mol}/\text{L}$ )		
	pH 4.0 (mean $\pm$ SD)	pH 7.0 (mean $\pm$ SD)	pH 10.0 (mean $\pm$ SD)
blank <sup>a</sup>	(ND) <sup>c</sup>	(ND)	(ND)
control <sup>b</sup>	(ND)	(ND)	(ND)
25	(ND)	(ND)	(ND)
30	(ND)	0.177 $\pm$ 0.023	(ND)
35	1.053 $\pm$ 0.035	2.035 $\pm$ 0.041	1.805 $\pm$ 0.043

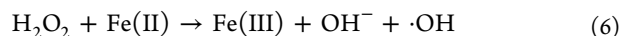
<sup>a</sup>Blank means the NaCl solution without any processing. <sup>b</sup>Control means NaCl solution passed through pipes of PEF without electric field and pulse on. <sup>c</sup>ND, not detected (below the detection level).

solution also had significant effects on the  $\text{H}_2\text{O}_2$  concentration. The maximal values of  $\text{H}_2\text{O}_2$  concentration were 1.053, 2.035, and 1.805  $\mu\text{mol}/\text{L}$  at pH 4.0, 7.0, and 10.0 for 35 kV/cm under test conditions, respectively. Similar with the ACS, the  $\text{H}_2\text{O}_2$  concentration of neutral solution was relatively higher.

Active oxygen species (AOS) is a generic name given to a variety of molecules and free radicals derived from molecular oxygen.  $\text{O}_3$  is an allotrope of  $\text{O}_2$ , which shows a higher oxidative ability, and was attained from the discharge of  $\text{O}_2$ . In the water environment,  $\text{H}_2\text{O}_2$  was produced through  $\text{O}_2$  photolysis and further reacted with  $\text{O}_3$  to form hydroxyl radical ( $\cdot\text{OH}$ ) with high reactivity. Apparently,  $\cdot\text{OH}$  is the main product of these kinds of reactions.<sup>31</sup> In this study,  $\text{O}_3$  has not been detected at all applied electric field strengths and different pH values. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a type of relatively stronger oxidant, which possesses a function of sterilization and anticorrosion. Meanwhile,  $\text{H}_2\text{O}_2$  is a type of mild reducing agent as well, which can form  $\text{O}_2$  and  $\text{H}_2\text{O}$  by means of disproportionation reaction spontaneously (eq 5).

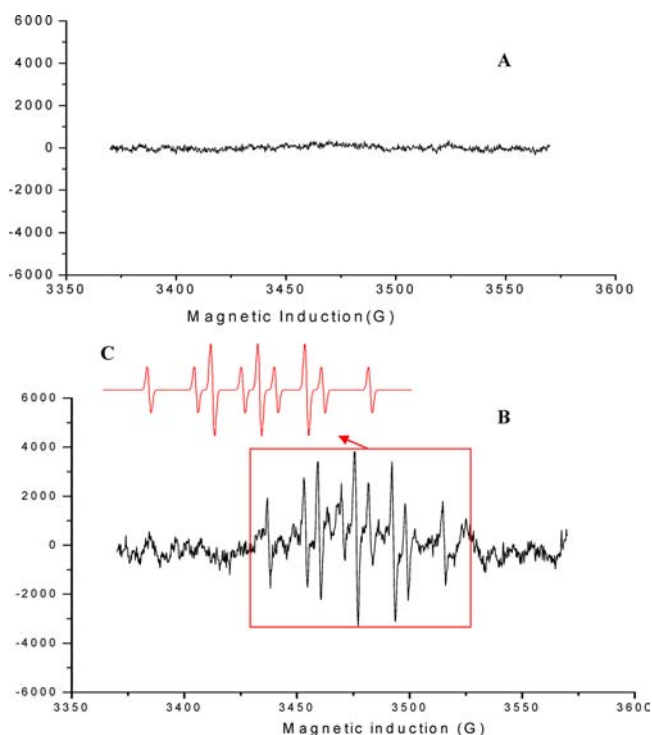


$\text{H}_2\text{O}_2$  can be formed by disproportionation reaction of superoxide anion radical. It can react with iron ion (from the electrode corrosion) to form  $\cdot\text{OH}$  of high reactivity (eq 6).



