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ORIGINAL PAPER

Antioxidant and Prooxidant Activity Behavior of Phospholipids in Stripped Soybean Oil-in-Water Emulsions

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Abstract Phospholipids have been reported to inhibit lipid oxidation in bulk oils, but very little is known about their influence on oxidation in oil-in-water emulsions. In the present study, the impact of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) on lipid oxidation was studied in 1% stripped soybean oil-in-water (O/W) emulsions as a function of DOPC concentration and pH (3 and 7). At pH 7.0, DOPC inhibited lipid oxidation in O/W emulsions, while DOPC was prooxidative at pH 3.0. DOPC did not affect emulsion droplet charge or size at either pH 3.0 or 7.0. The antioxidant activity at pH 7.0 was observed in a series of phospholipids (PL) that varied in fatty acid unsaturation level and chain length as well as type of phosphate head group. Overall, phosphatidylcholine with either oleic or palmitic acid were the most effective at inhibiting lipid hydroperoxide and hexanal formation of all of the PL tested. Antioxidant mechanism of PL could not be ascribed to their ability to decompose lipid hydroperoxides. It might be possible that, at pH 7.0, the PL antioxidant activity is related to their ability to form structures within the lipid phase of the emulsions droplets or to chelate metals.

Keywords Oil-in-water emulsions · Phospholipids · Soybean oil · Oxidation · pH · Particle size · Zeta potential · Antioxidant activity · Hydroperoxides · Hexanal

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Abbreviations

O/W	Oil-in-water emulsions
DBPC	1,2-Dibutyroyl-*sn*-glycero-3-phosphocholine
DOPC	1,2-Dioleoyl-*sn*-glycero-3-phosphocholine
DPPC	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine
DOPE	1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine
PL	Phospholipid(s)
MCT	Medium chain triacylglycerol(s)
EDTA	Ethylenediaminetetraacetic acid
SPME	Solid-phase microextraction

Introduction

Over the past few years, the vegetable oil utilization has been diversified beyond foods into numerous other applications. This has been driven by industrial firms choosing ingredients that derive from renewable sources and that are non-toxic, biodegradable, and non-persistent in the environment [1]. As oils find more and more commercial applications, the one thing that remains in common is their susceptibility to lipid oxidation which can lead to fatty acid decomposition into off-flavors and fatty acid polymerization that can cause equipment fouling. This is especially true in oil-in-water (O/W) emulsions which are extremely susceptible to oxidation. O/W emulsions are more prone to lipid oxidation than bulk oils, due to their emulsion droplet interfacial characteristics which influence the interactions between the lipids and water-soluble prooxidants. Oxidation of O/W emulsions are therefore strongly impacted by factors such as pH, ionic strength, the type, concentration and location of the surfactant and endogenous prooxidants in the food ingredients [2, 3].

Phospholipids are natural surface-active compounds that are widely used in the oil industry as O/W emulsion stabilizers. The ability of phospholipids to inhibit lipid oxidation in bulk oils has been known for several decades, but the mechanism of stabilization still remains controversial. Pokorny [4] reported that phosphatidylcholine reacts with peroxy radicals to yield trimethylammonium oxides, whereas phosphatidylamine can react with lipid hydroperoxides in the non-radical way to form imines suggesting that two possible mechanisms of inhibiting lipid oxidation are free radical scavenging and decomposition of lipid hydroperoxides into stable molecules. In addition, the phospholipids can chelate metals and increase the partitioning of other antioxidants at oil-water interfaces in bulk oils, increasing their effectiveness [5].

While there has been extensive research on the ability of phospholipids to impact lipid oxidation in bulk oils, very little has been reported in O/W emulsions. When phospholipids are the sole emulsifier used to stabilize O/W emulsions, they form an external anionic layer that accelerates lipid oxidation [6, 7]. However, this might not be the situation if low concentrations of phospholipids are added to an O/W emulsion along with another surface active agent that would be the primary emulsifier. The higher concentration of the primary emulsifier could, in fact, displace the phospholipid from the water-oil interface, thus increasing the partitioning of the phospholipid into the lipid core of the emulsion droplet. If this were the case, then the presence of the phospholipid in the emulsion droplet core could allow it to inhibit lipid oxidation in a manner similar to bulk oils.

