# Effects of Phospholipids on Lipid Oxidation of a Salmon Oil Model System

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**Total lipid (TL), neutral lipid (NL), and phospholipid (PL) fractions were extracted from bluefish *(Pomatomus saltatrix)* white and dark muscle with skin. The effects of each fraction on the oxidative stability of a refined salmon oil model system was measured by monitoring changes in the 2-thiobarbituric acid assay and decreases in the ratio of docosahexaenoic acid (DHA) to palmitic acid (C22:6/C16:0) following incubation at 55°C or 180°C. Phospholipid fractions at 2.5% and 5.0% (wt/wt) of oil improved the oxidative stability of oils incubated at both temperatures compared to controls, TL- and NL-supplemented oils at similar concentrations. Phospholipid fractions exhibiting antioxidant properties contained an average of 34% DHA as compared to only 15% in the NL and TL fractions.**

KEY WORDS: Antioxidant properties, bluefish, model system, oxidation, phospholipids, tissue lipids.

Oxidative deterioration of food lipids has been shown to be the single most important factor contributing to the development of off-flavors in fat-containing foods. Consumer demands for convenient items requiring little or no preparation have caused increased concern for off-flavors that may develop during storage of these processed foods. This problem is especially critical to pre-cooked fish products, which contain unsaturated fatty acids with chainlengths up to 22 carbon atoms and five to six double bonds.

In comparison to triglycerides (TG), phospholipids (PL) are generally thought to be the major lipid fraction responsible for the oxidative deterioration and off-flavor development of foods, due to their greater degree of unsaturation (1). Several studies involving the use of cooked beef, pork, lamb, and poultry concluded that triglycerides play only a minor role in the development of oxidative rancidity (2–5).

Research findings from studies involving fish have not been as conclusive about the role of PL in oxidation. Several studies (6–8) have reported that hydrolysis of PL fatty acids results in increased lipid oxidation; while other studies (9,10) demonstrated that PL had antioxidant properties. Shewfelt *et al.* (9) showed that the addition of phospholipase $A_2$ to flounder muscle microsomes led to increased PL hydrolysis and to the inhibition of both enzymatic and nonenzymatic lipid oxidation. A similar finding of an increased release of free fatty acids and reduced oxidation has also been shown in frozen mullet (11).

The antioxidant properties of extracted PL have also been demonstrated through their addition to processed vegetable oils and animal fats, such as sunflower, corn, cottonseed, soybean, and lard (12,13). The addition of 0.1% (by wt) soybean lecithin to lard extended the storage stability by 50%; whereas the addition of 1.0% was required to obtain an equivalent stability in sunflower oil (12). The variation in PL concentrations required to achieve the same level of oil stability was attributed to differences in the fatty acid composition of the two fats, the fatty acid composition of the PL, and the tocopherol content of the oil. Similar antioxidant properties of PL have been demonstrated in supercritical-extracted seed oils where the PL had been removed during the processing. The addition of commercially available phosphatides at a concentration of 0.16% (by wt) increased the stability of the heated seed oils (13).

Some of the inconsistency in understanding the role of PL in oxidation might be explained by the broad, perhaps oversimplified, application of *in vitro* test model systems to study oxidation of tissue lipids. Since it is generally accepted that most tissue PL exist in cells as bilayers in membranes and are frequently associated with a number of other cellular constituents (*e.g.*, enzymes, metal ions, heme, etc.) having both pro- and antioxidant activities, many *in vitro* test model systems involving extracted tissue or oils may not be truly representative of tissue oxidation under natural conditions (9).

Therefore, the objectives of this study were to investigate the role of phospholipids and the degree of fatty acid unsaturation on lipid oxidation in a fish oil test model system subjected to heated storage conditions. Since other model systems have measured the antioxidant properties of PL in mixed systems containing both pro- and antioxidant cellular components, the present system is designed to examine antioxidant properties of PL in a pure system in which only solvent-extracted components might be present.

