Effect of Fatty Acid Composition of Phospholipids on Their Antioxidant Properties and Activity Index

Chigozie Victor Nwosu[a], Leon C. Boyd[b,*], and Brian Sheldon[b]

[a]Alabama A&M University, Department of Food Science and Animal Industries, Normal, Alabama 35762, and
[b]North Carolina State University, Department of Food Science, Raleigh, North Carolina 27695

ABSTRACT: Various phospholipids may act as antioxidants or prooxidants. This study investigated the effects of three phospholipid classes and their fatty acid composition on antioxidant activity. Antioxidant properties of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine from salmon and menhaden oil were measured by oxidation induction time. An antioxidant activity index was determined in these systems with the Rancimat 617. Fatty acid profiles of the individual phospholipids and total oils were determined by gas–liquid chromatography before and after oxidation. The index was significantly ($P < 0.05$) influenced by the headgroup and fatty acid composition of the phospholipid. Lipids with a choline headgroup had oxidation induction times greater than 60 h in the salmon oil system. The choline-containing phospholipid also offered better ($P < 0.05$) protection from oxidation to the n-3 and total polyunsaturated fatty acids in salmon oil. Phospholipids containing more saturated fatty acids had longer oxidation induction times (>84 h) and higher antioxidant index (>9). Chainlength of the fatty acids may have contributed to the observed index, as phospholipids with longer chains (i.e., C_{18} and above) had longer oxidation induction times. Phospholipids tested in this study had little or no antioxidant activity in menhaden oil, nor did they offer protection to n-3 or total polyunsaturated fatty acids in this oil. These findings suggest that fatty acid profiles of individual oils may influence the antioxidant index of each phospholipid.
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KEY WORDS: Activity index, fatty acid profile, induction time, phospholipids.

Oxidation of lipids and the resulting off-flavors and off-odors are of major concern to the food industry. These off-flavors and off-odors significantly affect the sensory quality of foods, and their shelf life and nutritional quality. There also are health concerns related to the consumption of secondary oxidation products (1).

Lipid oxidation is influenced by factors such as the presence of oxygen, metal ions, light, temperature, and enzymes (1). These factors contribute to lipid oxidation, which generates hydroperoxides and eventually peroxide radicals. Subsequent chain reactions take place leading to the formation of secondary products that contribute to off-flavors and off-odors (2,3). Controlling the oxidative stability of oils and products that contain lipids is crucial to shelf life, storage stability, and consumer acceptability of those products (3,4).

Antioxidants often are added to lipid-containing products to delay or slow the rate of oxidation. Numerous studies have shown that individual phospholipids (PL) possess varying degrees of antioxidant activity (5–8). However, there is considerable disagreement among the reported antioxidant properties of individual PL. This variability has not been fully resolved. Some studies attributed antioxidant properties of PL to the chelating properties of their primary or free amino headgroup component (9). Other explanations included a synergism between tocopherol and PL (10), Maillard reaction products (11), or the physical barrier effects of PL located at the interface between oil and air.

Fatty acid composition of individual PL varies, depending on their source and their location in tissues. Environmental factors, such as diet, influence fatty acid composition of PL (12). Physical and structural differences also exist among and within each PL class, and may in part contribute to the differences observed in their antioxidant properties. The objectives of this study were to determine the effects of fatty acid composition and differences in headgroup composition of selected PL on their antioxidant property and activity index in fish oil model systems.

MATERIALS AND METHODS

Two types of fish oils, a salmon oil and a dietary grade of menhaden oil, were used to determine the effects of PL on oxidative stability. Oxidation was measured by a conductimetric procedure with the Rancimat 617 (METROHM, Herisau, Switzerland) and by measuring the change in fatty acid profiles by gas chromatography (GC). Menhaden oils were donated by Zapata Protein Inc. (Reedsville, VA); salmon oil was donated by Body Products Research Inc. (Van Nuys, CA). These oils were devoid of synthetic antioxidants and were stored under N_2 and –20°C and flushed with N_2 after every sampling to maintain freshness and oil quality. Individual PL that were evaluated included: phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SPH), lysophosphatidylcholine (LPC), dipalmitoyl phosphatidylethanolamine (diC16:0 PE), distearoyl phosphatidic acid (diC18:0 PA), distearoyl phosphatidylcholine

*To whom correspondence should be addressed.