Understanding how chemical properties of phospholipids impact lipid oxidation and the physical properties of emulsions could provide fundamental knowledge that could be used to improve the oxidative stability of oils in emulsions and other food dispersions. The aim of the present work was to study the effect of some phospholipids on the oxidative stability of stripped soybean oil-in-water emulsions stabilized with Tween 20. Phospholipids with three different chain lengths (butyric, oleic and palmitic acids) and two different headgroups (choline and ethanolamine) were used. The research work was divided into four steps: (a) investigation on the role of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) on emulsions' oxidation as related to its concentration and pH; (b) comparison of DOPC behavior with that of the other phospholipids having different levels of unsaturation (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC), fatty acid chain length (1,2-dibutyroyl-*sn*-glycero-3-phosphocholine; DBPC) and head group (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPE); (c) investigation on the interaction between cumene hydroperoxide and phospholipids.

Materials and Methods

Materials

Refined soybean oil was purchased from a local retail store (Amherst, MA). 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dibutyroyl-*sn*-glycero-3-phosphocholine (DBPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids Inc. (Alabaster, Al). Ethylenediaminetetraacetic acid (EDTA), potassium phosphate monobasic, potassium phosphate dibasic heptahydrate, silicic acid (100–200 mesh, 75–150 mm, acid washed), activated charcoal (100–400 mesh), polyoxyethylene sorbitan monolaurate (Tween 20), ammonium thiocyanate and iron (II) sulfate heptahydrate, were supplied by Sigma Chemical Co. (St. Louis, MO). Iso-octanol, *n*-hexane, iso-propanol, methanol and 1-butanol, were purchased from Fisher Scientific (Fair Lawn, NJ). All the chemicals used were at least of analytical grade. Glassware was incubated in 3 mM HCl overnight to remove metals, followed by rinsing with double-distilled water before use. Double-distilled water was employed throughout the study.

Preparation of Stripped Soybean Oil

Stripped soybean oil was prepared according to Boon et al. [8] and was used in all experiments. In short, silicic acid (100 g) was washed three times with a total volume of 3 L of distilled water, followed by filtering with Whatman filter paper (No.1; Whatman International Ltd., Maidstone, England) in a Buchner funnel and drying at 110 °C for 20 h. A chromatographic column (3.0 cm internal diameter × 35 cm height) was then packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of soybean oil was dissolved in 30 mL of *n*-hexane and passed through the column by eluting with 270 mL of *n*-hexane. To retard lipid oxidation during stripping, the container used to collect the triacylglycerols was held in an ice bath and covered with aluminum foil. The solvent present in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37 °C and traces of the remaining solvent were evaporated under nitrogen stream. The effectiveness of stripping was verified by measuring the removal of tocopherols to below detectable levels [8]. Three grams of the stripped oil were then transferred into 3-mL vials, flushed with nitrogen and kept at −80 °C for subsequent studies.

Emulsion Preparation and Storage Conditions

Oil-in-water (O/W) emulsions were prepared using 1.0% wt stripped soybean oil and 10 mM phosphate buffer

solution (pH 7.0 and 3.0). Tween 20 was used as the emulsifier at a 1:10 emulsifier:oil ratio. The emulsion was prepared by adding purified phospholipids in chloroform into a beaker and flushing with nitrogen gas to remove the solvent. Stripped soybean oil, Tween 20, and phosphate buffer were then added to the beaker and a coarse emulsion was made by blending with a hand-held homogenizer (M133/1281-0, Biospec Products Inc., Bartlesville, OK) for 2 min. The coarse emulsion was then homogenized with a microfluidizer (Microfluidics, Newton, MA) at a pressure of 9 kbar for three passes. During homogenization, ice was used to cover the homogenizer chamber and coil, in order to maintain the emulsion temperature at $\leq$25 °C. One milliliter of each emulsion was transferred into 10 mL GC vials (Supelco), capped with aluminum lids having PTFE/silicone septa and stored in the dark at 7 °C.

The sequence of the emulsions' experimental design is listed as follows: (a) O/W emulsions prepared with different concentrations of DOPC (0.0003, 0.003, 0.03 and 0.3 mmol/kg oil) at pH 7.0 and stored at 7 °C for 9 days; (b) O/W emulsions prepared with DOPC (0.0003, 0.003, 0.03 and 0.3 mmol/kg oil) at pH 3.0 and stored at 7 °C for 13 days; (c) O/W emulsions separately prepared with DOPC, DBPC, DPPC and DOPE (0.3 mmol/kg oil) at pH 7.0 and stored for 9 days at 7 °C; (d) medium chain triacylglycerol-in-water emulsions with and without cumene hydroperoxide, separately prepared with DOPC, DBPC, DPPC and DOPE (0.3 mmol/kg oil) at pH 7.0 and stored for 48 h at 27 °C. Each experiment included control samples (emulsions without added PL).