## MATERIALS AND METHODS

*Preparation of bluefish lipid fractions.* Three medium-size bluefish *(Pomatomus saltatrix)*, weighing approximately 4.4 kg each, were obtained from a local fresh fish market in Raleigh, North Carolina during the month of June, 1989. The fish were obtained from the market with head and viscera removed at the point of purchase, followed by washing and transporting on ice to the seafood laboratory at the Department of Food Science at North Carolina State University. Within one hour of purchase the fish were separated into white muscle and dark muscle with skin. In an effort to facilitate grinding of tissues without significant rise in temperature, all tissues were frozen in an air-circulated freezer for approximately 2–3 hr to obtain partially frozen samples (3°C). A sample from each tissue consisting of approximately 150 g was ground in a Cuisinart Food Processor (Cuisinarts, Inc., Greenwich, CT) at medium speed for 5 min. Total lipids (TL) were extracted from duplicate 50-g samples of white tissue and of dark tissue with skin by the procedure of Bligh and Dyer (14). Following concentration of the chloroform extracts under a nitrogen atmosphere at 35°C, total fat was determined gravimetrically. Aliquots (100 mg) of each type of tissue (white and dark muscle plus skin) were separated into neutral lipids (NL) and phospholipids (PL)

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by using Sep-pak silica gel cartridges (Waters Associates, Milford, MA) as described by Bitman *et al.* (15). The phosphorus assay, as described by Bartlett (16), was used to quantitate phospholipids and to check for completeness of the silica-pak separation of PL and NL. Because of the relatively small percentage of PL present in both tissues, multiple PL extractions (*i.e.,* 6–100 mg) were used to obtain sufficient PL for use in the storage stability model studies.

*Phospholipid class separation.* Phospholipid fractions from white and dark muscle with skin were separated into individual classes of PL by high-performance liquid chromatography (HPLC) (17). Total and individual phospholipid concentrations were estimated by multiplying their phosphorus concentrations from the Bartlett assay (16) by 25, except for lysophosphatidylcholine (LPC), which was estimated by multiplying by a factor of 17 (18). Phospholipids were identified by comparison to authentic standards obtained from Sigma Chemical Co. (St. Louis, MO).

*Model oil systems.* The antioxidant properties of total lipid, neutral lipid, and phospholipid fractions from both white and dark muscle with skin were determined by their addition to refined salmon oil in three different model systems. The salmon oil was obtained without the addition of antioxidants or stabilizers from Body Products Research, Inc. (Chatsworth, CA). Prior to use, the salmon oil, TL of each tissue, and individual fractions of NL and PL were analyzed for their tocopherol content by HPLC as described by Widicus and Kirk (19). In two of the model systems (*i.e.,* I and II), salmon oil was added in a chloroform solvent system, followed by the addition of selected fractions of extracted lipids from bluefish. All treatments were mixed on a vortex mixer at medium speed for 1 min, allowed to sit exposed to the atmosphere at room temperature (*i.e.,* 21.1°C) for 10 min, followed by closure with teflon-lined caps and heating in a forced-draft oven at selected temperatures.

*Model system I.* The first model system consisted of the addition of six fractions—white total lipids (WTL), white neutral lipids (WNL), white phospholipids (WPL), dark total lipids (DTL), dark neutral lipids (DNL), and dark phospholipids (DPL) at 5% (wt/wt) of the salmon oil. Fifty-mg aliquots of each fraction were added to 1 gm of salmon oil dissolved in 25 mL of chloroform in 50-mL test tubes with teflon-lined caps. All samples were heated in a forced-draft oven at 55°C for six weeks with analyses performed at 0, 12, 28, and 42 days. Duplicate 20-mg aliquots of oil were removed for chemical analyses at each interval, followed by recapping and continued heating over the duration of the study.

*Model system II.* The second model system was similar to model system I except that 0.02% (wt/wt) butylated hydroxytoluene (BHT) (Sigma Chemical Co.) was added to the salmon oil as a treatment and only WPL's were used. The four treatments were as follows: i) control-salmon oil; ii) salmon oil containing 0.02% BHT; iii) salmon oil containing 2.5% WPL; and iv) salmon oil containing 5.0% WPL. The BHT was added at 0.02% of lipid weight to simulate maximum levels that can be added under legal restrictions for food-type oils. The WPL were selected because fatty acid analyses of the different fractions had shown that the WPL contained the highest percentage of omega-3 polyunsaturated fatty acid(s) (PUFA) when compared to TL, NL, and DPL. All samples were incubated and analyzed in duplicate at periods of 0, 7, 14, 21, 28, 35, 42, and 60 days.

*Model system III.* The third model system was patterned after model system II in that TL, NL, and PL fractions from white muscle were tested in addition to the DPL fraction. This system differed from model systems I and II in that samples were heated in the absence of the chloroform solvent system at 180°C up to 180 min with duplicate 20-mg aliquots of salmon oil/treatment at each time interval. Chemical analyses were performed at intervals of 0, 15, 30, 60, 120, and 180 min. The treatments consisted of the following: i) control-salmon oil; ii) salmon + 5% WTL; iii) salmon + 5% WNL; iv) salmon + 5% WPL; and v) salmon oil + 5% DPL.