From the results discussed above,  $\text{H}_2\text{O}_2$  was constantly accumulated in the processing chamber with electric field strength rising up during PEF treatment. It is believed that many more reactive oxygens such as singlet oxygen ( $^1\text{O}_2$ ), superoxide anion radical ( $\text{O}_2^{\bullet-}$ ), and hydroxyl radical ( $\cdot\text{OH}$ ) would be produced in the PEF treatment chamber, which is vital for food lipid oxidation.

**Detection of Free Radicals in NaCl Solution under PEF Treatment.** The free radicals in NaCl solutions with an electrical conductivity of 830  $\mu\text{s}/\text{cm}$  and different pH values under PEF treatment were detected. Figure 3 shows the ESR spectra of radical adducts of DMPO in NaCl solutions. NaCl solutions with different pH values exhibited identified ESR spectra. Figure 3A illustrates the ESR spectrum of DMPO adducts of NaCl solution without PEF treatment. There was no ESR signal in the ESR spectrum of the control, while there were very intense ESR spectral signals of NaCl solution under PEF treatment (Figure 3B). The NaCl solution produced a DMPO spin adduct signal, indicating that hydrogen radicals were generated under PEF processing.



**Figure 3.** ESR spectra of radical adducts of DMPO in NaCl solution. (A) Without PEF treatment and (B) with 35 kV/cm PEF treatment for 400  $\mu$ s. (C) Computer-simulated spectra of DMPO adducts:  $a_N = 1.65$  mT,  $a_H^\beta = 2.23$  mT, and line width = 0.08 mT.

As shown in Figure 3B, the spectra contain a triplet with the same intensities, and each triplet line is further split into another triplet with intensities of 1:2:1. The two triplet patterns were both centered at  $g = 2.0098$ . It was concluded that the first triplet peaks arising from the hyperfine splitting were due to one nitrogen atom of the DMPO adduct with a hyperfine coupling constant ( $a_N = 1.65$  mT), and the second triplet peaks were caused by the two identical  $\beta$ -protons ( $a_H^\beta = 2.23$  mT) of the DMPO adduct. The ESR spectra of hydrogen radical adducts of DMPO were also simulated using Biomolecular EPR Spectroscopy Software (Figure 3C). The ESR spectra experimentally obtained (Figure 3B) were identical with the simulated ESR spectra of hydrogen radical adducts of DMPO, evidencing that hydrogen radicals were generated under PEF processing.

#### Effects of PEF Treatment on the Oxidation of Lecithin.

Lecithin is a phospholipid that widely exists in fauna and flora, which primarily includes phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid, and phosphoinositide (PI). In a narrow sense, lecithin only refers to PC. Lecithin is some kind of amphoteric molecule, which are essential cell membrane components. It is known that lecithin plays a role in emulsifying action because of both the lipid solubility and the hydrophilic property. Yolk lecithin mainly consists of palmitic acid, oleic acid, stearic acid, and linoleic acid. Most of them are unsaturated fatty acids. Because of having many polyunsaturated fatty acids, yolk lecithin is easily oxidized in the presence of an oxidizing substance. To be specific, fatty acids of long chain were hydrolyzed from lecithin to become free fatty acids. In the presence of oxidizing agents, unsaturated fatty acids are rather easily oxidized to form hydroperoxide (PCOOH). Through the course of chain transition, PCOOH could be degraded into many kinds of second oxidative products such as

aldehydes, alcohol, acid, and polymer.<sup>32,33</sup> Therefore, it is necessary to test the oxidation of yolk lecithin under PEF treatment.