(diC18:0 PC), and dibehenoyl phosphatidylcholine (diC22:0 PC). These PL were tested because of their reported effectiveness as antioxidants in the fish oil model systems (6,10). With the exception of diC18:0 PA purchased from Matreya Inc. (Pleasant Gap, PA), all PL were purchased from Sigma Chemical Company (St. Louis, MO). The PC and PE were obtained from egg yolk and ox brain, respectively, whereas all other sources of PL were synthetic in origin.

Rancimat procedure. Six replicate determinations of each sample were performed in duplicate by weighing 2.5 g of oil into thoroughly cleaned reaction vessels. Deionized water (50 mL) was added to the measuring vessels, which were maintained at room temperature. Electrodes were attached for measuring changes in conductivity. Air flow was maintained at approximately 18 L/h (300 mL/min). The conductivity of the distilled water, as influenced by the trapping of secondary oxidation products, was measured on a strip chart recorder in micro siemens/cm (μS/cm) with a chart speed of 1 mm/min. Oxidation induction time was determined from the tangent of the inflection point on the plot (3). Inflection point is defined as the location where the plot deviates from linearity. Oxidation induction time is defined as the time of heating required for lipids to generate a sufficient quantity of volatile compounds necessary to change the conductivity of deionized water in the measuring vessel. All oxidation studies were carried out at 100°C.

Changes in fatty acid profiles. Oxidation also was assessed by monitoring changes in fatty acid composition of the oils with and without added antioxidants. Fatty acid composition of the oils was determined prior to and after oxidation in the Rancimat. Oils (50 mg) were transesterified at 80°C overnight (13) with 5% methanolic hydrochloric acid. Crude fatty acid methyl esters (FAME) were extracted in hexane, dried over sodium sulfate, and purified by eluting with 95:5 (vol/vol) hexane/diethyl ether solution through Pasteur pipets packed with Florisil (2 cm) (14). Purified FAME were analyzed on an HP 5890 Hewlett-Packard (Avondale, PA) GC, equipped with a flame-ionization detector, an HP 3393A integrator, and containing a 30 m × 0.25 mm i.d. DB 225 fused-silica capillary column (J&W Scientific, Folsom, CA) as described by Boyd *et al.* (15). Column temperature was programmed from 180 to 230°C at 2°C/min with a final hold time of 2 min. Injector and detector temperatures were set at 250 and 275°C, respectively. Helium was used as the carrier gas. Fatty acids were identified by comparison of their relative retention time to authentic (C_{14}–C_{24}) standards (Nu-Chek-Prep, Elysian, MN) and a fatty acid internal standard (23:0). Fatty acid composition was expressed as a mole percentage of total FAME. Detector response correction factors, based on fatty acids in reference standards, were programmed into the integrator. The fatty acids in the reference standard were the same as those in the oils, except for a few isomers whose retention times were determined by their addition to the reference standard.

