Antioxidant Properties of Individual Phospholipids in a Salmon Oil Model System

M.F. King, L.C. Boyd* and **B.W. Sheldon**
Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-7624

The antioxidant properties of phospholipids (PL) in a refined salmon oil model system were measured by determining changes in the 2-thiobarbituric acid number and decreases in the ratio of docosahexaenoic acid (DHA)/palmitic acid (22:6/16:0) of a fish oil system incubated at 180°C for up to 3 h. The more phosphatidylcholine (PC) added to the oil system, the higher the oxidative stability obtained. The order of effectiveness of commercial phospholipids in inhibiting oxidation and the loss of polyunsaturated fatty acids was as follows: sphingomyelin (SPH) = lysophosphatidylcholine (LPC) = phosphatidylcholine (PC) = phosphatidylethanolamine (PE) > phosphatidylserine (PS) > phosphatidylinositol (PI) > phosphatidylglycerol (PG) > control salmon oil. Nitrogen containing PL, including PE, PC, LPC and SPH, were equally effective in exerting greater antioxidant properties than PS, PG and PI. The inverse relationship observed between the oxidation index (C22:6/C16:0) and color intensity for treatments following 2 h of heating suggests that Maillard-type reaction products may have contributed to the oxidative stability of PL-supplemented fish oils.

KEY WORDS: Antioxidant properties, browning reaction, fish oil, oxidation, phosphatidylcholine, phospholipids.

Phospholipids (PL) are believed to be major lipid components responsible for the development of off-flavors and odors in a number of food products during prolonged storage. When compared to triglycerides (TG), phospholipids are generally higher in polyunsaturated fatty acids, and for this reason they are believed to play a major role in the development of warmed-over flavors in poultry, mutton, beef and pork (1). However, because PL contain phosphorus, a nitrogen-containing moiety and polyunsaturated fatty acids (PUFA), their role as pro- or antioxidants in oxidation systems is far more complex than that of the neutral lipids. The phosphorus and nitrogen-containing moieties are possibly involved in stabilizing lipid systems, whereas the PUFA moiety is suspected of destabilizing lipids.

Antioxidant properties of phospholipids have been demonstrated through their addition to processed vegetable oils and animal fats, including those from sunflower, corn, cottonseed, soybean and lard (2,3). Though the exact mechanism of action of PL is still not fully established, four postulates have been proposed to explain their antioxidant activity: i) synergism between PL and tocopherol (4–6); ii) chelation of pro-oxidant metals by phosphate groups (7,8); iii) formation of Maillard-type products between PL and oxidation products (9); and iv) action as an oxygen barrier between oil/air interfaces (10,11).

Though current research is continuing to support the health benefits of increased consumption of lipids from fish and other seafood, the highly unsaturated nature of these lipids makes them extremely sensitive to oxidation, which results in the development of off-flavors and odors. In a previous investigation by the authors (12), phospholipid fractions extracted from bluefish *(Pomatomus saltatrix)* were shown to have greater antioxidant properties than neutral lipids or total lipids in spite of their higher polyunsaturated fatty acids (PUFA) content. The stability of the lipids was highly correlated with their phospholipids (PL) content, with no correlation observed between the degree of fatty acid unsaturation and antioxidant properties. The purpose of this study was to further investigate the antioxidant properties of individual PL in fish oil model systems. Since other studies have postulated a relationship between the antioxidant properties of PL and the formation of Maillard browning reaction products, this study investigated the oxidative stability of a heated salmon oil model system and the relationship between color intensity and the type of PL added to the system.

EXPERIMENTAL PROCEDURES

Materials. Phospholipid standards were obtained from Sigma Chemical Co. (St. Louis, MO) with each having a purity above 98%. The PL were as follows: L-α-phosphatidylcholine (PC), L-α-phosphatidylethanolamine (PE), L-α-lysophosphatidylcholine (LPC), L-α-phosphatidylglycerol (PG) and sphingomyelin (SPH) from egg yolk, L-α-phosphatidyl-L-serine (PS) from bovine brain, and L-α-phosphatidylinositol (PI) from bovine liver. Salmon oil was obtained without added antioxidants or stabilizers from Body Products Research, Inc. (Chatsworth, CA). Endogenous α-tocopherol levels in salmon oil were determined by high-performance liquid chromatography (HPLC) as described by Widicus and Kirk (13). The salmon oil was obtained as a refined product that had been cold-processed and deodorized prior to packaging in gelatin capsules. The purity of the PL was checked by HPLC analysis as described by Kaduce *et al.* (14), whereas the fatty acid composition of the PL and of the refined salmon oil was obtained by gas-chromatographic analysis of prepared fatty acid methyl esters (FAME) as described by Morrison and Smith (15).

The prepared FAME were dissolved in 0.1 mL of *iso*-octane and injected onto a Hewlett-Packard (HP) model 5890 gas chromatograph (GC) (Avondale, PA) equipped with a flame ionization detector, an HP 3393A integrator, and an IBM PC-2 computer (International Business Machine Corp., Rye, NY) for data storage and handling. The GC contained a DB-225, 30 m × 0.25 mm i.d. fused silica capillary column (J & W Scientific Co., Folsom, CA), which was temperature programmed from 180°C to 230°C at a rate of 2°C per min with injector and detector temperatures set at 250°C and 275°C, respectively. A column flow rate of 0.7 mL/min with a split flow of 10:1 was used to elute the FAME. Identification of FAME was based on comparison of retention times of unknown peaks to methyl ester standards (NuChek Prep, Elysian, MN).