The effects of electric field strength on the oxidation of lecithin are shown in Tables 2 and 3. As compared with the

**Table 2.** AV of Lecithin Extracted from Egg Yolk Initially after PEF Treatment and Throughout Storage Period ( $4 \pm 1$  °C)<sup>a</sup>

storage (days)	AV (mg KOH/g lipid)			
	control	25 kV/cm	30 kV/cm	35 kV/cm
0	0.67 a	0.66 a	0.67 a	0.71 a
3	0.67 a	0.65 a	0.67 a	0.72 a
6	0.68 a	0.66 a	0.68 a	0.72 a
9	0.74 b	0.69 a	0.72 a	0.77 b
12	0.78 b	0.73 a	0.78 b	0.80 b
15	0.83 c	0.79 b	0.82 b	0.85 c

<sup>a</sup>The PEF treatment time is 400  $\mu$ s at each strength. The value is the average of three replicates. The data marked with different letters in the same row were statistically different at  $P = 0.05$ .

**Table 3.** POV of Lecithin Extracted from Egg Yolk Initially after PEF Treatment and Throughout Storage Period ( $4 \pm 1$  °C)<sup>a</sup>

storage (days)	POV (Mequiv/kg lipid)			
	control	25 kV/cm	30 kV/cm	35 kV/cm
0	0.64 a	0.62 a	0.76 b	0.82 c
3	0.64 a	0.63 a	0.77 b	0.82 c
6	0.64 a	0.64 a	0.76 b	0.82 c
9	0.70 d	0.72 d	0.82 c	0.96 e
12	0.73 d	0.83 c	0.91 e	1.07 f
15	0.80 c	0.94 e	1.06 f	1.28 g

<sup>a</sup>The PEF treatment time is 400  $\mu$ s at each strength. The value is the average of three replicates. The data marked with different letters in the same row were statistically different at  $P = 0.05$ .

control, there was no significant difference in AV of lecithin initially after PEF processing at 0–35 kV/cm and throughout the storage period at 4 °C (Table 2), indicating that the electric field strength has no apparent effect on lecithin hydrolysis, whereas significant effects of electric field strength on POV of lecithin were found (Table 3). POV steadily increased with the electric field strength and storage time increasing. The effects of PEF treatment time on the oxidation of lecithin are shown in Tables 4 and 5. Similar with electric field strength, the PEF treatment time had no significant effect on AV of extracted lecithin initially after PEF processing at 35 kV/cm for 0–800  $\mu$ s and throughout the storage period at 4 °C (Table 4). Table 5 demonstrates the effect of PEF treatment time on POV of lecithin. Generally, POV of lecithin dramatically increased with the increase of PEF treatment time and storage time. Moreover, the carbonyl value was not detected at all applied electric field strengths after cold storage for 0–15 days.

These results confirmed the occurrence of oxidation of lecithin under PEF treatment. It has been shown that generation of active chlorine, active chlorine, and other free radicals took place in the NaCl solutions during PEF. The concentration of active chlorine and  $H_2O_2$  of NaCl solutions with different pH values increased with the increase of applied electric field strength, especially for those neutral solutions. Liquid egg belongs to neutral solution with a pH of 7.0–8.0. So,

**Table 4. AV of Lecithin Extracted from Egg Yolk Initially after PEF Treatment and Throughout Storage Period ( $4 \pm 1$  °C)<sup>a</sup>**

storage (days)	AV (mg KOH/g lipid)				
	control	200 $\mu$ s	400 $\mu$ s	600 $\mu$ s	800 $\mu$ s
0	0.71 a	0.70 a	0.72 a	0.70 a	0.71 a
3	0.71 a	0.70 a	0.72 a	0.71 a	0.71 a
6	0.72 a	0.72 a	0.73 a	0.70 a	0.72 a
9	0.73 a	0.73 a	0.73 a	0.72 a	0.74 a
12	0.73 a	0.73 a	0.74 a	0.74 a	0.75 a
15	0.75 b	0.74 b	0.76 b	0.75 b	0.76 b

<sup>a</sup>The PEF treatment strength is 35 kV/cm. The value is the average of three replicates. The data marked with different letters in the same row were statistically different at  $P = 0.05$ .

