


12-10-2008

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Abstract

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Keywords

egg lecithin, emulsion, fatty acid composition, lipid oxidation, oxidative stability, pH effect, phospholipids, soy lecithin, transition metal ions

Disciplines

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Oxidative Stability of Egg and Soy Lecithin as Affected by Transition Metal Ions and pH in Emulsion

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Oxidative stability of egg and soy lecithin in emulsion was evaluated with two transition metal ions, cupric and ferric ion, at two concentration levels (50 and 500 μM). The effect of pH on lipid oxidation was also examined under these two concentrations for each ion. Egg lecithin (EL) had similar peroxide value (PV) development pattern as soy lecithin (SL) when treated with cupric ion under both acidic and neutral pH. Acidic pH of 3 accelerated oxidation of both EL and SL, especially under high concentration of copper. When treated with ferric ion, EL oxidized much faster than SL did. EL had higher value of thiobarbituric acid-reactive substances (TBARS) than SL, possibly because of its higher content of long-chain polyunsaturated fatty acids (PUFA). Acidic pH accelerated TBARS development for both EL and SL, but EL had more significantly increased values. Cupric ion was more powerful than ferric in catalyzing oxidation of both EL and SL under both acidic and neutral pH conditions as measured by PV and TBARS. Linoleic acid may contribute to higher PV production, however, arachidonic acid and docosahexaenoic acid may have contributed more to TBARS production. Overall, SL showed better oxidative stability than EL under the experimental conditions. This study also suggests that using multiple methods is necessary in properly evaluating lipid oxidative stability.

KEYWORDS: Egg lecithin; emulsion; fatty acid composition; lipid oxidation; oxidative stability; pH effect; phospholipids; soy lecithin; transition metal ions

INTRODUCTION

Lipid oxidation and the generation of secondary oxidation products have always been serious concerns of food quality and consumer health (1). Studies have shown that fatty acid composition is the dominant factor affecting lipid oxidative stability in both bulk oil and emulsion systems (2–4). Several researchers reported that the lipid oxidation rate of linoleic acid was 20 to 40 times faster than that of oleic acid (5–7). In many food emulsions, n-3 and n-6 polyunsaturated fatty acids (PUFAs) represent significant amount of fatty acids in total lipid because of their natural existence and fortified formulation. The rancidity development of such foods is much faster compared with that of formulas containing more saturated lipids.

Transition metal ions, commonly found as copper and iron in food emulsions, are major pro-oxidants that catalyze lipid oxidation. Copper has received somewhat less interest than iron because of its lower content in food, but it was reported to be as effective as or even more active in accelerating the decomposition of primary oxidation products (8). For each ion, the cuprous and ferrous are much more effective than the cupric and ferric ion in catalyzing oxidation, but the higher oxidation state ions are more stable.

There are other important factors that contribute to lipid oxidative stability in emulsion systems, such as oxygen availability, storage temperature, and pH. These topics have been reviewed thoroughly (2).

Phospholipids (PLs) or lecithin are typically used as food emulsifiers because of their amphiphilic characteristic. Different

Table 1. Tocopherol Content in Soy Lecithin (SL) as Measured by HPLC

| tocopherol | SL, mg/100 g | |
|------------|--------------------|-------------------|
| | commercial | acetone washed |
| alpha | 1.989 \pm 0.096 | 0.032 \pm 0.002 |
| gamma | 10.976 \pm 0.255 | 0.328 \pm 0.018 |
| delta | 12.513 \pm 0.511 | 0.698 \pm 0.013 |

Table 2. Major Phospholipid Class and Fatty Acid Composition (%) of Soy and Egg Lecithin^a

| lecithin | PL, % | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | C20:4 | C22:6 | other |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| egg | | 30.4 | 15.9 | 26.4 | 16.2 | nd | 6.1 | 1.7 | 3.3 |
| PE | 18.1 | 21.2 | 30.5 | 21.1 | 13.7 | nd | 13.5 | nd | nd |
| PC | 78.7 | 35.1 | 12.2 | 32.5 | 16.9 | nd | 1.5 | nd | 1.8 |
| soy | | 20.6 | 4.3 | 11.4 | 56.6 | 7.1 | nd | nd | nd |
| PE | 25.7 | 20.5 | 6.4 | 10.0 | 56.2 | 6.9 | nd | nd | nd |
| PC | 35.3 | 16.6 | 3.0 | 10.9 | 59.9 | 6.6 | nd | nd | 3.0 |

^a Results were average of two preparations with maximal coefficient variation of determination of 7%; nd, not detectable.

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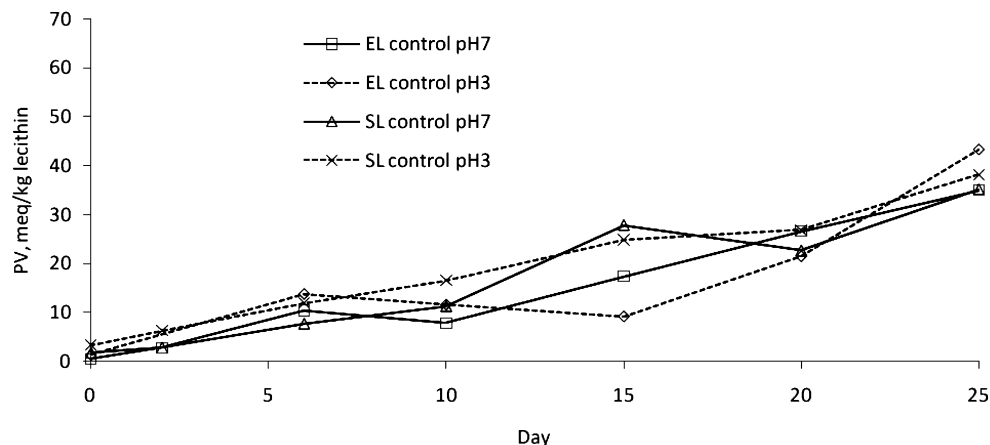


Figure 1. Peroxide value of egg and soy lecithin in emulsion under two pH conditions.