Model systems. Antioxidant activity of PL was compared to that of butylated hydroxytoluene (BHT) in salmon oil and dietary-grade menhaden oil. Though tertiary butylhydroquinone (TBHQ) is a more effective antioxidant, BHT was selected because it is a widely used synthetic antioxidant in the storage of oils and lipid-containing products. All PC were added at 0.5% (w/w) per 2.5 g oil, whereas BHT was added at the legal limit of 0.02% (w/w) of sample weight. Salmon and menhaden oils without antioxidants were used as controls. The efficacy of the PL to act as antioxidants was measured as the activity index (AI) and changes in fatty acid profile. AI is defined as the ratio of the oxidation induction time with and without the presence of an antioxidant. Model System 1: Treatments consisted of a control that contained only salmon oil, salmon oil with BHT, and salmon oil with each of the PL noted above. All treatments were oxidized in the Rancimat at 100°C with each assay terminated when the recorder plot deviated from linearity. The oil was then analyzed for fatty acid composition. Model System 2: This model system compared the effects of two PL (PC^1 vs. PC^2 and PE^1 vs. PE^2) with varying fatty acid profiles on the oxidation induction time and AI in salmon oil. the PC^2 and PE^2 used in this model were generally more saturated than PE^1 and PC^1 of Model System 1. The reaction conditions were the same as in Model System 1. Model System 3: This experiment compared the antioxidant properties of PL to BHT by using a dietary grade of menhaden oil with all other conditions maintained as described in Model System 1.

Statistical analysis. Six replicate analyses were performed per treatment to determine the antioxidant properties of PL and BHT by measuring changes in oxidation induction times, AI, and changes in fatty acid profile before and following heating. Three replicate determinations of fatty acid profile of individual PL were performed to evaluate the various component fatty acid groups. Data were analyzed with a one-way analysis of variance according to SAS (16). Waller-Duncan k ratio *t*-tests (17) were computed for the treatment main effects which were significantly different.

RESULTS AND DISCUSSION

Addition of PL improved the oxidative stability of salmon oil. Table 1 shows the oxidation induction times of salmon oil with and without added antioxidants. Salmon oil with SPH, LPC, diC22:0 PC, and diC18:0 PC had the longest induction times (>100 h) and the highest AI (11). This finding demonstrated that in salmon oil, PL that contained a choline headgroup possessed improved antioxidant activity compared to ethanolamine and phosphatide-containing PL. Synthetic diC22:0 PC, diC18:0 PC, and diC16:0 PE were significantly ($P < 0.01$) better than native PC and PE. These findings support earlier suggestions (19) that PL with saturated fatty acids were more effective antioxidants, especially if they contained a choline headgroup. When compared to PL, BHT-supplemented salmon oil had the shortest induction time (10 h) which was not significantly different from the control (9 h). This finding may have been associated with the low hydrophilic-lipophilic balance (HLB) value of BHT (1,19). Antioxidants with low HLB values are not effective in bulk oils because they do not concentrate at the surface (lipid–air interface) of the oil where reaction with molecular

TABLE 1
Oxidation Induction Times and Activity Indexes of Salmon Oil, with Added Antioxidants, After Accelerated Oxidation on the Rancimat

Treatments[a]	Induction time[b,f] (h)	Activity index[c]
Control	9.1[f]	1[f]
+BHT	10.1[f]	1.1[f]
+PE[1d]	21.0[f]	2.3[g]
+diC18:0 PA	30.7[h]	3.4[h]
+PC[1d]	61.7[i]	6.8[i]
+PE[2e]	84.0[j]	9.2[j]
+diC16:0 PE	85.8[j]	9.4[j]
+diC18:0 PC	<100[k]	11.0[k]
+SPH	>100[k]	11.0[k]
+LPC	>100[k]	11.0[k]
+diC22:0 PC	>100[k]	11.0[k]
+PC[2e]	>100[k]	11.0[k]
	LSD 0.01	LSD 0.01

[a]BHT, butylated hydroxytoluene; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SPH, sphingomyelin; LPC, lysophosphatidylcholine; diC18:0 PA, distearoyl phosphatidic acid; diC22:0 PC, dibehenoyl PC; diC16:0 PE, dipalmitoyl PE; LSD, least significant difference.
[b]Expressed as the mean of six replicate determinations.
[c]Activity index, ratio of oxidation induction time without/with an antioxidant.
[d,e]Phospholipids with different degrees of saturation as used in Model Systems 1 and 2 to evaluate effects of saturation within the class of phospholipids.
[f]Where superscripts are different in any given column, the values are significantly different at the level indicated by the LSO.