*To whom correspondence should be addressed.

Individual fatty acid concentration was expressed as a weight percent of the total FAME and was used to calculate the polyene index. A normalization technique was used to calculate absolute response factors for all identified fatty acids (16).

Model oil system. The antioxidant properties of PL were determined by their addition to refined salmon oil. To ensure proper mixture of salmon oil with PL, the PL were individually dissolved in 10 mL of chloroform-methanol (2:1) at a concentration of 5 mg/mL and mixed with 20 mg of salmon oil on a vortex mixer at medium speed for 1 min, followed by evaporation of solvent under a stream of prepurified nitrogen. The control salmon oil without PL was dissolved in chloroform-methanol, vortexed, capped and heated. All treatments were placed in screw-cap glass test tubes (16 mm × 100 mm) without caps, and heated in a Fischer forced-draft oven (Model 412, Lexington, MA) maintained at 180°C. Samples were taken for chemical analyses at 0, 15, 30, 60, 120, and 180 min of heating.

Experiment I. Four treatments were tested in the first experiment: i) control salmon oil, ii) salmon oil + 0.01% PC, iii) salmon oil + 0.1% PC, and iv) salmon oil + 1% PC.

Experiment II. The following combinations were used in the second experiment: i) control salmon oil, ii) salmon oil + 1% PG, iii) salmon oil + 1% PI, iv) salmon oil + 1% PS, v) salmon oil + 1% PE, vi) salmon oil + 1% PC, vii) salmon oil + 1% LPC, and viii) salmon oil + 1% SPH.

Test of oxidative stability. Oxidative stability was determined by measuring changes in the 2-thiobarbituric acid number (TBA), the polyene index and total changes in fatty acid composition after different times of heating. The TBA test involved the measurement of TBA-reactive substances (TBARS) as described by Ke and Woyewoda (17). The TBA analysis was performed on samples taken at intervals of 0, 15, 30, 60, and 120 min of heating and expressed as μmoles/g of oil. The polyene index, which measures the ratio of polyunsaturated fatty acids (PUFA) to saturated (SAT) fatty acids (18), was determined by gas chromatography (GC). Fatty acids containing two or more unsaturated double bonds were classified as PUFA. Since docosahexaenoic acid (DHA, 22:6) represents the most highly unsaturated fatty acid present in a fish oil model system, the loss of DHA relative to palmitic acid (*i.e.*, C22:6/C16:0) is often used as the most sensitive polyene index indicative of the oxidation of fish oil PUFA and was therefore used in the present study.

Prepared FAME were dissolved in 0.2 mL of *iso*-octane and 4 μL or less was used per injection. The FAME were analyzed on the same HP 5890 GC used to analyze the fatty acid content of PL under almost identical GC conditions. The two major exceptions were the higher flow rate of 1.2 mL/min and a split flow ratio of 70:1. The polyene index was determined on samples heated for 0, 60, 120, and 180 min.

Browning reaction. Samples of 20-mg aliquots from each treatment were removed after 0, 30, 60, and 120 min of heating and redissolved in 3 mL of chloroform for spectrophotometric measurements of color changes. Browning reaction products were measured at 430 nm with a Shimadzu Recording Spectrophotometer UV-240 (Schimadzu Corp., Kyoto, Japan) to detect changes in color intensity due to heating (9).

Statistical analysis. A randomized complete block design with treatments blocked with heating times and replications (two) was used in the analysis of all data. SAS (19) procedures were used to perform the analysis of variance with significant mean differences separated by Duncan's Multiple Range Test. Pearson correlation coefficients were used to compare chemical measurements (20). All analyses were performed in duplicate with each experiment replicated two times and $P < 0.05$ established as the minimum level of significance.

RESULTS AND DISCUSSION

Fatty acid composition of salmon oil and PL. Table 1 shows the fatty acid composition of salmon oil triglycerides and of each individual PL. Salmon oil contained n-3 fatty acids at a relatively high level of about 29% of the total fatty acids. The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) comprised 84% of the total n-3 fatty acids in the oil. The fatty acid composition of PG was similar to that of PC with both having a high content of palmitic (16:0) and oleic (18:1) acids. Both PI and PS contained high levels of stearic acid (18:0), whereas PS also contained a high level of oleic acid (18:1). The PI and PE lipids were high in arachidonic acid (20:4), while PS contained relatively high levels of 22:4 and 22:6. Both LPC and SPH contained high levels of palmitic acid (16:0).

Test of oxidative stability. Figure 1A shows the TBA numbers of Experiment I containing salmon oil supplemented with three different levels of PC. The addition of PC at all levels improved the overall oxidative stability of the salmon oil over the control containing no PC. Addition of PC at the 1% level was most effective in stabilizing the oil, whereas additions at the 0.01% and 0.1% did not differ from each other but resulted in significantly greater stability of the oil than the nonsupplemented control oil. The polyene index of fatty acid loss (Fig. 1B) showed a similar trend to that observed for TBA values in that 1% PC was the most effective treatment concentration in preventing the loss of PUFA whereas .01% PC did not differ from the control.