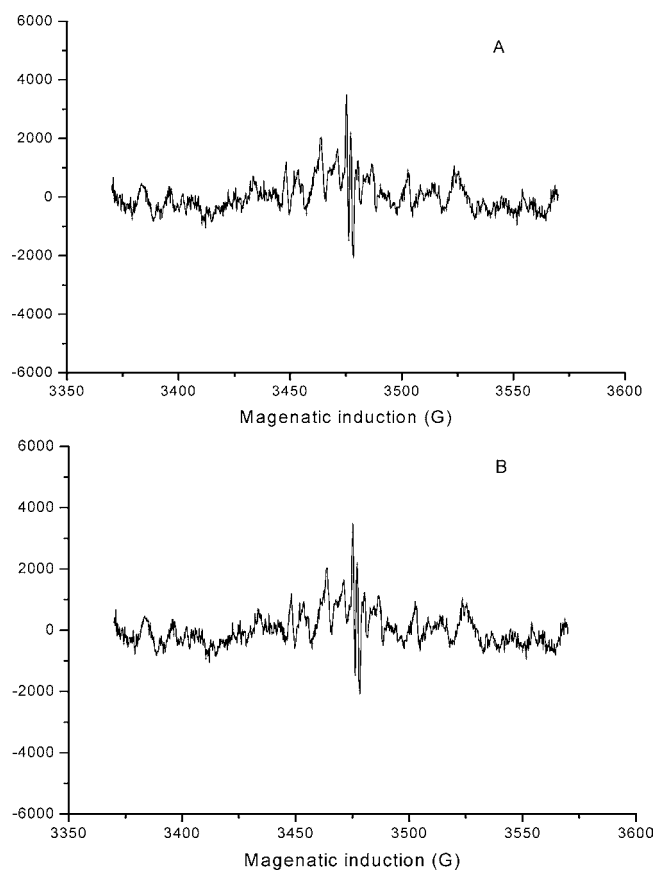
**Table 5. POV of Lecithin Extracted from Egg Yolk Initially after PEF Treatment and Throughout Storage Period ( $4 \pm 1$  °C)<sup>a</sup>**

storage (days)	POV (Mequiv/kg lipid)				
	control	200 $\mu$ s	400 $\mu$ s	600 $\mu$ s	800 $\mu$ s
0	0.64 a	0.76 b	0.76 b	0.88 c	1.02 d
3	0.68 a	0.77 b	0.78 b	0.92 c	1.19 e
6	0.72 a	0.80 b	0.81 b	0.98 g	1.36 f
9	0.75 b	0.83 b	0.93 c	1.34 e	1.75 f
12	0.78 b	0.87 c	0.99 g	1.57 h	1.93 i
15	0.80 b	0.96 g	1.06 d	1.86 i	2.24 j

<sup>a</sup>The PEF treatment strength is 35 kV/cm. The value is the average of three replicates. The data marked with different letters in the same row were statistically different at  $P = 0.05$ .

these oxidative agents induced by PEF might be responsible for the oxidation of extracted yolk lecithin.