Table 3. Particle Size of Different Treatments as Measured as Volume Mean Diameter (μm)^a

| | EL control | ELCu50 | ELCu500 | ELFe50 | ELFe500 |
|------|---------------|--------------|--------------|--------------|-------------|
| pH 7 | 6.2 ± 0.02 | 11.3 ± 0.02 | 18.0 ± 0.02 | 11.6 ± 0.01 | 16.4 ± 0.05 |
| pH 3 | 53.2 ± 0.19 | 16.5 ± 0.11 | 118.0 ± 0.45 | 29.2 ± 0.08 | 18.3 ± 0.02 |
| | SL control | SLCu50 | SLCu500 | SLFe50 | SLFe500 |
| pH 7 | 8.9 ± 0.01 | 7.8 ± 0.02 | 6.4 ± 0.01 | 10.7 ± 1.82 | 8.1 ± 0.01 |
| pH 3 | 253.4 ± 10.40 | 227.4 ± 7.27 | 8.7 ± 0.01 | 171.1 ± 7.50 | 7.5 ± 0.02 |

^a Data represents mean ± SD from two assays. LSD_{0.05}, least significant difference at 95% confidence level, is 5.5. EL, egg lecithin stabilized emulsion; SL, soy lecithin stabilized emulsion; Cu50 and Cu500, 50 μM and 500 μM cupric sulfate in aqueous phase; Fe50 and Fe500, 50 μM and 500 μM ferric chloride in aqueous phase.

PLs may have different oxidative stability not only because of their difference in fatty acid composition but also because of their PL class composition. Regarding fatty acid composition, egg phospholipids or egg lecithin (EL) contains less linoleic and nearly no linolenic acid but more long-chain polyunsaturated fatty acids (PUFA) than soy lecithin (SL) that has mainly linoleic acid and low amount of linolenic acid. In terms of PL class, EL contains mainly zwitterionic phosphatidylethanolamine (PE, 18.1%) and phosphatidylcholine (PC, 78.7%), whereas a relatively high level of anionic PL such as phosphatidylinositol (PI, 17–24%) (9) and phosphatidic acid (PA, 6%) (9) is also present in SL along with 25.7% PE and 35.3% PC. The difference in electric charge of PLs is hypothesized to contribute to its oxidative stability, especially in emulsions and under different pH conditions. It is believed that negatively charged emulsifiers will attract positively charged transition metal ions and thus stimulate formation and decomposition of lipid hydroperoxides. It is unknown how the oxidation of EL and SL as PL mixtures is affected by the two transition metal ions under different pH environments. A preliminary study conducted in our research showed that cupric ion did not accelerate oxidation of EL in emulsion, but it did accelerate oxidation of SL in emulsion when the concentration of cupric ion was only 1 ppm.

The objectives of this research were to determine how oxidative stability of egg and soy lecithin is affected by type and amount of transition metal ions, i.e. cupric and ferric ion, in emulsions, as measured by the formation of primary and secondary oxidation products; and to evaluate the effect of pH of emulsion on the oxidative stability of lecithins in the presence of transition metal ions.

MATERIALS AND METHODS

Materials. EL was purchased from Q.P. Corporation (Tokyo, Japan) with claimed purity of near 100%. SL was obtained from Fisher Scientific (Fair Lawn, NJ) with purity of 99%, and it was further washed by using acetone (AOCS Official Method JA 4-46) to remove neutral lipids and residual tocopherols. Mineral oil was obtained from Fisher Scientific (Fair Lawn, NJ). Cupric sulfate, ferric chloride, isooctane, 2-propanol, methanol, chloroform, 2-thiobarbituric acid, hexane and other reagents were all purchased from Sigma-Aldrich (St. Louis, MO).

Lecithin Characterization. Initial peroxide value (PV) of both EL and SL was determined by using a modified ferrous iron method as described by Wang and others (10). Briefly, a series amount of slightly oxidized soybean oil with PV of 7.45 meq/kg as measured by the AOCS Official Method Cd 8-53 (11) was used to establish the standard quantification curve. Lecithin, 10 mg, was dissolved in 5 mL of chloroform:methanol (7:3, v/v). Ammonium thiocyanate solution (15 μL , 3.75 M) and freshly prepared ferrous chloride solution (15 μL , approximately 0.014 M) were added into the test tube, and the mixture was stirred well. After 10 min, the absorbance at 500 nm (for the purple-red color) was measured by a Genesys 20 spectrophotometer (Cambridge, U.K.), and PV was calculated using the standard curve equation.

Determination of natural cupric and ferric content in two lecithins was conducted by following the atomic absorption spectrophotometric method of AOAC 990.05 (12). To prepare sample for analysis, lecithin sample of 5 g was ashed in a Thermolyne 1400 laboratory furnace until white ash formed. The ash was then dissolved in 10 mL of nitric acid (10%). The samples were then analyzed by the Soil & Plant Analysis Laboratory at Iowa State University.

To measure tocopherol content in both lecithin samples, a HPLC method modified from AOCS official method Ce 8-89 (11) was used. Lecithin, 5 g, was first subjected to saponification by following AOCS official method Ca 6a-40 (10), and the unsaponifiable matter was extracted. The extract was used for HPLC quantification for tocopherols. Alpha-, gamma-, and delta-tocopherols purchased from Sigma-Aldrich were used as external standards for quantification.