Evaluation of Particle Size Distributions and Zeta Potential (ζ)

Samples for droplet size distribution and zeta potential measurements were prepared by diluting the emulsion into 10 mM phosphate buffer at the same pH (pH 7.0 and 3.0) as the emulsions, keeping an emulsion:buffer ratio at 1:50. Both particle size distributions and zeta potential of the emulsions were analyzed in a ZetaSizer Nano-ZS (Malvern Instruments, Worcestershire, UK). The particle size and zeta potential were determined right after emulsion preparation and every day during each experiment. Each measurement was repeated twice at room temperature.

Evaluation of Lipid Oxidation

Lipid hydroperoxides, which are primary lipid oxidation products, were determined using a modified version of the method reported by Shantha and Decker [9]. Each sample (0.3 mL) was vortexed three times (10 s each) with 1.5 mL of an iso-octanol:iso-propanol (3:1, v/v) solution. The samples were then centrifuged for 2 min at 3,400 g (Centrific TM Centrifuge, Fisher Scientific, Fairlawn, NJ) and 0.2 mL of the upper organic layer was mixed with 2.8 mL of methanol:butanol solution (2:1, v/v), 15 μL of 3.94 M ammonium thiocyanate and 15 μL of a ferrous iron solution. The ferrous iron solution was prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$. Twenty min after iron addition, the absorbance of the samples was measured at 510 nm, using a Genesys 20 spectrophotometer (Thermo-Spectronic, Waltham, MA). Hydroperoxide levels were quantified on the basis of a cumene hydroperoxide standard calibration curve (0.8–800 mmol/kg oil; $y = 0.0497x + 0.0832$, $r^2 = 0.996$).

Headspace hexanal was determined as a secondary lipid oxidation product by solid-phase microextraction-head space gas chromatography with flame ionization detection (SPME-GC-FID), according to Boon et al. [8]. The instrument was a GC-17A Shimadzu model (Shimadzu, Kyoto, Japan), which was equipped with an AOC-5000 autosampler (Shimadzu) and a split-splitless injector. An Equity DB-1 column (30 m × 0.32 mm × 1 mm film thickness, Supelco, Bellefonte, PA), was used for separation of volatiles. Samples were shaken and heated at 55 °C for 13 min in an autosampler heating block before measurement. A 50/30 mm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber needle (Supelco, Bellefonte, PA) was introduced into the vial for 1 min to absorb volatiles and then was transferred to the injector port to allow volatile desorption for 3 min at 250 °C. Oven temperature was 65 °C. The injector and detector temperatures were both set at 250 °C. Helium was used as carrier gas at a flow rate of 1.0 mL/min with a split ratio of 1:5. Hexanal concentrations were determined from peak areas using a calibration curve prepared with hexanal standard solutions (0.2–750 mmol/kg oil; $y = 43.98x + 5100$ and $r^2 = 0.981$).

Both lipid hydroperoxides and headspace hexanal were determined right after the emulsions' preparation (t 0), after 6 h and then every 24 h.

Statistical Analysis

All experiments were performed with triplicate samples and were repeated at least twice. Data are presented as means ± standard deviation. All data results were treated by one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., Chicago, IL). The differences between mean values were compared using Tukey's multiple range test with a significance level defined as $P \leq 0.05$.

Results and Discussion

Effect of 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine Concentrations on the Physical and Chemical Properties of O/W Emulsions at pH 7.0 and 3.0

Transition metals are abundant in nature and, thus, can end up in food from a variety of sources, such as water, packaging material, processing equipment and ingredients including fats and oils [10]. Transition metals, such as iron, primarily accelerate lipid oxidation by promoting the lipid hydroperoxide decomposition into highly reactive alkoxy and peroxy radicals, which can abstract hydrogen from unsaturated fatty acids, thus further propagating oxidation [11]. Iron is, in fact, the major prooxidant in most O/W emulsions [12]. Iron reactivity in emulsions can be influenced by pH, since its solubility increases with decreasing pH. Another factor that influences the prooxidant activity of iron in emulsions is its physical location, which influences its ability to interact with lipid hydroperoxides. For instance, iron-lipid hydroperoxide interactions and, thus, lipid oxidation rates increase dramatically in negatively charged emulsion droplets, where iron is attracted to the emulsion droplet surface [12, 13]. Thus pH could impact lipid oxidation in O/W emulsions containing DOPC by modifying iron solubility and/or the charge of the phosphate head group on DOPC. Alteration of the DOPC charge could change emulsion droplet charge if DOPC is at the droplet interface or could alter the ability of DOPC to chelate metals and inhibit their reactivity. Therefore, the effect of DOPC on lipid oxidation of O/W emulsions was tested at both pH 7.0 and 3.0. Since the ability of compounds to impact lipid oxidation rates in O/W emulsions depends on their concentration, DOPC was, therefore, added to the emulsions prior to homogenization at concentrations ranging from 0.0003 to 0.3 mmol/kg oil.