Increasing PC concentrations resulted in a progressive increase in the color intensity (430 nm) of the heated salmon oil (Fig. 2). The 0.1% and 1% levels of PC produced higher ($P < .05$) color intensities, whereas the 0.01% PC level did not differ significantly from the control. Correlation coefficients between TBA numbers, polyene index, color intensity, and percentage of added PC (Table 2) revealed significant relationships among several of the parameters over the course of heating. TBA values showed the greatest inverse correlation to the PC level at the 30- and 60-min heating periods, reflecting the rapid formation of TBARS followed by decreases at the 120-min heating period. In contrast, the polyene index was strongly correlated to the level of PC added toward the end of the heating cycle, reflecting the generally slower loss of PUFA over time. The change in color intensity showed high positive correlations to percentage of PC added at several periods of heating, indicative of the formation of Maillard-type browning reaction products formed during the heating.

The antioxidant effects (polyene index and TBA) of individual PL on salmon oil of Experiment II are summarized in Table 3 and Figure 3. The TBA numbers from the control oil increased rapidly after 15 min of heating and reached a maximum level at 1 h of heating followed

TABLE 1

Fatty Acid Composition of Salmon Oil Triglycerides and of Phospholipids Added as Antioxidants[a]

Fatty acids	Salmon oil (TG)	Phospholipids[b]						
		PG	PI	PS	PE	PC	LPC	SPH
C14:0	7.6	—	—	—	—	—	—	—
C16:0	18.5	32.5	3.5	0.3	16.1	31.1	69.6	75.8
C16:1	6.2	—	—	—	—	—	—	1.1
C18:0	3.1	16.1	52.6	47.8	27.7	14.9	24.5	6.8
C18:1	21.6	30.7	8.8	30.2	17.6	33.2	1.3	—
C18:2	1.8	14.7	5.0	—	12.1	13.6	—	—
C18:4(n-3)[c]	2.7	—	—	—	—	—	—	—
C20:0	—	—	—	4.1	—	—	—	—
C20:1	6.0	—	—	—	—	—	—	—
C20:2	—	—	0.3	—	—	—	—	—
C20:3(n-6)	—	3.1	8.7	—	—	2.6		—
C20:4(n-6)	—	3.0	11.3	2.8	14.8	2.4	4.7	3.3
C21:0	—	—	3.5	—	—	—	—	—
C20:3(n-3)	—	—	1.1	—	3.5	—	—	—
C20:5(n-3)	11.7	—	2.3	—	—	—	—	—
C22:0	6.0	—	—	—	—	—	—	2.5
C22:2	—	—	—	2.5	—	—	—	—
Unknown	—	—	—	2.5	—	—	—	—
C22:4(n-6)	—	—	0.5	8.7	4.5	1.1	—	0.4
C22:5(n-3)	2.0	—	—	—	—	0.4	—	—
C22:6(n-3)	12.9	—	2.4	8.1	3.7	0.3	—	—
C24:0	—	—	—	—	—	—	—	4.8
C24:1	—	—	—	—	—	—	—	5.3
Saturates	35.2	48.6	59.7	52.2	43.8	46.1	94.1	87.4
Monoenes	33.7	30.7	8.8	30.2	17.6	33.2	1.3	6.4
Polyenes	31.0	20.7	29.2	24.7	38.6	20.4	4.7	6.2
Total (n-3)	29.2	0.0	5.8	8.1	3.7	0.7	0.0	2.5

[a]Fatty acid concentration expressed as wt% of individual fatty acid methyl esters in the total lipids.

[b]PL from egg yolk: PG = phosphatidylglycerol; PC = phosphatidylcholine; SPH = sphingomyelin; LPC = lysophosphatidylcholine; PE = phosphatidylethanolamine. PL from bovine: PI = phosphatidylinositol; PS = phosphatidylserine.

[c]n-x = position of double bond farthest removed from carboxyl group.

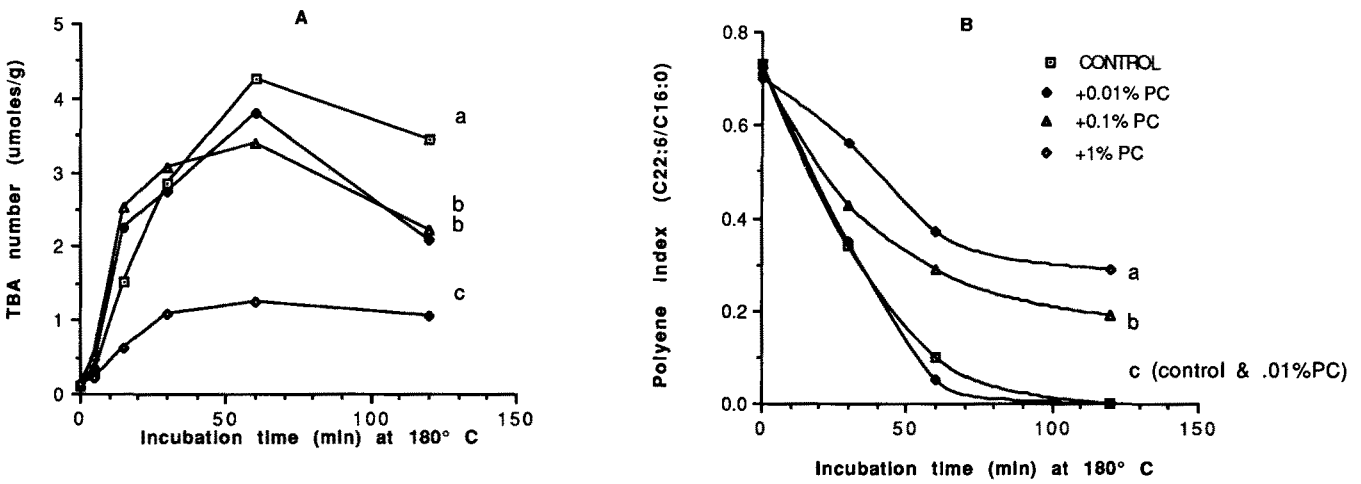

FIG. 1. Model system I—effects of 0.01, 0.1 and 1.0% (w/w) additions of phosphatidylcholine (PC) on (A) thiobarbituric acid (TBA) numbers and (B) polyene index of salmon oil heated to 180°C for 180 min. Small letters a, b, c, indicate significant differences between treatments ($P < 0.05$).