TABLE 2
Fatty Acid Profiles of Individual Phospholipids Before Oxidation[a]

Phospholipid	Saturates	Monoenes	PUFA
PC[1]	50.7[e,f]	41.9[g]	7.4[e,f]
PE[1]	44.3[e]	14.0[e,f]	38.7[g]
SPH	87.7[g]	6.3[e]	6.3[e,f]
LPC	94.0[g]	6.0[e]	n.d.
diC16:0 PE	100[g]	n.d.	n.d.
diC18:0 PA	100[g]	n.d.	n.d.
diC18:0 PC	100[g]	n.d.	n.d.
diC22:0 PC	100[g]	n.d.	n.d.
PC[2]	56.6[e]	37.2[g]	6.0[e,f]
PE[2]	57.9[e]	20.8[f]	21.3[e]
	LSD 0.05	LSD 0.05	LSD 0.05

[a]Each column value is a mean of three replicate determinations; n.d., not detected, PUFA, polyunsaturated fatty acids. See Table 1 for other abbreviations and explanation of superscripts.

oxygen is prevalent (1). Treatments that contained diC18:0 PA, PE, PC, and diC16:0 PE were not so effective as SPH, LPC, diC22:0 PC, and diC18:0 PC, but they were more effective antioxidants than BHT. Although both diC18:0 PA and diC18:0 PC contained the same fatty acids, the diC18:0 PC had an oxidation induction time of >100 h compared to 30.7 h for diC18:0 PA. Therefore, the observed differences between classes could only be attributed to the polar headgroup.

Comparison of oxidation induction times and AI of individual PL (Table 1) to their fatty acid profiles (Table 2) showed that PL with a greater proportion of saturated fatty acids had greater antioxidant activity, except diC18:0 PA (Table 2). For example, SPH, LPC, diC18:0 PC, diC22:0 PC, and diC16:0 PE were significantly ($P < 0.05$) more saturated than PC[1] or PE[1]. The ineffectiveness of diC18:0 PA to prolong oxidation induction times longer than PC1 could be attributed to the phosphatidic acid headgroup, which lacked an amine alcohol moiety. Salmon oil samples with diC16:0 PE had significantly shorter oxidation induction times than those with diC18:0 PC and diC22:0 PC. The results of Model System 2 clearly showed the effects of saturation and polyunsaturated fatty acids (PUFA) content on the antioxidant activity of the PL (Tables 1 and 2). Both PC[2] and PE[2] of Model System 2 were more saturated (not significantly for PC) than those used in Model System 1, and they were more effective antioxidants as noted by significantly ($P < 0.01$) higher induction times and AI.

These results are in agreement with our previous results (7,18). Thus, it has been consistently shown that antioxidant properties of these PL differ, despite similarity in amine composition of their headgroup. Other investigators (5,8,9) have attributed antioxidant activity of PL to the primary or free amine headgroups. Because naturally-derived PC[2] and PE[2] used in Model System 2 contained similar choline and ethanolamine headgroups, both would be expected to give similar antioxidant activity, yet they did not. Data from this research clearly indicated that degree of saturation is a critical factor in the antioxidant activity of PL. However, differences in chainlength of PL fatty acids may also be a contributing factor to the variations detected in their antioxidant properties. Differences in chainlength have been shown to affect the thermodynamic properties of lipids, especially PL (8,9,20). Evaluation of the thermophysical properties of the fish oils with and without the addition of PL could provide more information on how PL function as antioxidants or prooxidants in fish oils.