The droplet size of all emulsions used in this study was measured immediately after the emulsion's preparation and every 24 h throughout storage. At pH 7.0, emulsion droplets diameters ranged from 170 to 188 nm while, at pH 3.0, emulsion droplets sizes were higher (260–328 nm). At both pH values, droplet sizes did not significantly change during the entire length of all experiments. The stability of the emulsions was also confirmed by no visual observation of creaming during storage (data not shown). The steady trends of these parameters indicated that the emulsions were stable against droplet aggregation, flocculation, or coalescence [2].

Table 1 shows the droplet surface charge or zeta potential (ζ) of the emulsions with varying concentrations of DOPC. At pH 7.0, the zeta potential of control emulsions had a surface charge of −5.64 mV, while the samples with DOPC ranged from −5.45 to −6.25 mV. Tween 20-stabilized O/W emulsions have been previously reported to be negatively charged [14–17]. No consistent reduction in surface charge ($P > 0.05$) with increasing DOPC concentrations was observed, suggesting that the negatively charged phosphate groups of DOPC did not migrate and concentrate at the lipid-water interface of the emulsion droplet. Lack of the ability of the DOPC to change emulsion droplet charge could be due to its low concentrations compared to Tween 20, which would result in Tween 20 displacing DOPC from the emulsion droplet interface [2].

Table 1 The droplet surface charge or zeta potential (ζ) of 1.0% stripped soybean oil-in-water emulsions without (control) and with addition of 0.0003, 0.003, 0.03, 0.3 mmol/kg oil DOPC at pH 7.0 and 3.0

Sample	pH 7.0	pH 3.0
Control	−5.64 ± 0.1a	−1.85 ± 0.0ab
O/W + 0.0003 mmol of DOPC/kg oil	−6.25 ± 0.1b	−1.49 ± 0.0a
O/W + 0.003 mmol of DOPC/kg oil	−6.23 ± 0.2b	−1.96 ± 0.1ab
O/W + 0.03 mmol of DOPC/kg oil	−6.23 ± 0.3b	−1.67 ± 0.2a
O/W + 0.3 mmol of DOPC/kg oil	−5.45 ± 0.2a	−2.52 ± 0.6b

Each value represent the mean ($n = 3$) ± standard deviations. Different letters within a column (a, b) are statistically different means (Tukey's test; $P \leq 0.05$)

Lipid oxidation rates of 1.0% stripped soybean O/W emulsions with DOPC at pH 7.0 were evaluated by lipid hydroperoxide and hexanal as indicators of primary and secondary oxidation products (Fig. 1a, b). Increasing DOPC concentrations significantly decreased both lipid hydroperoxides and headspace hexanal. After 3 days of storage, control emulsions exhibited a significantly higher hydroperoxide concentration than the other emulsions. After 6 days of storage, a marked hydroperoxide increase was observed in all the emulsions that contained DOPC (Fig. 1a). At 0.003 mmol/kg oil, DOPC was less effective than higher DOPC concentrations. However, the ability of the other DOPC concentrations (0.003–0.3) to inhibit lipid hydroperoxide formation was similar. An analogous trend in inhibition of lipid oxidation by DOPC was also observed when hexanal was used as oxidation marker (Fig. 1b).