*Test of oxidative stability.* Oxidative stability was determined by measuring changes in the 2-thiobarbituric acid number (TBA) (20) and polyene index (PI). The polyene index (PI) was calculated as the weight ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SAT) (PUFA/SAT). Since eicosapentaenoic acid [C20:5 (EPA)] and docosahexaenoic acid [C22:6 (DHA)] represent the two most highly unsaturated fatty acids present in marine fish oils, the loss of DHA to palmitic acid (*i.e.,* C22:6/C16:0) was used as the major index of PUFA loss in this study (21). Fatty acid methyl esters (FAME) were prepared by the method of Morrison and Smith (22). The FAME were determined on a Hewlett Packard model 5890A gas chromatograph (GC) (Avondale, PA) equipped with a flame ionization detector and HP 3393A integrator; interfaced to an IBM PC-2 computer (IBM Corp., Rye Brook, NY) for data storage and handling. Separation was accomplished with a 30 m × 0.25 mm i.d. DB 225 fused silica 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. The injector and detector temperatures were 250°C and 275°C, respectively. Total column flow was 1.2 mL/min with a split flow of 70:1. All samples were dissolved in 0.4 mL HPLC-grade iso-octane with 0.6 μL or less injected onto the GC.

Identification of FAME was based on comparisons of retention times of unknown peaks to authentic FAME (NuChek Prep, Elysian, MN). Fatty acid composition was expressed as weight percent of total fatty acid methyl esters and was used to calculate the polyene index. Normalization technique was used to calculate response factors for all identified fatty acid peaks.

*Statistical analyses.* A General Linear Model was used to analyze all data as a randomized complete block design with treatments blocked with heating times. Mean differences were determined by using Duncan's Multiple Range Test for separation of means showing significant differences (23). All analyses were performed in duplicate.

## RESULTS AND DISCUSSION

The addition of crude bluefish lipid fractions including TL, NL, and PL to a refined salmon oil model system improved the oil's stability as measured by changes in the TBA values and polyene index (Tables 1 and 2). The control samples, which contained no added lipid fractions, oxidized rapidly over the first 12 days of heating while the added lipid fractions extended the stability of the salmon oil beyond 42 days of heating (Table 1). The DNL fraction exhibited the least antioxidant properties, whereas the

TABLE 1

**Model System I: Effect of Bluefish Lipid Extracts on TBA Values (μmoles/g) of Heated Salmon Oil[a]**

| Treatments | Days of heating | | | |
|---|---|---|---|---|
| | 0 | 12 | 28 | 42 |
| Control | 0.17 aA | 0.43 bB | 26.30 cB | 22.78 dC |
| 5% DTL | 0.18 aA | 0.20 aA | 0.28 aA | 0.38 bA |
| 5% DNL | 0.16 aA | 0.25 aA | 0.38 aA | 0.58 bB |
| 5% DPL | 0.23 aA | 0.24 aA | 0.35 aA | 0.35 aA |
| 5% WTL | 0.15 aA | 0.21 aA | 0.28 aA | 0.33 aA |
| 5% WNL | 0.17 aA | 0.23 aA | 0.32 aA | 0.28 aA |
| 5% WPL | 0.18 aA | 0.21 aA | 0.27 aA | 0.28 aA |

[a]Model contained 1 g of oil and 5% of each fraction incubated at 55°C.

A, B and C, means within columns with common capital letters are not significantly different between treatments at $P < 0.05$. a, b and c, Means within rows with common lower case letters are not significantly different at $P < 0.05$. TBA, thiobarbituric acid; DTL, dark muscle total lipid; DNL, dark muscle neutral lipid; DPL, dark muscle phospholipid; WTL, white muscle total lipid; WTL, white muscle total lipid; WNL, white muscle neutral lipid; and WPL, white muscle phospholipid.