The lecithin fatty acid profile was determined by using a capillary gas chromatography (GC) equipped with Hewlett-Packard model 5890 series II gas chromatograph with flame ionizer detector. Lecithin of 10 mg was first dissolved in 4 mL of sodium methoxide (1 M in methanol). Reaction was conducted for 30 min under ambient temperature (25 °C) and was stopped by adding a few drops of water. Fatty acid methyl esters (FAME) were extracted by using hexanes and applied to GC for analysis. The conditions used for GC were as follows: injection temperature, 230 °C; detector temperature, 230 °C; oven temperature was programmed from 110 to 220 °C with heating rate of 10 °C/min. The column used was a Supelco SP-2330 (Bellefonte, PA) capillary column, 15 m (length) × 0.25 mm (i.d.) × 0.2 μm (film thickness). In addition, fatty acid profile of each PL classes was measured by first using thin layer chromatography (TLC) to separate PL classes, and then using the GC method to determine fatty acid composition after the PL bands were transesterified as described above. The conditions used for TLC were as follows: TLC plate, Alltech

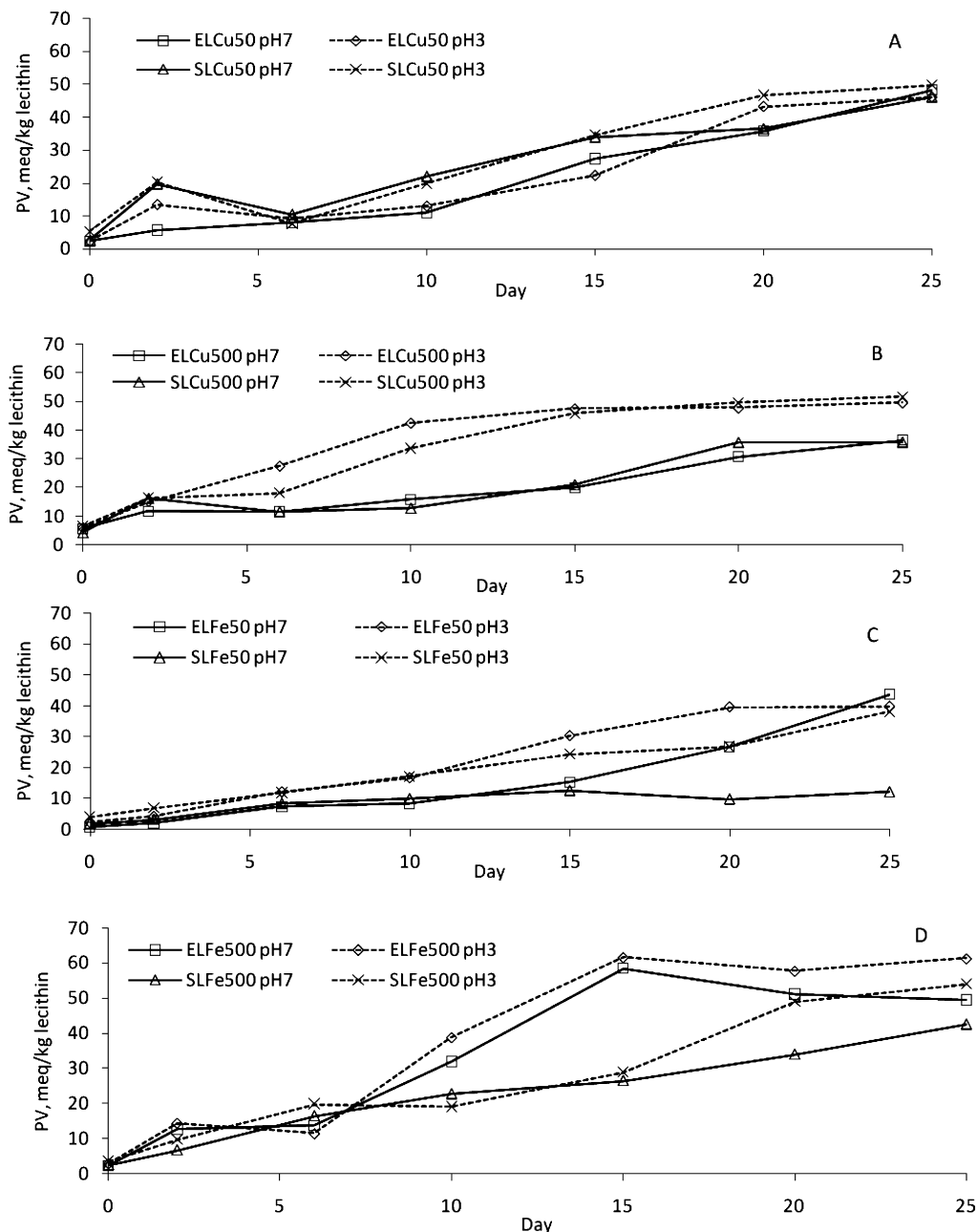


Figure 2. Peroxide value of EL and SL in emulsion under different pH, type and concentration of transition metal ions. Refer to footnote of **Table 3** for abbreviations.

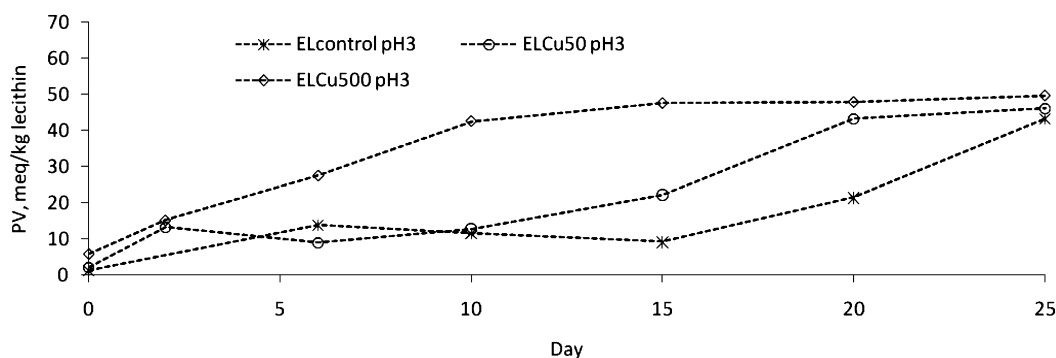


Figure 3. Peroxide value of EL in emulsion as affected by different amount of cupric addition under acidic pH.