As is known, as compared with conventional thermal pasteurization methods, foods were less affected initially after PEF processing and were maintained in higher quality throughout the storage period.<sup>7,34,35</sup> A large number of literatures have fully demonstrated that small molecular compounds in plant-based foods, mainly aroma compounds and health-related phytochemicals, were not significantly affected by PEF.<sup>34–36</sup> However, recent studies have looked at some side effects of PEF processing and the influences on food qualities and food components such as proteins<sup>7,37</sup> and anthocyanin.<sup>38</sup> How to minimize the impact of PEF treatment on food qualities remains unknown. In this study, vitamin C (0.01 mg/mL) was added into NaCl and lecithin solutions to quench free radicals and inhibit oxidation. Figure 4 shows ESR spectra of radical adducts of DMPO in NaCl solution with 0.01 mg/mL vitamin C. The ESR spectra without (Figure 4A) and with 35 kV/cm PEF treatment for 400  $\mu$ s (Figure 4B) were identified, only displaying some bands of vitamin C. It suggests that vitamin C completely quenched the free radicals under PEF treatment. The oxidation of lecithin solutions during PEF processing was also inhibited by vitamin C. There was no significant change in AV and POV of lecithin initially after PEF processing at 0–35 kV/cm and throughout storage period at 4 °C. Vitamin C as a natural antioxidant is widely contained in foods, especially in plant-based foods. It could effectively quench free radicals and inhibit oxidation of lipid in NaCl and lecithin solutions as model systems under PEF processing. In the same way, other radical quenchers like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and



**Figure 4.** ESR spectra of radical adducts of DMPO in NaCl solution with 0.01 mg/mL vitamin C. (A) Without PEF treatment and (B) with 35 kV/cm PEF treatment for 400  $\mu$ s.

tocopherol should have effects on the inhibition of lipid oxidation induced by PEF, but this is an area that has not been fully elucidated, and further research is required.

PEF processing is a promising nonthermal food preservation technology, which is ongoing from laboratory and pilot plant scale levels to the industrial level. More studies are needed to investigate the effects of PEF treatment on the food quality and food components. The present study confirmed that the electrochemical reaction could occur during PEF processing, producing many oxidizing substances and further causing oxidation of lipids in foods. Vitamin C could effectively quench free radicals and inhibit oxidation of food lipids under PEF processing.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Tel/Fax: 86 510 85919150. E-mail: zhaow@jiangnan.edu.cn (W.Z.) or ruijinyang@yahoo.com.cn (R.Y.).

### Funding

We gratefully acknowledge the financial support provided by Project 31000829 of the National Natural Science Foundation of PR China, the Project BK2010148 of Jiangsu Provincial Natural Science Foundation. This study was also supported by National “863” project (2011AA100801-02).

### Notes

The authors declare no competing financial interest.