TABLE 2

**Model System I: Effects of Bluefish Lipid Extracts on Polyene Index of Heated Salmon Oil[a]**

| Treatments | Days of heating | | | |
|---|---|---|---|---|
| | 0 | 12 | 28 | 42 |
| Control | 0.59 aC | 0.50 bC | 0.32 cC | 0.15 dB |
| +5% DTL | 0.60 aBC | 0.53 bBC | 0.53 bB | 0.60 aA |
| +5% DNL | 0.60 aBC | 0.53 bBC | 0.57 abAB | 0.60 aA |
| +5% DPL | 0.65 aA | 0.60 abA | 0.58 bA | 0.65 aA |
| +5% WTL | 0.64 aAB | 0.53 cBC | 0.59 bA | 0.62 aA |
| +5% WNL | 0.62 aABC | 0.56 bAB | 0.59 abA | 0.61 aA |
| +5% WPL | 0.64 aAB | 0.60 aA | 0.60 aA | 0.64 aA |

[a]Model contained 1 g of oil and 5% of each fraction incubated at 55°C.

A, B, and C, means within columns with common capital letters are significantly different between treatments at $P < 0.05$. a, b, and c, Means within rows with common lower case letters are not significantly different between heating times at $P < 0.05$. Polyene index, C22:6/C16:0; DTL, dark muscle total lipid; DNL, dark muscle neutral lipid; DPL, dark muscle phospholipid; WTL, white muscle total lipid; WNL, white muscle neutral lipid; and WPL, white muscle phospholipid.

WNL and WPL yielded similar antioxidant effects. The change in polyene ratio (Table 2) showed a similar pattern to TBA changes. Control oils showed significant losses in DHA, as noted by a drop in the polyene index (PI) after 28 days of heating. The change in fatty acid composition as indicated by PI does not appear to be as sensitive to oxidation as the TBA assay, as noted by the lack of significant treatment differences among the lipid-supplemented treatments. Polyene index showed no major change in the control values until 28 days of heating as compared to differences noted in the TBA assay after only 12 days of heating.

Because of the unexpected antioxidant properties detected in the neutral lipid fraction, all fractions of each tissue including TL, NL, and PL of each tissue were examined for tocopherol content (Table 3). Higher tocopherol levels were present in the neutral lipid fractions compared to PL fractions, which may have contributed to the antioxidant properties of the NL fraction and the lack of significant differences noted between WNL and WPL fractions (Tables 1 and 2).

Considerable variation was detected in the PUFA composition of the refined fish oil, TL, NL, and PL fractions (Table 4). The salmon fish oil contained only 19.21% EPA and DHA compared to 37.16% and 28.05% for WTL and DTL, respectively. Dark and white muscle PL contained similar concentrations of PUFA which were significantly higher than that of the NL fraction. The DNL fraction contained the lowest PUFA content. Therefore, when the relative percentages of PUFA found in salmon fish oil, TL, NL, and PL fractions are examined and compared to the oxidation pattern measured by TBA values and decreasing PI, these comparisons fail to show the anticipated prooxidant activity of the PL, considering the higher PUFA content of both dark and white muscle PL.

Total PL were separated by HPLC into seven fractions that included solvent front, PI, phosphatidylserine (PS), phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), LPC, phosphatidylcholine (PC), and sphingomyelin (SPH). The PL composition of white tissue and dark tissue with skin (Table 5) were similar, with few differences observed between the two tissues. Based on phosphorus analyses, higher concentrations of PE and SPH were found in dark muscle with skin than in white muscle. The PC was the predominant PL class in both muscles, followed by PE, SPH, LPC, solvent front, PI, PS, and LPE.

Because the WPL appeared to have one of the highest antioxidant activities, as well as the highest PUFA content (53.42%), model system II was designed to further study the antioxidant activity of PL in comparison to the synthetic antioxidant BHT. When compared with the control, both the 2.5% and 5.0% PL additions were equally effective in preventing oxidation as noted by lower TBA

TABLE 3

**α-Tocopherol and Phosphorus Content of Salmon Oil and Bluefish Lipid Extracts[a]**

| Sample[a] | α-Tocopherol (mg/g lipid) | Phosphorus (mg/100 g of tissue) |
|---|---|---|
| Salmon oil | 146 AB | 1.95 F[b] |
| DTL | 1.200 C | 494.29 A |
| DNL | 0.766 B | 47.43 E |
| DPL | 0.102 A | 406.36 B |
| WTL | 0.642 B | 345.47 C |
| WNL | 0.432 AB | 43.22 E |
| WPL | 0.163 A | 238.30 D |

[a]Numbers followed by the same letter were not significantly different at $P < 0.05$. DTL, dark muscle total lipid; DPL, dark muscle phospholipid; WTL, white muscle total lipid; WNL, white muscle neutral lipid; WPL, white muscle phospholipid; and DNL, dark muscle neutral lipid.

[b]Phosphorus content in mg/100 g oil.