Addition of PL and BHT to dietary-grade menhaden oil revealed an antioxidant pattern different from that observed for the salmon oil model system. Induction times and AI for menhaden oil treatments are shown in Table 3. None of the antioxidant treatments evaluated was effective in protecting the menhaden oil from oxidation, as indicated by short oxidation induc-

TABLE 3
Oxidation Induction Times and Activity Indexes of Menhaden Oil (dietary grade), with Added Antioxidants, Following Accelerated Oxidation on the Rancimat

Treatments	Induction time[a] (h)	Activity index
Control	0.19[c,d]	1.0[c,d]
+diC18:0 PA	0.05[d]	0.3[d]
+diC22:0 PC	0.05[d]	0.3[d]
+diC18:0 PC	0.05[d]	0.3[d]
+LPC	0.05[d]	0.3[d]
+diC16:0 PE	0.08[d]	0.4[d]
+PC	0.09[c]	0.5[c]
+PE	0.25[c]	1.3[c]
+SPH	0.30[c]	1.6[c]
+BHT	1.59[b]	8.5[b]
	LSD 0.05	LSD 0.05

[a]Each column value is a mean of three replicate determinations. See Table 1 for abbreviations and explanation of superscripts.

TABLE 4
Comparison of Fatty Acid Profiles of Salmon Oil Treatments Prior to and After Accelerated Oxidation[a]

Treatments	Saturates	Monoenes	Total PUFA	n-3 PUFA
Unoxidized oil	26.2^{e}	48.2^{e,f}	25.2^{g}	21.2^{g,h}
Oxidized oil	37.3^{f,g}	58.1^{g,h}	4.6^{e}	2.0^{e}
+BHT	31.4^{e,f}	54.5^{f,g}	14.1^{f,g}	11.2^{f}
+PC[1]	34.7^{f}	60.2^{h}	4.9^{e}	1.2^{e}
+PE[1]	39.0^{g}	58.0^{g,h}	3.0^{e}	0.6^{e}
+SPH	27.3^{e}	46.5^{e}	26.3^{h}	23.4^{g,h}
+LPC	25.3^{e}	50.7^{f,g}	23.8^{h}	19.9^{g,h}
+diC18:0 PA	31.1^{e,f}	49.5^{e,f}	17.6^{g}	13.6^{f}
+diC18:0 PC	28.3^{e}	46.6^{e}	25.1^{h}	20.9^{g,h}
+diC22:0 PC	27.6^{e}	46.6^{e}	26.8^{h}	23.8^{h}
+diC16:0 PE	34.9^{f,g}	56.9^{g,h}	9.1^{e,f}	3.8^{e}
+PC[2]	27.7^{e}	48.3^{e,f}	24.0^{h}	19.7^{g,h}
+PE[2]	27.9^{e}	48.7^{e,f,g}	22.0^{g,h}	17.4^{f,g}
	LSD 0.05	LSD 0.05	LSD 0.05	LSD 0.05

[a]Each column value is a mean of six replicate determinations. See Tables 1 and 2 for abbreviations and Table 1 for explanation of superscripts.

tion times. BHT produced significantly ($P < 0.05$) longer oxidation induction times and improved AI as compared to the PL treatments in this oil system. Differences ($P < 0.05$) in oxidation induction times were observed between PE and SPH treatments in comparison to PC, LPC, diC22:0 PC, diC18:0 PC, diC18:0 PA, and diC16:0 PE treatments, with PE and SPH equal to the control.

Table 4 summarizes the changes in fatty acid classes of salmon oil after heating at 100°C for 100 h with and without added antioxidants. A comparison of oxidation induction times to loss of PUFA indicates that induction time is not totally explained by a loss of PUFA. For example, the BHT treatment yielded the shortest oxidation induction times among the antioxidants (Table 1), but offered better protection to n-3 PUFA and total PUFA than PE[1] and PC[1]. Treatments with PC[2], PE[2], SPH, diC18:0 PC, diC22:0 PC, and LPC were the most effective at protecting PUFA from oxidation. PC[2] and PE[2] are PL with less PUFA than PC[1] and PE[1] as indicated in Table 2.