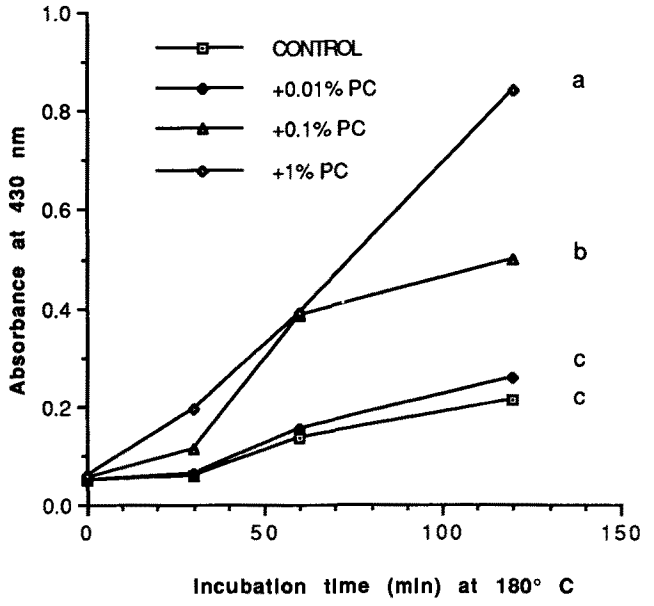

FIG. 2. Model system I—effects of 0.01, 0.1 and 1.0% (w/w) additions of phosphatidylcholine (PC) on absorbance values of salmon oil measured at 430 nm heated to 180°C for 180 min. Small letters a, b, c, indicate significant differences between treatments ($P < 0.05$).

TABLE 2

Simple Correlation Coefficients Between TBA Numbers, Polyene Index, and Browning Color of Salmon Oil and of Salmon Oil + 0.01%, 0.1% and 1% Phosphatidylcholine over Different Heating Times at 180°C

Heating time (min)		Polyene ratio (22:6/16:0)	Browning	% of PC added
0	TBA numbers	−0.56	−0.52	0.45
	Polyene ratio		−0.44	−0.47
	Browning			0.84[a]
30	TBA numbers	−0.72	−0.82[a]	−0.93[a]
	Polyene ratio		0.94[a]	0.87[a]
	Browning			0.95[a]
60	TBA numbers	−0.78	−0.64	−0.95[a]
	Polyene ratio		0.83[a]	0.74[b]
	Browning			0.57
120	TBA numbers	−0.66	−0.69	−0.70
	Polyene ratio		0.90[a]	0.84[a]
	Browning			0.86[a]

[a]($P < 0.01$). [b]($P < 0.05$).

thereafter by a decline. The addition of each PL extended the oxidative induction period of the supplemented salmon oil. Nitrogen-containing PL, including PE, PC, LPC and SPH, yielded the highest antioxidant activity, whereas PG and PI exhibited the least activity. Both PG and PI had the lowest mean polyene indices at 120 and 180 min (Table 3) and highest TBA values, which were not different from the control after 3 h of heating (Fig. 3A). A comparison of the antioxidant effects (polyene index, 180 min) of the nitrogen-containing PL indicated that PS and PI were less effective than PE, PC, LPC or SPH. No

TABLE 3

Polyene Index (C22:6/C16:0) for Salmon Oil and for Salmon Oil + 1% Commercial Phospholipids Heated (0, 60, 120, and 180 min at 180°C)[a]

	Heating time (min)			
	0	60	120	180
Oil	0.70[a]	0.29[d]	0.07[b]	0.00[b]
+1% PG	0.65[a]	0.51[c]	0.22[b]	0.07[b]
+1% PI	0.74[a]	0.69[a,b]	0.20[b]	0.00[b]
+1% PS	0.78[a]	0.69[a,b]	0.56[a]	0.11[b]
+1% PE	0.71[a]	0.70[a]	0.54[a]	0.27[a]
+1% PC	0.70[a]	0.61[a,b,c]	0.49[a]	0.32[a]
+1% LPC	0.64[a]	0.53[b,c]	0.47[a]	0.35[a]
+1% SPH	0.75[a]	0.62[a,b,c]	0.46[a]	0.42[a]

[a]Means with different letter superscripts within heating time indicate a significant difference between treatments ($P < 0.05$). Footnotes as in Table 1.

differences ($P > 0.05$) were observed in antioxidant activity (*i.e.*, TBA values) for PE, PC, LPC and SPH additions, whereas TBA values of PG, PI and PS were significantly lower than the control oil at 60 min of heating but did not differ significantly from the control after 120 min of heating.