Adsorbosil Plus 1 with dimensions 20 × 20 cm, 500 μm (Deerfield, IL); the mobile phase was chloroform:methanol:water (25:10:1, v/v/v).

The PL class composition was determined by a HPLC method as described by Wang and others (13). Briefly, a Beckman-Coulter Gold

HPLC system equipped with a model 508 autosampler and model 126 delivery pumps was connected with Alltech 2000 evaporative light scattering detector (ELSD). A Pholipidec normal phase silica column (250 mm × 4.6 mm i.d., 5 μm particle size) with an integrated guard

Table 4. Zeta Potential (mV) of Lecithin Stabilized O/W Emulsions as Affected by Addition of Various Amounts of Metal Ions^a

| | EL control | ELCu50 | ELCu500 | ELFe50 | ELFe500 |
|------|-------------|-------------|-------------|-------------|-------------|
| pH 7 | -44.4 ± 5.7 | -37.5 ± 0.6 | -36.5 ± 0.2 | -38.1 ± 2.5 | -34.4 ± 2.3 |
| pH 3 | | -66.0 ± 1.6 | -57.2 ± 3.0 | -51.4 ± 3.0 | -52.7 ± 0.7 |
| | SL control | SLCu50 | SLCu500 | SLFe50 | SLFe500 |
| pH 7 | -39.1 ± 5.3 | -33.8 ± 1.5 | -33.3 ± 0.4 | -32.1 ± 3.9 | -30.7 ± 1.0 |
| pH 3 | | | | -57.5 ± 1.3 | -50.8 ± 1.2 |

^aData represents means of two assay ± SD. Certain data under acidic pH were missing because the samples were discarded due to instability at the time of sampling. Refer to notes in **Table 3** for abbreviations.

column was used for separation (Advanced Separations Technologies, Whippany, NJ). A gradient program with two mobile phases at a flow rate of 1 mL/min was used. Phase A was chloroform/methanol/ammonia (80:19:1, by volume) and phase B was chloroform/methanol/ammonia/water (50:48:1:1, by volume). Phase B increased to 100% during the first 25 min and maintained there for 15 min, then it went back to 0% in 2 min. Phase A stayed for another 6 min at 100% before the next analysis was run.

Emulsion Preparation and Sampling. All glassware was immersed in a nitric acid solution (10%, v/v) for 4 h and then rinsed with deionized water before use to ensure glassware were free from metal ion contamination. Oil-in-water (o/w) emulsions were prepared by the following procedure: Lecithin, 4.5 g, was dispersed in 45 mL of mineral oil under 45–50 °C heating. Cupric sulfate and ferric chloride were predissolved in deionized water with concentration of 500 μM. Varied amount of each solution was added to the water phase to obtain desired concentrations of 0, 50, 500 μM ions. Emulsions were prepared by using a Polytron homogenizer equipped with a 3012/2T generator at the speed setting of 20,000 rpm for 60 s in an ice–water bath. All treatments were stored in a dark oven at 60 °C for 25 days. Samples of 10 mL were taken at day 0, 2, 6, 10, 15, 20, 25. All treatments were rehomogenized every 24 h and before sampling time. All samples were stored in a -20 °C freezer for further lipid extraction and analysis.

Emulsion Droplet Size Analysis. Particle size distribution of the oil droplets was measured immediately after emulsion preparation by using a Hydro 2000 MU Laser Scattering Particle Size Analyzer (Malvern, U.K.). The result was expressed as the volume mean diameter (VMD) $D[4,3]$, which measures the average diameter based on the unit volume (mass) of a particle and was calculated as $D[4,3] = \sum n_i d_i^4 / \sum n_i d_i^3$. To measure $D[4,3]$, emulsion (about 0.2–1 mL) was placed into 1 L of water with stirring until obscuration (%) reached a level between 10 and 15, which indicates ideal count of particle for accurate measurement. The final concentration of the emulsion was estimated as between 0.02% and 0.1%. Each sample was measured three times and results were presented as the average.

Zeta Potential. Surface charge of the oil droplets as expressed by zeta potential was measured for each treatment after the emulsion was made. Zeta potential characterizes electric potential difference between the charged emulsion droplet and the surrounding liquid continuous phase. A Malvern Zetasizer Nano Z potential (Malvern, U.K.) was used. Each sample was diluted 1000 times, and well mixed, and placed into a conductive cell. Each sample was prepared twice for this measurement and each preparation was measured three times to obtain the mean values.

Lecithin Oxidation. Emulsion of 10 mL containing 300 mg of lecithin and 3 mL of mineral oil was added to 40 mL of a mixture of isooctane/2-propanol (3:1). Each sample was vortexed and mixed thoroughly for lipid extraction, followed by centrifugation for 5 min at 1000g (14). The organic phase was rotary evaporated with temperature below 45 °C, and the residual solvent was removed by using a vacuum oven for overnight drying. All samples were filled with nitrogen and stored in a freezer until analysis.

To measure PV change with time, the colorimetric method mentioned previously was used. An oil sample of 100 μL containing 10 mg of lecithin was used. To measure secondary oxidation products, a method determining the amount of 2-thiobarbituric acid-reactive substances

(TBARS) modified from refs 15, 16 was used. Briefly, 15% (w/v) trichloroacetic acid (TCA) and 0.375% (w/v) thiobarbituric acid (TBA) were dissolved in 0.25 M hydrochloric acid aqueous solution by mild heating and agitation. Butylated hydroxytoluene (BHT), 3 mL of 2% in absolute ethanol, was added to 100 mL of the TCA/TBA stock solution. An oil sample of 400 μL containing 40 mg of lecithin was added to 4 mL of TCA/TBA stock solution in a test tube. The mixture was mixed thoroughly with a Vortex mixer, and it was put in a boiling water bath for 15 min, and then cooled to room temperature. All samples were then centrifuged at 1000g for 5 min. TBARS were measured at 535 nm with a blank containing only TCA/TBA reagent and mineral oil of 0.4 mL. Concentrations of TBARS were determined using a standard curve prepared using 1,1,3,3-tetraethoxypropane.