Changes in the fatty acid classes of dietary-grade menhaden oil are shown in Table 5. There were no differences in saturates, monoenes, n-3 PUFA, and total PUFA between menhaden oil samples that contained LPC, diC18:0 PA, and the oxidized menhaden oil that contained no antioxidant. The oil with BHT differed from oxidized control menhaden oil for only the monoenes class. Treatments with LPC and diC18:0 PA were significantly ($P < 0.05$) more protected than those with other PL for total PUFA and n-3 PUFA. Though SPH and PE (Table 3) had better AI than the other PL, LPC and diC18:0 PA offered comparatively better protection to the PUFA. No significant differences in n-3 PUFA were observed between the control and treatments with diC18:0 PA and LPC, whereas diC18:0 PC, diC22:0 PC, and diC16:0 PE appeared to act as prooxidants in the menhaden oil system.

TABLE 5
Comparison of Fatty Acid Profiles of Menhaden Oil (dietary grade) Treatments Prior to and After Accelerated Oxidation[a]

Treatments	Saturates	Monoenes	Total PUFA	n-3 PUFA
Unoxidized oil	33.7^{c}	25.8^{c}	40.9^{h}	34.6^{j}
Oxidized oil	39.9^{d}	28.3^{d}	31.9^{f,g}	25.5^{h,i}
+BHT	39.6^{d}	30.7^{e}	29.9^{f}	23.4^{h}
+PC	44.4^{e}	32.1^{e}	23.5^{f}	18.8^{g}
+PE	46.5^{b,e}	33.1^{e,f}	20.5^{e,f}	14.8^{e,f}
+SPH	44.0^{e}	32.2^{e}	23.8^{f}	16.6^{f,g}
+LPC	38.6^{d}	27.0^{d,c}	34.4^{g}	28.1^{i}
+diC18:0 PA	39.6^{d}	27.7^{d}	32.8^{g}	26.4^{h,i}
+diC18:0 PC	48.1^{f}	34.1^{f}	17.5^{d,e}	12.2^{d,e}
+diC22:0 PC	49.2^{f}	34.8^{f}	16.0^{d}	10.5^{d}
+diC16:0 PE	54.6^{g}	41.4^{g}	4.0^{c}	1.0^{c}
	LSD 0.01	LSD 0.01	LSD 0.01	LSD 0.01

[a]Each column value is a mean of six replicate determinations. See Tables 1 and 2 for abbreviations and Table 1 for explanation of superscripts.

None of the PL improved the stability of the menhaden oil relative to that observed for salmon oil. This finding may be attributed to differences in the fatty acid profiles of both oils resulting from the species difference. Oxidative stability of fish lipids has been reported to differ from species to species and to be significantly higher in red meat species than in white meat species (8,21). However, other studies have reported that white muscle tissues are more stable to oxidation than dark muscle tissues (22–24). Because both oils are from dark-muscle fish species, differences in their stability and AI of the PL could be due to positional distribution of component fatty acids on the triacylglycerols. Both oils had similar initial peroxide values of less than 5 μeq/kg; thus the quality of the oils, though not discounted, was not a major contributor.

The results obtained from this study indicate that the antioxidant property of PL depends on the degree of unsaturation and chainlength of the fatty acids that constitute the PL. These results support other studies that reported that the antioxidant properties of PL depend on their fatty acid composition, which affects the thermodynamic properties of the PL and oils containing them (20, 25). Our results, however, differ from those of Chen and Nawar (5) who reported that the antioxidant property of PL was due to the chelating function of the amine headgroup of the PL. The results from this study support the theory that the antioxidant property of PL is affected by the headgroup and by the saturation and chainlength of the PL fatty acids. The ability of PL to form reverse micelles in a nonaqueous medium needs to be evaluated, because such micelles could influence oxidation induction time.

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