The polyene indices (Table 3) of Experiment II showed a pattern similar to that observed for TBA values, except that TBA values changed more rapidly (*i.e.*, within 15 min of heating), whereas changes in the polyene index started occurring after 60 min of heating. The antioxidant properties of SPH, LPC, PC and PE were not significantly different from each other at the end of polyene measurements (180 min), whereas PS, PI, PG and the control oil were alike and of lower polyene index.

Figure 3B shows changes in color absorbance of Experiment II in which salmon oil was supplemented with different classes of PL. The addition of either PE or PS showed a rapid increase in color intensity after 1 h of heating, followed thereafter by more gradual increases in color absorbance up to 2 h of heating. In contrast, LPC, PC and SPH showed gradual increases in color intensity during the first hour of heating, followed thereafter by sharp increases in color intensity with further heating. Both LPC and PC produced more ($P < 0.05$) color than SPH, PE and PS after 2 h of heating. Although PG showed a moderate absorbance reading after 2 h of heating, the color produced in PG, PI and control samples was generally lower than some of the nitrogen-containing PL. Thus, color intensity appears to be a good secondary index of the antioxidant activity of individual PL subjected to thermal oxidation.

Correlation coefficients between TBA numbers, polyene index, and browning color intensity of salmon oil and salmon oil + 1% PL during heating indicate an inverse correlation between TBA values and polyene index, with the 60-min heating period showing the greatest negative correlation (Table 4). Increases in browning color intensity varied with the polyene index. The general correlation pattern appears to be similar to that observed in Experiment I in that TBA values are negatively correlated with the formation of browning reaction products and the polyene index, whereas the polyene index is positively correlated to color intensity at 60 and 120 min of heating.

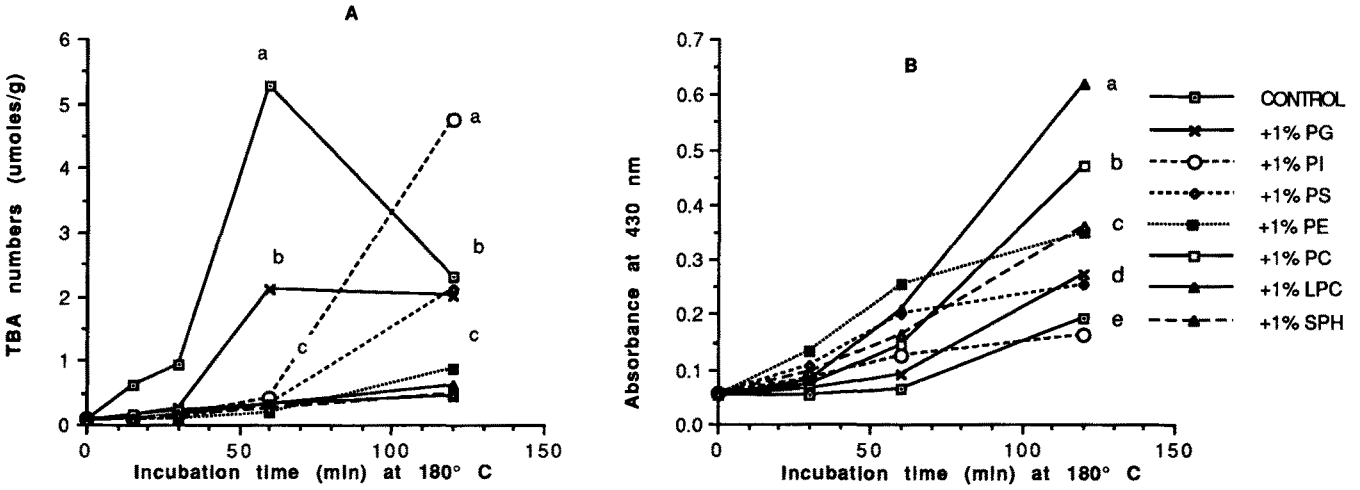

FIG. 3. Model system II—effects of 1% (w/w) additions of different classes of phospholipids on (A) thiobarbituric acid (TBA) number and (B) absorbance values of salmon oil heated to 180°C for 180 min. PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine; PC = phosphatidylcholine; LPC = lysophosphatidylcholine; SPH = sphingomyelin. Small letters a, b, c, d, e indicate significant differences between treatments ($P < 0.05$).

TABLE 4

Simple Correlation Coefficients Between TBA Numbers, Polyene Index, and Browning Color for Salmon Oil and for Salmon Oil + 1% Commercial Phospholipids at Different Heating Times

Heating time (min)		Polyene index (22:6/16:0)	Browning
0	TBA numbers	0.24	−0.16
	Polyene ratio		0.40[a]
30	TBA numbers	−0.07	−0.36[a]
	Polyene ratio		−0.25
60	TBA numbers	−0.69[b]	−0.67[b]
	Polyene ratio		0.63[b]
120	TBA numbers	−0.55[b]	−0.70[b]
	Polyene ratio		0.51[b]

[a]($P < 0.01$). [b]($P < 0.05$).

These results indicate that increases in the formation of the browning reaction products were contributing to reduced oxidation as indicated by (a) reduced TBA numbers and (b) higher polyene ratios.