Table 1 shows that the droplet surface charge of the emulsions at pH 3.0 was lower than at pH 7.0. Waraho and co-workers [14] found that the zeta-potential of Tween 20-stabilized O/W diminished with decreasing pH. No significant differences were found in emulsion droplet charge in the presence or absence of varying concentrations of DOPC with the surface charge ranging from −1.49 to

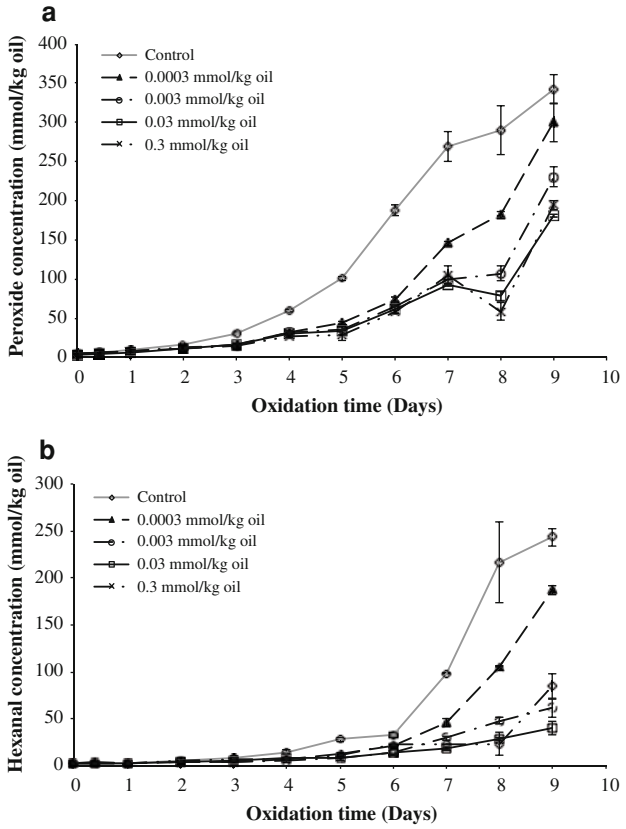

Fig. 1 Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions at pH 7.0, without (control) and with addition of 0.0003, 0.003, 0.03 and 0.3 mmol/kg oil DOPC during storage at 7 °C in the dark for 9 days. *Data points* represent means ($n = 3$) ± standard deviations. Some *error bars* lie within the data points

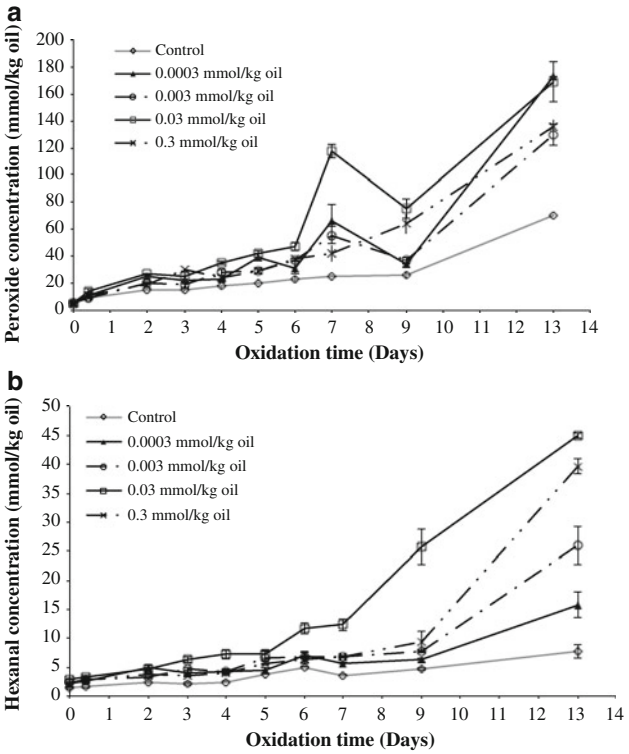

Fig. 2 Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions at pH 3.0, without (control) and with addition of 0.0003, 0.003, 0.03 and 0.3 mmol/kg oil DOPC during storage at 7 °C in the dark for 13 days. *Data points* represent means ($n = 3$) ± standard deviations. Some *error bars* lie within the data points

−2.52 mV. This again suggests that the DOPC did not concentrate at the emulsion droplet surface, where it could impact droplet charge or that, at pH 3.0, DOPC itself was not charged. The intrinsic pK_a of phosphatidylcholine has been reported to range from 0.8 to 3.0 in monolayers [18, 19] and from 2.0 to 4.5 in phospholipid bi-layers [20]. The pK_a variations are likely due to differences in the physical environment where the phospholipid head group resides. Therefore, it is possible that pH 3.0 is below or near the pK_a of DOPC and, thus, it would not be highly charged and could not change the emulsion droplet charge, even if it was on the emulsion droplet surface.