**TABLE 4**

**Fatty Acid Composition of Salmon Oil and Bluefish Lipid Extracts[a]**

| Fatty acid | Salmon oil | DTL | DNL | DPL | WTL | WNL | WPL |
|---|---|---|---|---|---|---|---|
| 14:0 | 5.77[b] | 4.32 | 4.64 | — | 3.00 | 2.01 | — |
| 16:0 | 15.92 | 23.25 | 22.78 | 25.03 | 25.31 | 21.48 | 29.24 |
| 16:1 | 5.08 | 7.74 | 9.14 | 1.55 | 3.68 | 3.75 | 2.01 |
| 17:1 | — | 0.62 | — | — | 0.86 | — | — |
| 18:0 | 2.63 | 7.80 | 8.17 | 9.08 | 6.12 | 7.01 | 4.17 |
| 18:1 | 21.16 | 18.72 | 21.15 | 11.37 | 12.73 | 12.19 | 11.16 |
| 18:2 | 1.66 | 1.30 | 1.44 | — | 1.14 | — | — |
| 19:1 | 0.48 | — | — | — | — | — | — |
| 18:4 | 2.07 | 1.67 | 2.17 | — | 0.94 | — | — |
| 20:1 | 10.85 | 0.54 | — | — | — | — | — |
| 20:4 | — | 2.09 | 1.56 | 4.17 | 4.23 | 3.43 | 5.44 |
| 21:1 | 0.58 | — | — | — | — | — | — |
| 20:5 | 8.96 | 9.21 | 9.79 | 8.72 | 8.82 | 7.28 | 9.65 |
| 22:0 | 8.76 | — | — | — | — | — | — |
| 22:1 | 0.62 | — | — | — | — | — | — |
| 22:4 | — | 0.51 | — | 2.20 | 1.67 | 2.24 | 2.09 |
| 22:5 | 5.22 | 3.40 | 3.50 | 4.30 | 3.16 | 13.60 | 3.20 |
| 22:6 | 10.24 | 18.84 | 15.66 | 33.57 | 28.34 | 27.01 | 33.05 |
| SAT[c] | 33.09 A | 35.99 A | 35.59 A | 34.11 A | 34.43 A | 34.96 A | 33.40 A |
| MON | 38.76 A | 27.00 B | 30.29 B | 12.92 D | 17.26 C | 18.29 C | 13.17 D |
| PUFA | 28.15 D | 37.01 C | 34.12 C | 52.97 A | 48.30 AB | 46.75 B | 53.42 A |
| (n-3)[d] | 26.50 C | 3.12 B | 31.12 BC | 46.59 A | 41.27 A | 41.91 A | 45.89 A |
| (Sln-3)[e] | 19.21 E | 28.05 D | 25.45 D | 42.29 AB | 37.16 C | 38.48 BC | 42.70 A |

[a]DTL, dark muscle total lipid; DNL, dark muscle neutral lipid; DPL, dark muscle phospholipid; WTL, white muscle total lipid; WNL, white muscle neutral lipid; WPL, white muscle phospholipid; SAT, saturated; MON, monounsaturated; PUFA, polyunsaturated.

[b]Percent of total fatty acids.

[c]Groups of fatty acids followed by same capital letter are not significantly different at $P < 0.05$.

[d]Total n-3 or omega-3 fatty acids.

[e]Selective n-3 fatty acids, including 20:5 and 22:6 only.

**TABLE 5**

**Phospholipid Composition of White and Dark Muscle with Skin of Bluefish[a]**

| | Dark muscle | | White muscle | |
|---|---|---|---|---|
| | Mg PL/100 g | | Mg PL/100 g | |
| Phospholipid fraction | Tissue | Percent[b] | Tissue | Percent |
| Solvent front | 18.94 ± 3.84 A | 5.15 ± 1.14 c | 10.13 ± 0.84 B | 3.14 ± 0.24 d |
| Phosphatidylinositol | 11.65 ± 3.44 A | 3.14 ± 0.86 c | 11.35 ± 2.58 A | 3.53 ± 0.86 c |
| Phosphatidylserine | 4.70 ± 1.29 A | 1.27 ± 0.33 c | 3.88 ± 0.22 A | 1.21 ± 0.13 c |
| Phosphatidylethanolamine | 55.08 ± 8.78 A | 14.90 ± 1.99 c | 42.17 ± 4.07 B | 13.08 ± 1.12 c |
| Lysophosphatidylethanolamine | 4.34 ± 0.30 A | 1.18 ± 0.12 c | 3.85 ± 0.80 A | 1.20 ± 0.29 c |
| Phosphatidylcholine | 229.91 ± 28.35 A | 62.05 ± 2.89 d | 219.64 ± 22.27 A | 67.92 ± 0.87 c |
| Lysophosphatidylcholine | 17.05 ± 2.79 A | 4.59 ± 0.30 c | 14.21 ± 2.31 A | 4.38 ± 0.43 c |
| Sphingomyelin | 28.57 ± 4.15 A | 7.71 ± 0.72 c | 17.87 ± 2.09 B | 5.54 ± 0.53 d |

[a]Mean ± standard deviation of two replicates.