Table 5 summarizes the changes in fatty acid composition of treatments at different periods of heating. The oxidation pattern was similar to that observed for Experiment I in that the control oil and some PL-supplemented oils were less effective in stabilizing salmon oil to heat abuse than were the other PL. For example, after 3 h of heating, the control oil and PG-supplemented oil had lost approximately 93% of their PUFA. However, the LPC- and SPH-supplemented treatments had lost only 35% and 28% of their PUFA, respectively. The percentage of monoenes (16:1, 18:1, and 20:1) and saturates (14:0, 16:0, 18:0, and 22:0) for each treatment increased in proportion to the reduction in n-3 fatty acids and linoleic acid. The polyene ratio (n-3/SAT) provides further evidence of the greater antioxidant properties of SPH, LPC, PC and PE than of the control oil, PG, PI and PS.

Comparison of the slope values of the oxidation of the most effective PL (SPH), against the least effective PL (PI), and the control, revealed that the PL were effective in reducing the rate of oxidation and that the rate of oxidation was also affected by its degree of unsaturation. Tables 5 and 6 show that during the 3-h heating period, the concentration of n-3 fatty acids containing more than four double bonds (Table 5; Sln-3) was reduced at a more rapid rate than was linoleic acid (18:2). The addition of SPH to the oil reduced the oxidation rate of n-3 fatty acids by a factor of 3 and the n-6 fatty acids by a factor of 25 as compared to the control. Even though the addition of PI was least effective, it also reduced the oxidation rate (slopes) of n-3 and n-6 fatty acid by 47% and 24%, respectively. As expected, the oxidation rate of 18:2 was less than that observed for the more unsaturated n-3 fatty acids. Treatments containing PE, PC, LPC and SPH were more effective in stabilizing the PUFA of PL-supplemented oils than were PG, PI or PS. No significant treatment differences ($P < 0.05$) were detected in the total PUFA content at time zero. However, by 120 min of heating, the antioxidant activities of PE, PC, LPC and SPH were quite apparent, and by 180 min of heating, lower TBA numbers, higher polyene indexes and slower rates of oxidation of n-3 fatty acids and of 18:2 were observed.

The fatty acid composition of individual PL appeared to play little or no role in the antioxidant activity of PL. For example, the fatty acid composition of PG and PC are similar, yet they appeared to have different antioxidant activities. In general, the antioxidant activity of PL appeared to be more related to their functional group than to their fatty acid composition. These findings are in agreement with the results from a previous study (12), which showed that the addition of PL from bluefish to a salmon oil model system resulted in improved oxidative stability unrelated to their fatty composition. The antioxidant activity does appear to be related to the polarity of the PL, as SPH is the most polar and retained the highest polyene ratio, followed by LPC, PC and PE with

TABLE 5

Changes in Fatty Acid Composition of Salmon Oil and of Oil Plus 1% Commercial Phospholipids During Heating (180°C for 3 h)[a]

Time (min)	Treatment	SAT (%)	MONO (%)	PUFA (%)	C18:2 (%)	Total n-3 (%)	Sln-3 (%)	n-3/SAT
0	Oil	35.23^{a,b}	33.75^{a}	31.02^{a}	1.79^{b}	29.23^{a}	24.62^{a}	0.83^{a,b}
	+PG	36.14^{a,b}	33.90^{a}	29.96^{a}	2.30^{a}	27.66^{b}	23.96^{a}	0.77^{b}
	+PI	36.19^{a,b}	33.22^{a}	30.59^{a}	1.88^{b}	28.71^{a,b}	24.24^{a}	0.79^{a,b}
	+PS	35.17^{a,b}	33.30^{a}	31.53^{a}	1.77^{b}	29.76^{a}	25.01^{a}	0.85^{a}
	+PE	35.44^{a,b}	33.27^{a}	31.28^{a}	2.21^{a}	29.07^{a,b}	24.55^{a}	0.82^{a,b}
	+PC	35.35^{a,b}	33.52^{a}	31.13^{a}	2.25^{a}	28.88^{a,b}	24.33^{a}	0.82^{a,b}
	+LPC	36.66^{a}	32.89^{a}	30.45^{a}	1.81^{b}	28.64^{a,b}	23.95^{a}	0.78^{a,b}
	+SPH	34.73^{b}	33.54^{a}	31.72^{a}	1.85^{b}	29.88^{a}	25.08^{a}	0.86^{a}
60	Oil	43.47^{a}	38.64^{a}	17.89^{c}	1.47^{c}	16.41^{c}	13.68^{c}	0.39^{d}
	+PG	37.26^{b,c}	36.43^{b}	26.31^{b}	2.25^{a}	24.06^{b}	20.12^{b}	0.65^{c}
	+PI	39.06^{b}	34.29^{c,d}	26.65^{b}	1.83^{b}	24.82^{b}	20.53^{b}	0.64^{c}
	+PS	35.25^{c}	34.52^{b,c,d}	30.24^{a,b}	1.80^{b}	28.44^{a,b}	23.85^{a,b}	0.81^{a,b}
	+PE	35.40^{c}	32.71^{d}	31.86^{a}	2.13^{a}	29.73^{a}	24.83^{a}	0.84^{a}
	+PC	35.63^{c}	34.97^{b,c}	29.40^{a,b}	2.08^{a}	27.31^{a,b}	22.82^{a,b}	0.77^{a,b,c}
	+LPC	37.96^{b,c}	34.60^{b,c,d}	27.44^{a,b}	1.76^{b}	25.68^{a,b}	21.30^{a,b}	0.68^{b,c}
	+SPH	35.50^{c}	34.95^{b,c}	29.55^{a,b}	1.83^{b}	27.71^{a,b}	23.00^{a,b}	0.78^{a,b,c}
120	Oil	51.18^{a}	42.97^{a}	5.85^{c}	0.92^{d}	4.94^{c}	4.14^{c}	0.10^{c}
	+PG	44.48^{b}	41.39^{a}	14.13^{b}	1.89^{a,b,c}	12.24^{b}	10.57^{b}	0.28^{b}
	+PI	44.35^{b}	38.48^{b}	17.17^{b}	1.19^{c,d}	15.98^{b}	13.27^{b}	0.38^{b}
	+PS	36.66^{c}	37.31^{b,c}	26.03^{a}	1.74^{a,b,c}	24.29^{a}	20.11^{a}	0.67^{a}
	+PE	38.28^{c}	34.44^{c}	27.28^{a}	2.17^{a,b}	25.11^{a}	21.01^{a}	0.66^{a}
	+PC	38.48^{c}	36.27^{b,c}	25.25^{a}	2.21^{a}	23.04^{a}	19.43^{a}	0.60^{a}
	+LPC	39.47^{c}	35.67^{b,c}	24.86^{a}	1.35^{b,c,d}	23.51^{a}	19.47^{a}	0.60^{a}
	+SPH	39.06^{c}	37.61^{b}	23.32^{a}	1.78^{a,b,c}	21.55^{a}	17.97^{a}	0.58^{a}
180	Oil	56.10^{a}	41.73^{a}	2.16^{d}	1.01^{a,b}	1.16^{d}	1.16^{d}	0.02^{d}
	+PG	51.88^{a}	39.31^{a}	8.28^{c}	1.54^{a,b}	6.74^{c}	3.34^{c}	0.13^{d}
	+PI	54.98^{a}	42.83^{a}	2.19^{d}	0.58^{b}	1.61^{d}	1.16^{d}	0.03^{d}
	+PS	52.00^{a}	40.50^{a}	7.50^{c}	0.73^{a,b}	6.76^{c}	6.76^{c}	0.13^{d}
	+PE	45.17^{b}	38.78^{a}	16.05^{b}	1.73^{a,b}	14.32^{b}	12.55^{b}	0.32^{b,c}
	+PC	42.56^{b,c}	38.63^{a}	18.81^{a,b}	1.91^{a}	16.90^{b}	14.37^{a,b}	0.40^{c}
	+LPC	41.93^{b,c}	38.42^{a}	19.65^{a,b}	1.60^{a,b}	18.05^{a,b}	15.33^{a,b}	0.43^{a,b}
	+SPH	39.77^{c}	37.47^{a}	22.76^{a}	1.87^{a}	20.89^{a}	17.25^{a}	0.53^{a}