## REFERENCES

- (1) Donsi, F.; Ferrari, G.; Fruiolo, M.; Pataro, G. Pulsed Electric Field-Assisted Vinification of Aglianico and Piediroso Grapes. *J. Agric. Food Chem.* **2010**, *58* (22), 11606–11615.
- (2) Guderjan, M.; Elez-Martinez, P.; Knorr, D. Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil. *Innovative Food Sci. Emerging Technol.* **2007**, *8* (1), 55–62.
- (3) Barbosa-Canovas, G. V.; Pothakamury, U. R.; Palou, E.; Swanson, B. G. *Nonthermal Preservation of Foods*; Marcel Dekker: New York, 1998; p 276.
- (4) Morren, J.; Roodenburg, B.; de Haan, S. W. H. Electrochemical reactions and electrode corrosion in pulsed electric field (PEF) treatment chambers. *Innovative Food Sci. Emerging Technol.* **2003**, *4* (3), 285–295.
- (5) Soliva-Fortuny, R.; Balasa, A.; Knorr, D.; Martín-Belloso, O. Effects of pulsed electric fields on bioactive compounds in foods: A review. *Trends Food Sci. Technol.* **2009**, *20*, 544–556.
- (6) Toepfl, S.; Mathys, A.; Heinz, V.; Knorr, D. Review: Potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Rev. Int.* **2006**, *22*, 405–423.
- (7) Zhao, W.; Yang, R.; Zhang, H. Q. Recent advances in the action of pulsed electric fields on enzymes and food component proteins. *Trends Food Sci. Technol.* **2012**, *27*, 83–96.
- (8) Hye, W. Y.; Streaker, C. B.; Zhang, Q. H.; Min, D. B. Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *J. Agric. Food Chem.* **2000**, *48*, 4597–4605.
- (9) Barsotti, L.; Cheftel, J. C. Food processing by pulsed electric fields. II. Biological aspects. *Food Rev. Int.* **1999**, *15*, 181–213.
- (10) Turk, M. F.; Baron, A.; Vorobiev, E. Effect of Pulsed Electric Fields Treatment and Mash Size on Extraction and Composition of Apple Juices. *J. Agric. Food Chem.* **2010**, *58* (17), 9611–9616.
- (11) Vallverdú-Queralt, A.; Oms-Oliu, G.; Odriozola-Serrano, I.; Lamuela-Raventós, R. M.; Martín-Belloso, O.; Elez-Martinez, P. Effects of Pulsed Electric Fields on the Bioactive Compound Content and Antioxidant Capacity of Tomato Fruit. *J. Agric. Food Chem.* **2012**, *60* (12), 3126–3134.
- (12) Amiali, M.; Ngadi, M. O.; Smith, J. P.; Raghavan, G. S. V. Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in liquid egg yolk. *J. Food Eng.* **2007**, *79*, 689–694.
- (13) Cortes, C.; Esteve, M. J.; Frigola, A.; Torregrosa, F. Quality characteristics of horcata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. *Food Chem.* **2005**, *91*, 319–325.
- (14) Fernandez-Diaz, M. D.; Barsotti, L.; Dumay, E.; Cheftel, J. C. Effects of pulsed electric fields on ovalbumin solutions and on liquid egg white. *J. Agric. Food Chem.* **2000**, *48*, 2332–2339.
- (15) Monfort, S.; Gayan, E.; Saldana, G.; Puertolas, E.; Condon, S.; Raso, J.; et al. Inactivation of *Salmonella typhimurium* and *Staphylococcus aureus* by pulsed electric fields in liquid whole egg. *Innovative Food Sci. Emerging Technol.* **2010**, *11*, 306–313.
- (16) Jin, T.; Zhang, H. Q.; Hermawan, N.; Dantzer, W. Effects of pH and temperature on inactivation of *Salmonella typhimurium* DT104 in liquid whole egg by pulsed electric fields. *Int. J. Food Sci. Technol.* **2009**, *44*, 367–372.
- (17) Li, S. Q.; Zhang, Q. H. Inactivation of *E. Coli* 8739 in enriched soymilk using pulsed electric fields. *J. Food Sci.* **2004**, *69*, 169–174.
- (18) Peña, M. M. I.; Salvia-Trujillo, L.; Rojas-Graü, M. A.; Martín-Belloso, O. Impact of high intensity pulsed electric field on antioxidant properties and quality parameters of a fruit juice-soymilk beverage in chilled storage. *LWT Food Sci. Technol.* **2010**, *43*, 872–881.
- (19) Bligh, G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.
- (20) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Virginia, 1995.
- (21) Azad, S. A. K. M.; Tokunaga, C.; Kurihara, H.; Takahashi, K. Changes in lipids and their contribution to the taste of migaki-nishin (dried herring fillet) during drying. *Food Chem.* **2009**, *115* (3), 1011–1018.