Figure 2 shows the oxidative stability of stripped soybean O/W emulsions with and without DOPC during 13-days of storage at pH 3.0. As reflected by an increase in lag phase of both hydroperoxide and hexanal formation (Fig. 1), lipid oxidation rates were slower at pH 3.0 compared to pH 7.0, even though the emulsion droplets at pH 3.0 were larger than pH 7.0 and thus would have less surface area. A reduction in lipid oxidation rates in Tween 20-stabilized emulsion with decreasing pH has also been reported by Waraho et al. [14] and Mancuso et al. [13]. In contrast to pH 7.0, DOPC exhibited a prooxidant effect at pH 3.0, as hydroperoxide in DOPC-containing emulsions was higher at all phospholipid concentrations with respect to that of the control (Fig. 2a). Similar trends were observed for hexanal formation (Fig. 2b). The prooxidant activity of DOPC rose with increasing PL concentrations, except for 0.3 mmol DOPC/kg oil where lipid oxidation was not as high as in samples with 0.03 mmol DOPC/kg oil.

Effect of Phospholipid Head Group and Fatty Acid Type on the Physical and Chemical Properties of O/W Emulsions

Another aspect that could influence the oxidative behavior of PL-containing emulsions is the degree of unsaturation of the fatty acid bound to the PL molecules. To determine if the presence of a double bond could affect the ability of PL to impact oxidation kinetics, phosphatidylcholine with palmitic (DPPC) or oleic (DOPC) acid were separately added to the pH 7.0 stripped soybean O/W emulsions (1.0% oil) at a concentration of 0.3 mmol/kg oil. The different PL

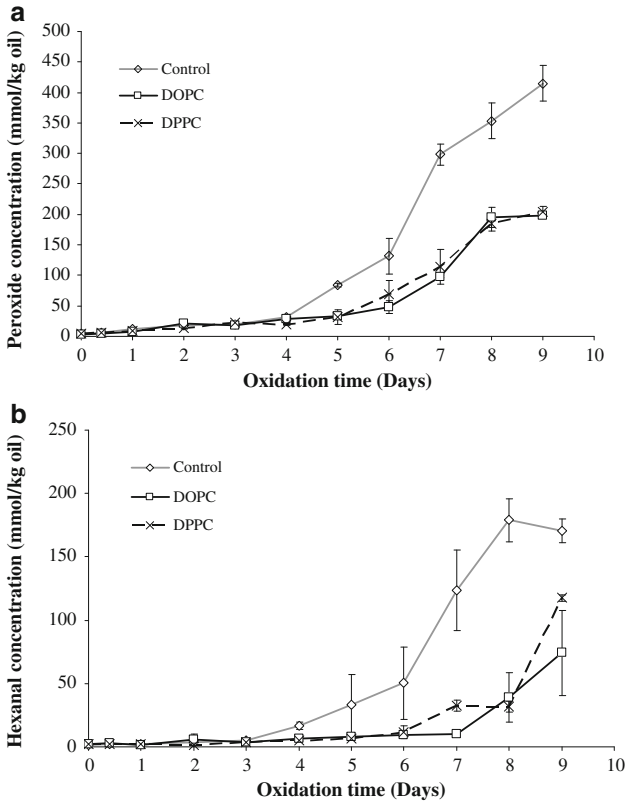

Fig. 3 Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions at pH 7.0, without (control) and with addition of 0.3 mmol/kg oil phosphatidylcholine bonded to oleic (DOPC) or palmitic (DPPC) acid during storage at 7 °C in the dark for 9 days. *Data points* represent means ($n = 3$) ± standard deviations. Some *error bars* lie within the data points