[a]Means with different letter superscripts within heating time and fatty acid category indicate a significant difference between treatments ($P < .05$). SAT = saturated, MONO = monounsaturated; n-3 = total of all fatty acids with n-3 double bonds; Sln-3 = combined C20:5 and C22:6; PUFA = polyunsaturated fatty acids. Footnotes as in Table 1.

TABLE 6

Effects of Selected Phospholipids on the Rate of Oxidation of n-3 and n-6 Fatty Acids of Salmon Oil[a]

Treatment	Slope	r Value
Fish oil:n-3	0.693	0.999
Fish oil:n-6	0.405	0.977
Fish oil + PI:n-3	0.370	0.952
Fish oil + PI:n-6	0.306	0.804
Fish oil + SPH:n-3	0.232	0.929
Fish oil + SPH:n-6	0.027	0.87

[a]Salmon oil was used at 20 mg/treatment with each treatment containing 1% phospholipids (w/w). Regression analyses were run on the loss of n-3 and n-6 fatty acids after heating for 180 min at 180°C. Fish oil:n-3 and fish oil:n-6 = fatty acid losses for control salmon oil. Fish oil + PI:n-3 and + PI:n-6 = fatty acid losses for salmon oil +1% phosphatidylinositol. Fish oil + SPH:n-3 and + SPH:n-6 = fatty acid losses for salmon oil + 1% sphingomyelin.

no differences observed between the control and PG, PI and PS ($P < 0.05$).

The results of this study demonstrated that the nitrogen-containing PL had better antioxidant activity than did PL containing glycerol (PG) and a reducing sugar (PI) as their major reactive components. Tsai and Smith (21) detected antioxidant activity for serine and ethanolamine in the presence of a pair of free electrons from the nitrogen molecule of an amine (-NH_2 state), while choline [a quaternary amine group, $(CH_3)_3N$)] did not affect the autoxidation rate of methyl linoleate at pH 7.9 or 10.2. Since the current study was conducted in a complex oil system, it can be reasonably concluded that the experimental conditions favored a nucleophilic amino group (-NH_2). Hildebrand *et al.* (22) indicated that additions of PI, PE and PC increased the oxidative stability of soybean oil. They suggested that the amine group of PE and PC and the reducing sugar of PI can facilitate hydrogen or electron donation to tocopherol, a free-radical terminator, thereby delaying the oxidation of the oil. Because the salmon oil used in the present study contained a considerable amount of tocopherol (280 ppm), the added PL may have acted synergistically with the endogenous tocopherol in the oil to enhance the oxidative stability of the system.