Duplicate assays were used for each treatment for both PV and TBARS determinations.

Statistical Analysis. Data analyses were done by using SAS program (version 9.1, SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) was used, and least significant differences were calculated at $P = 0.05$.

RESULTS AND DISCUSSION

Lecithin Characterization. The initial oxidation state of lecithin is important because it can affect the rate of further oxidation of lipids. The PV of EL was measured as 0 meq/kg by the AOCS official method and as 0.054 meq/kg by a modified iron colometric method. The PV of SL was 2.29 and 4.91 meq/kg by these two methods. The results from the two oxidation quantification methods showed that the colorimetric method gave slightly higher measurement than the AOCS method. Since we had a very limited amount of PLs for oxidation evaluation in the emulsion system, and the colorimetric method is a highly sensitive and convenient method to use, we chose this method for PV quantification. We see that evaluating oxidation trend is more important in this study than determining the absolute state of oxidation, therefore, the slight difference in the measurement using the two methods was not a serious concern for this study. Nonetheless, the difference in PV was suspected to be caused by the color difference when the oil and PL hydroperoxides reacted with the reagents.

Elemental iron and copper in EL were 3.4 and 2.6 ppm, and they were measured as 11.4 and 1.2 ppm in SL.

Tocopherol quantification by HPLC analysis showed that no tocopherols were detected in EL while three tocopherols, alpha, gamma, and delta, existed in the commercial SL, and their contents are presented in **Table 1**. It is shown that considerable amounts of gamma (about 11 mg/100 g) and delta (about 13 mg/100 g) tocopherols were contained in the commercial SL. Acetone washing removed almost all of these tocopherols, a total of 96% reduction.

Fatty acid profiles of both EL and SL are shown in **Table 2**. EL contained much less linoleate, 16.2%, and linolenate (not detectable) compared with SL, which had 56.6% and 7.1% of these fatty acids. On the other hand, EL had significant amount of other PUFA, such as arachidonic acid (AA, C20:4 ω6, 6.1%), and docosahexaenoic acid (DHA, C22:6 ω3, 1.7%). Fatty acid identification from C14 to C18 was verified by standard samples from Nu-CHEK-PREP, Inc. (Elysian, MN). C20:4 and C22:6 were confirmed by comparing to EL specification and analytical report provided by our supplier. The long chain PUFAs totaled to 9.4% in EL. All fatty acids were relatively uniformly distributed in PC and PE fraction of SL. For EL, higher amount of AA was found in PE than in PC, and its PE fraction had less oleic, linoleic, palmitic but more stearic acid than PC. HPLC analysis showed that PC/PE ratio in EL was 4.3, while it was

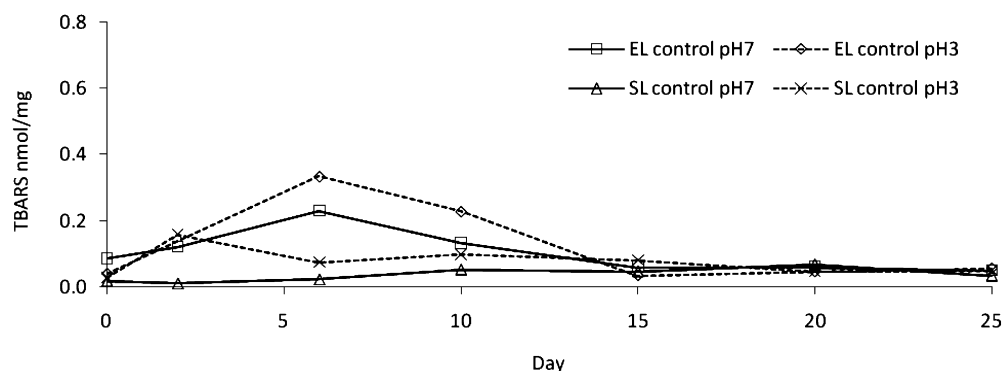


Figure 4. TBARS development of EL and SL in emulsion under different pH conditions. Refer to footnote in Table 3 for abbreviations.

1.4 for SL. The total quantifiable PLs in SL were 61%. SL contained a significant amount of PI too, but it was not quantified because of some unexpected technical difficulties.

Effect of pH and Cupric Concentration on PV Development of EL and SL. For EL and SL oxidation in emulsion without any metal ion addition, no differences were observed for type of lecithin and under the two pH conditions, as shown in Figure 1. All four treatments were slowly oxidized and PV reached to 40 meq/kg at the end of 25 days.

To examine if pH affects emulsion stability, we measured particle size of the oil droplets. It was found that, under acidic pH, neither EL nor SL emulsion was as stable as they were under neutral pH as shown in Table 3. The particle size of EL control under neutral and acidic conditions was 6.2 and 53.2 μm , respectively, and it was 8.9 and 253.4 μm for SL control. And for all the treatments with metal ions added, the particle diameter values under acidic conditions were all much higher than those under neutral conditions. The reason for this observation is unknown.

Addition of 50 μM (about 3.2 ppm) cupric sulfate did not change overall trend of PV for all treatments though SL showed slightly higher PV than EL under pH 7 and 3 (Figure 2A). pH did not show a significant effect for both EL and SL at this ion concentration. This slight more oxidation of SL than EL at low cupric concentration agrees with our previous research results, where 1 ppm cupric under neutral pH was used to compare oxidative stability of the two lecithins. As the concentration of cupric sulfate was increased to 500 μM (32 ppm), pH effect became significant. For both EL and SL emulsions, low pH significantly increased degree of oxidation of PLs as shown in Figure 2B. PVs of low pH EL and SL quickly increased to above 40 meq/kg after 10 to 15 days, and PVs of neutral pH EL and SL were less than 20 meq/kg at the same time. Under both pH conditions, EL had similar oxidative stability as SL. Therefore, at high cupric concentration, EL and SL were oxidized faster at acidic pH than at neutral pH, and the two lecithins behaved similarly for their oxidative stability under the two cupric and two pH conditions, as measured by lipid hydroperoxide formation.