- (22) Saulis, G.; Lape, R.; Praneviciute, R.; Mickevicius, D. Changes of the solution pH due to exposure by high-voltage electric pulses. *Bioelectrochemistry* **2005**, *67*, 101–108.
- (23) Meneses, N.; Jaeger, H.; Knorr, D. pH-changes during pulsed electric field treatments-numerical simulation and in situ impact on polyphenoloxidase inactivation. *Innovative Food Sci. Emerging Technol.* **2011**, *12*, 499–504.
- (24) Jeong, J.; Kim, J. Y.; Cho, M.; Choi, W.; Yoon, J. Inactivation of *Escherichia coli* in the electrochemical disinfection process using a Pt anode. *Chemosphere* **2007**, *67*, 652–659.
- (25) Kim, W. T.; Lim, Y. S.; Shin, I. S.; Park, H.; Chung, D.; Suzuki, T. Use of electrolyzed water ice for preserving freshness of Pacific saury (*Cololabis saira*). *J. Food Prot.* **2006**, *69*, 2199–2204.
- (26) Park, H.; Hung, Y. C.; Chung, D. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Int. J. Food Microbiol.* **2004**, *91*, 13–18.
- (27) Urano, H.; Ishikawa, H.; Fukuzaki, S. Involvement of radical species in inactivation of *Vibrio parahaemolyticus* in saline solutions by direct-current electric treatment. *J. Biosci. Bioeng.* **2006**, *102*, 457–463.
- (28) Pudtikajorn, K.; Shin, I. S.; Jeong, W. S.; Chung, D. Kinetic modeling of active chlorine generation by low-amperage pulsating direct current in a circulating brine solution. *J. Food Eng.* **2009**, *92* (4), 461–466.
- (29) Chen, X.; Guo, S.; Yuan, X. Study on Electrolytic Production of Active Chlorine in Electrolytic Cell. *Chem. World* **2001**, *8*, 395–398 (in Chinese).
- (30) Czarnetzki, L. R.; Janssen, L. J. J. Formation of hypochlorite, chlorate and oxygen during NaCl electrolysis from alkaline solutions at an RuO<sub>2</sub>/TiO<sub>2</sub> anode. *J. Appl. Electrochem.* **1992**, *22* (4), 315–324.
- (31) Covarrubias, L.; Hernández-García, D.; Schnabel, D.; Salas-Vidal, E.; Castro-Obregón, S. Review: Function of reactive oxygen species during animal development: Passive or active? *Dev. Biol.* **2008**, *320* (1), 1–11.
- (32) Hui, S.; Chiba, H.; Sakurai, T.; Asakawa, C.; Nagasaka, H.; Murai, T.; Ide, H.; Kurosawa, T. An improved HPLC assay for phosphatidylcholine hydroperoxides (PCOOH) in human plasma with synthetic PCOOH as internal standard. *J. Chromatogr., B* **2007**, *857* (1), 158–163.
- (33) Zhang, J.; Cazars, A. R.; Lutzke, B. S.; Hall, E. D. HPLC-Chemiluminescence and thermospray LC/MS study of hydroperoxides generated from phosphatidylcholine. *Free Radical Biol. Med.* **1995**, *18* (1), 1–10.
- (34) Soliva-Fortuny, R.; Balasa, A.; Knorr, D.; Martín-Belloso, O. Effects of pulsed electric fields on bioactive compounds in foods: a review. *Trends Food Sci. Technol.* **2009**, *20*, 544–556.
- (35) Schilling, S.; Schmid, S.; Jäger, H.; Ludwig, M.; Dietrich, H.; Toepfl, S.; Knorr, D.; Neidhart, S.; Schieber, A.; Carle, R. Comparative Study of Pulsed Electric Field and Thermal Processing of Apple Juice with Particular Consideration of Juice Quality and Enzyme Deactivation. *J. Agric. Food Chem.* **2008**, *56* (12), 4545–4554.
- (36) Vallverdú-Queralt, A.; Odriozola-Serrano, I.; Oms-Oliu, G.; Lamuela-Raventós, R. M.; Elez-Martinez, P.; Martín-Belloso, O. Changes in the Polyphenol Profile of Tomato Juices Processed by Pulsed Electric Fields. *J. Agric. Food Chem.* **2012**, *60* (38), 9667–9672.
- (37) Sui, Q.; Roginski, H.; Williams, R. P. W.; Wooster, T. J.; Versteeg, C.; Wan, J. Effect of the Ionic Strength of Pulsed Electric Field Treatment Medium on the Physicochemical and Structural Characteristics of Lactoferrin. *J. Agric. Food Chem.* **2010**, *58* (22), 11725–11731.
- (38) Zhang, Y.; Sun, J.; Hu, X.; Liao, X. Spectral Alteration and Degradation of Cyanidin-3-glucoside Exposed to Pulsed Electric Field. *J. Agric. Food Chem.* **2010**, *58* (6), 3524–3531.