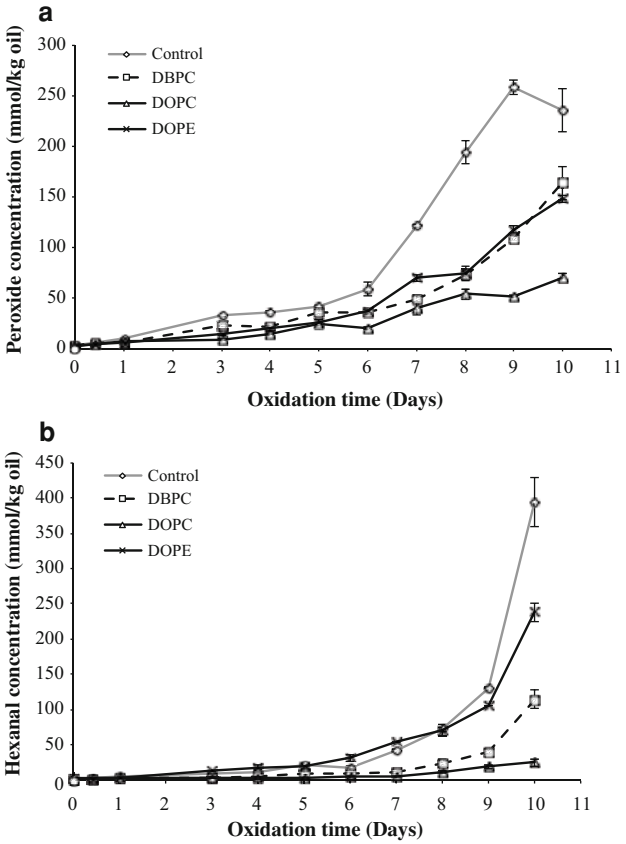

Fig. 4 Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions at pH 7.0, without (control) and with addition of phosphatidylcholine bonded to butyric (DBPC) or oleic (DOPC) acid and phosphatidylethanolamine bonded to oleic acid (DOPE) during storage at 7 °C in the dark for 10 days. *Data points* represent means ($n = 3$) ± standard deviations. Some *error bars* lie within the data points

did not have any effect on droplet size (175–190 nm) and surface charge with zeta potentials of -3.39 ± 0.3 and -4.45 ± 0.3 mV for the DPPC and DOPC containing emulsions, respectively. In the control sample, hydroperoxide formation began to increase after 4 days of storage, whereas it increased after 6 days of storage in both PL-containing emulsions (Fig. 3a). Similar trends were noticed for hexanal (Fig. 3b). No significant differences in the lipid hydroperoxide and hexanal formation were observed between DPPC and DOPC emulsions. These data suggest that the presence of a double bond in the FA structure has no effect on the antioxidant activity of phospholipids at pH 7.0. These results differ from bulk oils, where Nwosu et al. [21] reported that PL antioxidant properties depend on their degree of unsaturation.

To determine how phosphatidylcholine with different fatty acid chain length and head group type impact lipid oxidation of O/W emulsions, phosphatidylcholine with butyric acid (DBPC) and oleic acid (DOPC) and phosphatidylethanolamine with oleic acid (DOPE) were added to the O/W emulsions at 0.3 mmol/kg oil at pH 7.0. The zeta potential of control emulsions (-8.78 ± 0.1 mV) was similar to those containing DBPC, DOPC and DOPE (-7.74 ± 0.1, -6.77 ± 0.4 and -6.96 ± 0.2 mV, respectively). Lack of decreasing zeta potentials in the PL-containing emulsions again suggested that these negatively charged PL could not migrate to and concentrate at the lipid-water interface of the emulsion droplet.

As with the other emulsions at pH 7.0, control emulsions without PL showed the highest lipid hydroperoxides and headspace hexanal concentrations (Fig. 4). DOPC-containing emulsions consistently had the lowest levels of hydroperoxides and hexanal. DOPE- and DBPC-containing emulsions had similar concentrations of lipid hydroperoxides, while hexanal concentrations were lower in the presence of DBPC than DOPE. These results differ from bulk oil, where Hidalgo et al. [22] reported that the antioxidant activity of phospholipids containing a primary amino group, such as phosphatidylethanolamine (PE), is higher than those of PL without primary amino groups in

their structures, such as phosphatidylcholine (PC). The ability of DOPC to decrease lipid oxidation more than DBPC suggests that the difference is due to a physical effect, since both would have the same choline head group. Differences between DOPC and DBPC could be due to differences in their partitioning, as the smaller DBPC could partition more into the water phase or could be due to differences in their ability to form physical structures such as reverse micelles within the oil phase. Chen and coworkers [23] found that DOPC can form reverse micelles and promote lipid oxidation in bulk oils, while DBPC did not form structures or alter lipid oxidation rates.

The Ability of Different Phospholipid to Impact Hydroperoxide Decomposition Stability on O/W Emulsions

The previous results indicate that phospholipids are antioxidative in O/W emulsions at pH 7.0. The antioxidant activity of the phosphatidylcholine seems to be independent of its ability to change the surface charge of emulsion droplets as its addition to the emulsions has no impact on zeta potential. Another potential antioxidant mechanisms could include the ability of the DOPC to promote the decomposition of hydroperoxides into non-radical species [24, 25].