To illustrate PV's response to cupric concentration under acidic pH, we plotted Figure 3, which shows EL oxidation. It seems that as cupric concentration increased, the induction period for EL oxidation was shortened.

Effect of pH and Ferric Concentration on PV Development of EL and SL. Different from cupric treatments, for treatments with 50 μM (2.8 ppm) ferric chloride, both EL and SL at pH 3 oxidized slightly faster than that at pH 7 at early stage of the oxidation, as shown in Figure 2C. In addition, under both pH conditions, EL started to show higher PV than SL after day 10. For treatments containing 500 μM (28 ppm) ferric

chloride, EL at both pH conditions oxidized much faster than SL, as shown in Figure 2D. PV of EL reached peak value about 60 meq/kg under both pHs at day 15, and it was only about 25 meq/kg for SL at two the pHs. The effect of pH was not as significant as that shown in lecithin oxidation with 500 μM cupric ion.

Cross-comparing Figures 2C and 2D, it was shown that EL oxidation positively responded to the increasing ferric ion concentration. It is also important that our data show that EL was more sensitive to ferric ion catalyzed oxidation than that by cupric ion at both pHs.

Many studies have been conducted to examine the relationship between oxidative stability and droplet size of oil-in-water emulsion. A general belief is that small particle size increases surface area and thus increases the chance of oil droplet being contacted with pro-oxidant transition metal ions, leading to fast lipid oxidation (2, 17). Others reported that particle size of oil droplets did not affect lipid oxidation rate of o/w emulsion (18, 19), or decreased particle size improved oxidative stability of various o/w emulsion (20). Our study seems to suggest that particle size did not significantly affect PL oxidation. As shown in column 1 in Table 3 all four treatments had a wide range of particle size while they had similar oxidation trend in terms of PV developments. To have a definitive answer of how oil droplet size affects lipid oxidation, a better designed and controlled experiment needs to be carried out.

Electrical charge of emulsion droplet can be an important factor in lipid oxidation (16). For PL emulsifier, PE and PC are zwitterionic ions, and PI is the anionic type. Phosphorus group is negatively charged while choline (in PC) and ethanolamine (in PE) groups are positively charged under neutral condition and, therefore, PC and PE do not carry net charge. Since inositol group in PI has no charge, PI under neutral conditions is negatively charged. In o/w emulsion where transition metal ions are positively charged, the ions will migrate toward the oil droplets that have net negatively charged PLs, causing rapid lipid oxidation. Theoretically, SL should oxidize faster than EL under neutral pH conditions, since SL is negatively charged due to its relatively high PI content. However, the fatty acid composition of lecithin is another important factor in lipid oxidation. Therefore, the comparison of the oxidative stability of the two lecithins in emulsion is complex, and experimental data should reflect the effect of combination of influencing factors.

The surface charge of the oil droplets was measured as zeta potential. As shown in Table 4, under neutral conditions both EL and SL showed a decrease in zeta potential as transition metal ion was added, and this is as expected. EL consists of PE (18.1%) and PC (78.7%), whereas PE and PC were only 61% in total SL, and the other 40% is mainly the anionic types,