Additional antioxidant activity from the PL may also have been provided by the reaction of the amino group of the nitrogen-containing PL with aldehydes formed during the thermal oxidation of unsaturated fatty acids. The

increasing color intensity of the heated PL-supplemented salmon oil appears to have been dependent on the PC and PL concentration (Experiment I and II) and associated with the nitrogen-containing PL. The brown-colored products produced may have affected the oxidative stability of the salmon oil. The colored products formed in the present study appear to be similar to Maillard reaction products formed from reactions of reducing sugars and amino acids (9,23,24). These colored compounds have been reported to form melanophosphatides, which have the ability to inactivate hydroperoxides formed during oxidation (7,9,24). Evans *et al.* (25) also reported that the high sugar-containing fractions (PG and PI) from soybean lecithin had no effect on the color or the oxidative stability of soybean oil.

Husain *et al.* (9) reported that the insoluble fractions resulting from acetone/hexane (4:1) extracts of color compounds from PC and PE showed antioxidant activity at 24 h of incubation with methyl linoleate. They suggested that the antioxidant activity of PL may depend upon the chelation of metals with polymerized products rather than any hydrogen-donating ability. In contrast to the results of Kashima *et al.* (6), the detected antioxidant activity of PS in the present experiment appeared to be less than those of PC and PE, which were approximately equal. However, in the Kashima *et al.* (6) study, the perilla oil was tested at significantly lower temperatures (37°C) compared to the 180°C used in our study. In the present study, PS maintained a high antioxidant activity level during the initial stage of heating, which was lost after 2 h of heating. The lack of antioxidant activity of PG and PI observed in the present study may be related to their functional groups. Tsai and Smith (21) found that the phosphoryl and β-hydroxy groups of PL exhibited no effect on the oxidation rate of methyl linoleate emulsions.

ACKNOWLEDGMENTS

Paper number (FSR-92-34) of the journal series of the Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-7624. Research reported in this publication was funded by the North Carolina Agricultural Research Service.

REFERENCES

1. Lea, C.H., *J. Sci. Fd. Agric. 8*:1 (1957).
2. Nasner, A., in *Lipid Oxidation-Biological and Food Chemical Aspects: Contributions at a Lipid Forum/Sik Symposium*, edited by R. Marcuse, Scandinavian Forum for Lipid Research and Technology, Gotenborg, Sweden, 1986, p. 187.
3. List, G.R., and J.P. Friedrich, *J. Am. Oil Chem. Soc. 66*:98 (1989).
4. Hudson, B.J.F., and S.E.O. Mahgoub, *J. Sci. Fd. Agric. 32*:208 (1984).
5. Yamaguchi, K., and M. Toyomizu, *Bull. Soc. Sci. Fish. 50*:1897 (1984).
6. Kashima, M., G.S. Cha, Y. Isoda, J. Hirano and T. Miyazawa, *J. Am. Oil Chem. Soc. 68*:119 (1991).
7. Privett, O.S., and F.W. Quackenbush, *Ibid. 31*:225 (1954).
8. Jewell, N.E., and W.W. Nawar, *Ibid. 57*:398 (1980).
9. Husain, S.R., J. Terao and S. Matsushita, in *Amino-Carbonyl Reactions in Food and Biological Systems*, edited by M. Fugimaki, M. Namiki and H. Kato, Elsevier Press, New York, NY, 1984, p. 301.
10. Porter, W.L., in *Recent Trends in Food Applications of Antioxidants*, edited by M.G. Simic, and M. Karel, Plenum Press, New York, NY, 1980, p. 295.
11. Moberger, L., in *Lipids Oxidation-Biological and Food Chemical Aspects: Contributions at a Lipid Forum/Sik Symposium*, Gotenborg, Sweden, edited by R. Marcuse, Scandinavian Forum for Lipid Research and Technology, Gotenborg, Sweden, 1986, p. 114.
12. King, M.F., and L.C. Boyd, Abstract, Institute of Food Technologists' Annual Conference, Anaheim, CA, 1990, p. 219.
13. Widicus, W.A., and J.R. Kirk, *Assoc. Off. Anal. Chem. 62*:637 (1979).
14. Kaduce, T., K.C. Norton and A.A. Spector, *J. Lipid Res. 24*:1398 (1983).
15. Morrison, W.R., and L.M. Smith, *Ibid. 5*:600 (1964).
16. Sampugna, J., L.A. Pallansch, M.E. Enig and M. Keeney, *J. Chromatogr. 24*:245 (1982).
17. Ke, P.J., and A.W. Woyewoda, *Analytica Chimica Acta 106*:279 (1979).
18. Shono, T., and M. Toyomizu, *Bull. Jap. Soc. Sci. Fish 22*:290 (1972).
19. Helwig, J.T., and K.A. Council, *SAS User's Guide*, edited by SAS Institute, Inc., Cary, NC, 1982.
20. SAS, *Statistical Analysis System. User's Guide: Statistics*, SAS Institute Inc., Cary, NC, 1988.
21. Tsai, L., and L.M. Smith, *Lipids 6*:196 (1972).
22. Hildebrand, D.H., J. Terao and M. Kito, *J. Am. Oil Chem. Soc. 61*:552 (1984).
23. Corlis, G.A., and J.R. Dugan, *Lipids 5*:846 (1970).
24. Bratkowska, I., *Acta Alim. Pol. 4*:256 (1978).
25. Evans, C.D., P.M. Cooney, C.R. Scholfield and H.J. Dutton, *J. Am. Oil Chem. Soc. 31*:295 (1954).

[Received October 29, 1991; accepted April 14, 1992]