To investigate the ability of different PL to inhibit lipid oxidation by decomposing lipid hydroperoxides, Tween 20-stabilized O/W emulsions (pH 7.0) were prepared with elevated levels of hydroperoxides by adding cumene hydroperoxide. In this study, 1% medium chain triglycerides (MCT) were used instead of stripped soybean oil to provide a non-oxidizable lipid medium, which would not produce additional hydroperoxides via oxidation that would make data interpretation difficult. These emulsions also contained 200 μM EDTA, in order to chelate the metal ions and thus reduce metal-promoted hydroperoxide decomposition. As reported in Table 2, addition of cumene hydroperoxide to the emulsions increased lipid hydroperoxide concentrations by approximately 1.0 mmol/kg oil compared to the control samples (without cumene hydroperoxide). None of the PL were able to decrease hydroperoxide concentrations in the emulsions for the 48-h incubation, thus suggesting that in O/W emulsions they were not able to decompose lipid hydroperoxides. In fact, lipid hydroperoxides were observed to increase in all of the emulsions, including the control. This could be due to the oxidation of Tween 20, which has been reported to form hydroperoxides during oxidation [26]. These results differ from those of Yoshimoto et al. [24], who suggested that the mechanisms of hydrogen peroxide decomposition by PC in bulk oils involve the polarization of the oxygen–oxygen bond in the hydroperoxide by the phosphate group present in PC, followed by nucleophilic attack of an oxygen atom in another hydroperoxide molecule.

Table 2 Hydroperoxide degradation (mmol/kg oil) in 1.0% MCT oil-in-water emulsions at pH 7.0, without (control) and with addition of DBPC, DOPC, DOPE and cumene hydroperoxide at 1.3 mmol/kg oil during storage at 27 °C in the dark for 48 h

Time (h)	0	24	48
Control	2.69 ± 0.09b	2.44 ± 0.44b	3.00 ± 0.18c
Control with cumene	3.63 ± 0.13a	3.69 ± 0.06a	3.88 ± 0.13b
DBPC with cumene	3.63 ± 0.13a	3.63 ± 0.00a	3.88 ± 0.13b
DOPC with cumene	3.76 ± 0.13a	3.80 ± 0.07a	4.01 ± 0.00b
DOPE with cumene	3.63 ± 0.13a	3.94 ± 0.06a	4.34 ± 0.07a

Different letters within a column (a, b, c) are statistically different means (Tukey's test; $P \leq 0.05$)

Conclusions

In O/W emulsions at pH 3.0, DOPC was a prooxidant unlike its consistently observed antioxidant activity reported in bulk oils. However, at pH 7.0, PC was able to inhibit lipid oxidation in O/W emulsions irrespective of whether its fatty acids differed in degree of unsaturation or chain length. In O/W emulsions, the surface charge of the emulsion droplet can be extremely important as a negatively charged droplet can attract prooxidant metals and accelerate oxidation rates. However, in the Tween 20-stabilized emulsions used in this study, PC was not able to decrease the zeta potential of the emulsion droplet and thus alterations in the surface charge of the droplets could neither explain the prooxidant activity of PC at pH 3.0 nor its antioxidant activity at pH 7.0.

The properties of PL in bulk oils proposed to explain their antioxidant activity include their ability to increase the activity of tocopherols, chelate prooxidant metals, form antioxidative Maillard-type products and decompose hydroperoxides into non-radical compounds such as fatty acid alcohols [25, 27]. In the O/W emulsions used in this study, PC could not have inhibited lipid oxidation by increasing the activity of tocopherols as stripped oils where used in all studies. In addition, it is unlikely that Maillard products were involved as reaction temperatures were low and reducing sugars and other carbonyls were absent. Finally, the PL were not able to decompose cumene hydroperoxide in O/W emulsions suggesting that this pathway was also not responsible for the observed antioxidant activity. Of the proposed antioxidant mechanism for phospholipids, metal chelation seems to be the most probable mechanism. PL could represent a unique chelator in O/W emulsions if they reside in the lipid phase where they can inactive metals. This could provide an additional

antioxidant strategy that could be used instead of or in addition to traditional metal chelators (such as EDTA), which primarily reside in the water phase. A deeper understanding of how PL affects lipid oxidation in O/W emulsions could provide important knowledge to determine if phospholipids could be used as an effective strategy to inhibit rancidity development in emulsions and other food dispersions.

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