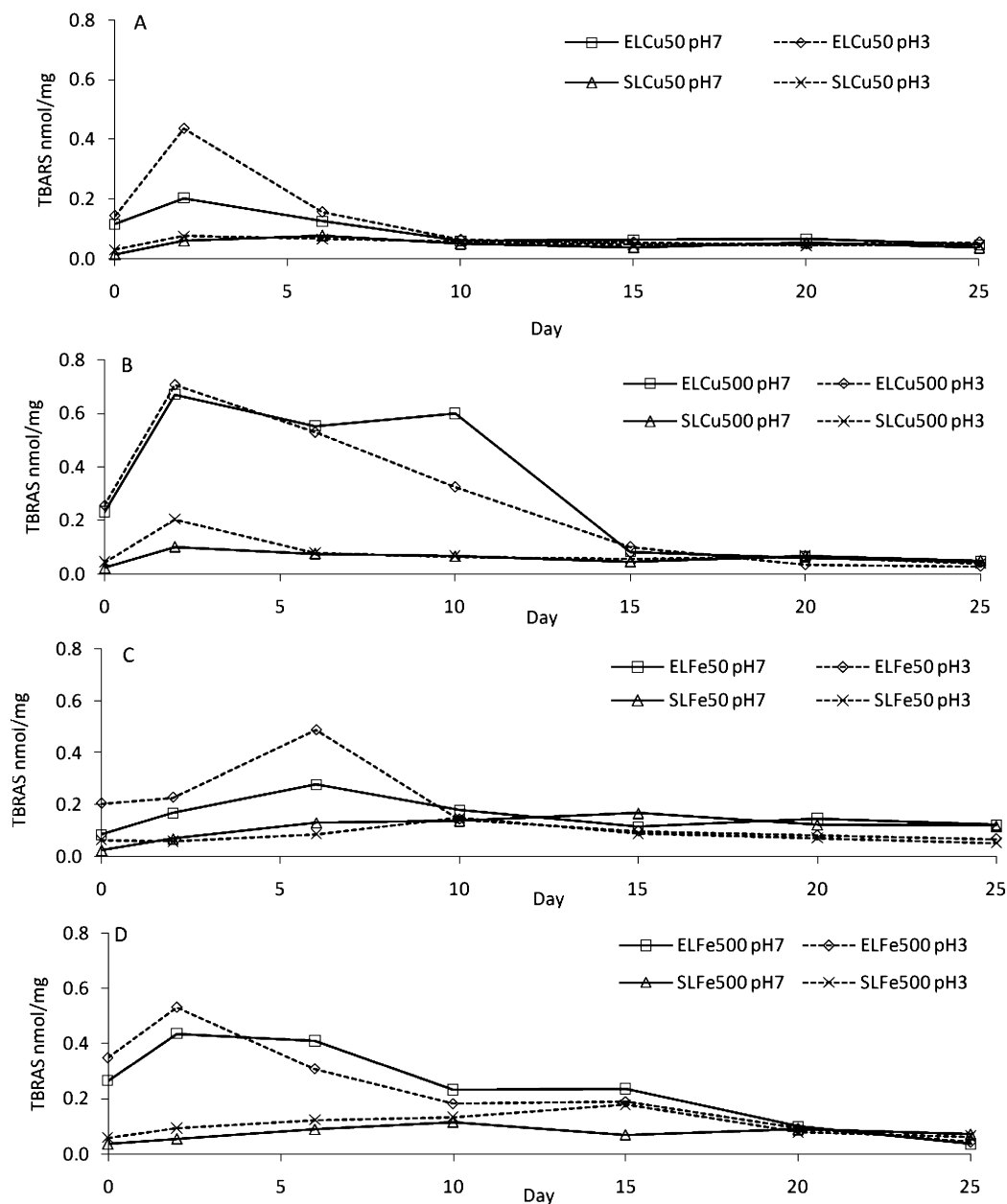


Figure 5. TBARS development of EL and SL in emulsion under different pH, type and concentration of transition metal ions. Refer to footnote in **Table 3** for abbreviations.

i.e. PI and PA (not quantified). Theoretically, SL control should have more negative zeta potential than EL control, but the data did not show this. Similar results were also reported by Sørensen and others (21). **Table 4** also shows that acidic pH has increased zeta potentials significantly compared to that at pH 7. The increased zeta potential at low pH may have contributed to the increased oxidation rate when metal ions were added, because they should be more attracted to the oil droplet surface.

Effect of pH and Cupric Concentration on TBARS Development of EL and SL. The TBARS method measures the end products of lipid hydroperoxidation decomposition. As shown in **Figure 4**, EL had higher TBARS development over time than SL, and low pH tended to promote more TBARS generation. As presented in **Table 2**, SL contains much less C20:4 and C22:6 than EL though SL had more linolenic acid. It was reported that TBARS developed from C20:4 was 8 times higher than that from linolenic acid (22). EL reached a peak TBARS value at day 6 with 0.20 and 0.35 nmol/mg for treatments under pH 7 and pH 3, respectively. SL at pH 7 had

no obvious peak throughout storage, but SL at pH 3 formed TBARS much faster and reached the peak value of 0.16 at day 2. The effect of pH may be because of its catalytic activity in breaking the primary products to secondary products.

For treatments with addition of 50 μM cupric sulfate, EL and SL showed a similar pattern as the controls. The pH effect became more significant for EL where EL at pH 7 had a peak value of 0.2 and at pH 3 it was 0.42. However, SL did not show a difference between two pH levels (**Figure 5A**).

With higher cupric concentration of 500 μM , as seen in **Figure 5B**, TBARS of EL at both pH levels increased to about 0.7 at day 2, and pH effect was no longer significant. Also, SL showed increased TBARS, but the TBARS difference between the two lecithins was further increased by the high ion concentration. SL at pH 3 reached peak value of TBARS of 0.2, and it was 0.1 at pH 7.

A further cross-examination of **Figures 4, 5A**, and **5B** helps to understand the effect of cupric concentration on TBARS change of each treatment. For EL in emulsions under both pHs,

addition of 50 μM cupric moved their peak value from day 6 to day 2 although its TBARS value was only slightly increased; further increasing cupric sulfate (500 μM) caused much faster formation of TBARS during the first 2 days. A similar phenomenon was also observed for SL in emulsion at pH 7. However, adding a large amount of cupric ion did not change SL TBARS as much as in EL under acidic pH.

Effect of pH and Ferric Concentration on TBARS Development of EL and SL. For treatments with 50 μM ferric chloride addition, EL at pH 3 oxidized 2 times faster than that at pH 7 at day 6, but low pH did not accelerate formation of TBARS for SL, as seen in **Figure 5C**. Still, EL had much higher TBARS than SL under both pH conditions. With higher ferric concentration, 500 μM (**Figure 5D**), EL at pH 3 showed slightly higher TBARS than that at pH 7. And SL at pH 3 also had overall slightly higher TBARS than that at pH 7. Examining **Figures 4, 5C, and 5D** together, we observed that adding 50 μM ferric chloride into EL emulsion did not change its time to reach maximal TBARS value, which was 6 days. Also, adding 500 μM ferric ion did not cause much increase in TBARS as it was seen in cupric treatments (**Figures 5B and 5D**). This phenomenon was also observed for SL emulsions. This indicated that cupric ion had a more powerful prooxidant effect than ferric ion with respect to TBARS development, and EL was more sensitive to cupric ion catalyzed oxidation as determined by TBARS.

For all the treatments, it was observed that TBARS decreased after it reached a peak value. The possible reason for this change of concentration of TBARS over time is that malondialdehyde (MDA) was first produced to a maximal level and it went down when available fatty acid for MDA formation was depleted (23). Because of this reason, if only simply recording TBARS value at the end of certain storage time, one may miss its peak value and give an improper conclusion. A full examination of oxidation which includes both peak value and the time to reach the value presents a better insight for lipid oxidation.

Relationship between Fatty Acid Composition, PL Class Composition and PV and TBARS Formation. As we discussed, lipid oxidation is a process initialized from unsaturated fatty acids. Usually it is believed that lipid-containing foods with high content of PUFAs deteriorate much faster. However, depending on what indicator is used for measuring oxidative stability, the results can be different. With respect to PV measurement, many researchers have agreed that linoleic and linolenic acids are oxidized much faster than oleate. But for those long-chain PUFAs, such as AA and DHA, they were found to have lower PV than linoleate in aqueous micelles and certain emulsions (22, 24). In our case, since SL contained 56.6% linoleate while EL had only 16.2% linoleate (**Table 2**), we would assume that SL had lower oxidative stability than EL. However, this was found not true. This indicates that there are many other factors, such as PL class composition, interaction between PL and transition metal ions, that all affect oxidative stability of lecithin in emulsion.

Factors influencing TBARS are very complicated. This oxidation indicator is not only governed by fatty acid profile but also determined by PL class composition. Pikul and others (25, 26) found that PL contributed about 90% of the total TBARS in a chicken meat containing PLs, TAG, and cholesterol ester. And further study on individual PL fraction revealed that PC and PE produced most of TBARS (77% and 90%) while only about 20% was formed by PS and PI. Our study confirmed this result with respect to TBARS difference between EL and SL. For the effect of fatty acid profile on TBARS development,

Visioli and others (22) also pointed out that TBARS production was maximal for DHA and AA which were about 8–10 times higher than that for linoleate. EL contains 1.7% DHA and 6.1% AA while none was found in SL. This could explain why EL samples had always shown much higher TBARS compared to SL.

LITERATURE CITED

- (1) Berliner, J. A.; Subbanagounder, G.; Leitinger, N.; Watson, A. D.; Vora, D. (2001) Evidence for a role of phospholipid oxidation products in atherogenesis. *Trends Cardiovasc. Med.* **2001**, *11*, 142–147.
- (2) McClements, D. J.; Decker, E. A. Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **2000**, *65*, 1270–1282.
- (3) Decker, E. A.; Warner, K.; Richards, M. P.; Shahid, F. Measuring antioxidant effectiveness in food. *J. Agric. Food Chem.* **2005**, *53*, 4303–4310.
- (4) Osborn, H. T.; Akoh, C. C. Effect of natural antioxidants on iron-catalyzed lipid oxidation of structured lipid-based emulsions. *J. Am. Oil Chem. Soc.* **2003**, *80*, 847–852.
- (5) Kolakowska, A. *Chemical and functional properties of food lipid*; CRC Press: New York, 2003; pp 133–166.
- (6) Frankel, E. N. *Lipid oxidation*; The Oily Press: Dundee, U.K., 1998.
- (7) Fatemi, S. H.; Hammond, E. G. Analysis of oleate, linoleate and linolenate hydroperoxides in oxidized ester mixtures. *Lipids* **1980**, *15*, 379–385.
- (8) Yoshida, Y.; Niki, E. Oxidation of methyl linoleate in aqueous dispersions induced by copper and iron. *Arch. Biochem. Biophys.* **1992**, *295*, 107–114.
- (9) Sikorski, Z. E.; Kolakowska, A. *Chemical and functional properties of food lipids*; CRC Press: New York, 2003.
- (10) Wang, T.; Jiang, Y.; Hammond, E. G. Effect of randomization on the oxidative stability of corn oil. *J. Am. Oil Chem. Soc.* **2005**, *82*, 111–117.
- (11) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998.
- (12) AOAC. *Official Methods of Analysis of AOAC International*, 18th ed.; AOAC International: Gaithersburg, MD, 2007.
- (13) Wang, L.; Wang, T.; Fehr, W. R. HPLC quantification of sphingolipids in soybeans with modified palmitate Content. *J. Agric. Food Chem.* **2006**, *54*, 7422–7428.
- (14) Shantha, N. C.; Decker, E. A. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxides values of food lipids. *J. AOAC Int.* **1994**, *77*, 421–424.
- (15) McDonald, R. E.; Hultin, H. O. Some characteristics of the enzymic lipid peroxidation systems in the microsomal fraction of flounder muscle. *J. Food Sci.* **1987**, *52*, 15–21.
- (16) Mei, L.; Decker, E. A.; McClements, D. J. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *J. Am. Oil Chem. Soc.* **1998**, *46*, 5072–5077.
- (17) Gohtani, S.; Sirendi, M.; Yamamoto, N.; Kajikawa, K.; Yamano, Y. Effect of droplet size on oxidation of docosahexanoic acid in emulsion system. *J. Dispersion Sci. Technol.* **1999**, *20*, 1319–1325.
- (18) Lethuaut, L.; Metro, F.; Genot, C. Effect of droplet size on lipid oxidation rates of oil-in-water emulsions stabilized by protein. *J. Am. Oil Chem. Soc.* **2002**, *70*, 425–430.
- (19) Osborn, H. T.; Akoh, C. C. Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chem.* **2004**, *84*, 451–456.
- (20) Nakaya, K.; Ushio, H.; Matsukawa, S.; Shimizu, M.; Ohshima, T. Effect of droplet size on the oxidative stability of oil-in-water emulsions. *Lipids* **2005**, *40*, 501–507.
- (21) Sørensen, A. M.; Haahr, A.; Becker, E.; Skibsted, L. H.; Bergenstahl, B.; Nilsson, L.; Jacobsen, C. Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-

- enriched oil-in-water emulsions. *J. Agric. Food Chem.* **2008**, *56*, 1740–1740.
- (22) Visioli, F.; Colombo, C.; Galli, C. Oxidation of individual fatty acids yields different profiles of oxidation markers. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 487–489.
- (23) Hendriks, W. H.; Cottam, Y. H.; Thomas, D. V. The effect of storage on the nutritional quality of meat and bone meal. *Anim. Feed Sci. Technol.* **2006**, *127*, 151–160.
- (24) Miyashita, K.; Nara, E.; Ota, T. Oxidative stability of polyunsaturated fatty acids in aqueous solution. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1638–1640.
- (25) Pikul, J.; Leszczynski, D. E.; Kummerow, F. A. Relative role of phospholipids, triacylglycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *J. Food Sci.* **1984**, *49*, 704–708.
- (26) Pikul, J.; Kummerow, F. A. Thiobarbituric acid reactive substance formation as affected by distribution of polyenoic fatty acids in individual phospholipids. *J. Agric. Food Chem.* **1991**, *39*, 451–457.

Received for review July 24, 2008. Revised manuscript received September 27, 2008. Accepted October 3, 2008. This research is supported by the Iowa Egg Council and the Midwest Advanced Food Manufactures Alliance.

JF8022832