[b]Percent of total phospholipid.

A and B, numbers within PL fraction followed by the same capital letter were not significantly different within mg of phospholipid per 100 g of tissue at $P < 0.05$. c and d, Numbers within PL fraction followed by the same lower case letter were not significantly different within percent of total phospholipid at $P < 0.05$.

values (Fig. 1) and higher PI values (Fig. 2). The formation of TBARS in the control sample indicated that oxidation had occurred after 21 days, though significant change in the PI was not detected until day 42. The BHT treatment appeared to extend the TBA oxidation induction period only slightly, as indicated by the rapid increase in oxidation detected around day 28. However, the polyene index of the BHT-supplemented treatment did not change until the end of the study (60 days).

The oxidation of model system III, heated at 180°C, followed a pattern similar to model systems I and II. However, due to the higher temperature used in this study, more rapid changes in PI and TBA values were detected. Increases in TBA values (Fig. 3) were significantly higher for the control, WTL, and WNL-supplemented oils after 15 min of heating, whereas both 5% WPL- and 5% DPL-supplemented oils remained relatively low throughout the entire heating period. Decreases in the PI index (Fig. 4) for control, WTL, and WNL occurred within 30 min of heating, with no measurable amount of DHA remaining in the control after 180 min of heating. Oil samples containing the PL fractions from white and dark muscle retained more than 50% of their DHA following the same

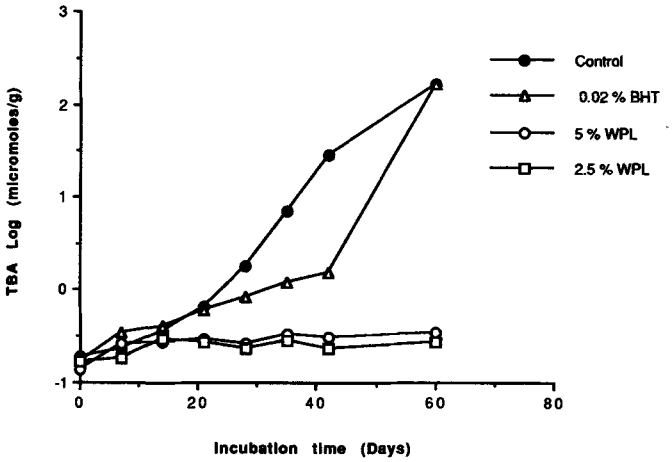


FIG. 1. Changes in TBA (log μmoles/g) of model system II. Model system II contained control oil (salmon oil), supplemented with 0.02% BHT (butylated hydroxytoluene) or 2.5% or 5% WPL (white muscle phospholipid), and was incubated at 55°C.

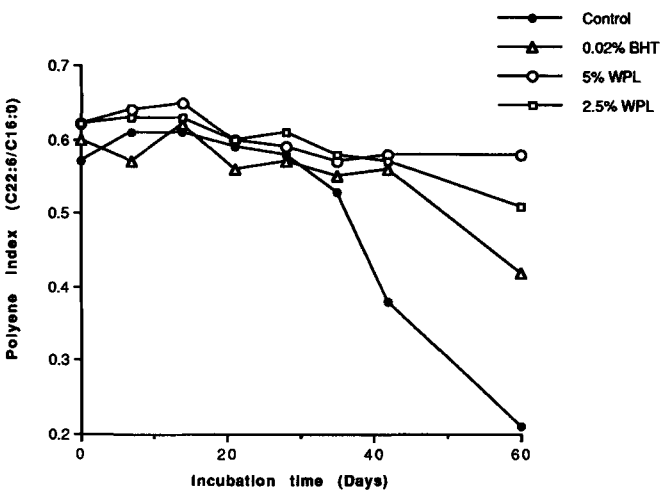


FIG. 2. Changes in polyene index (C22:6/C16:0) of model system II. Model II contained control oil (salmon oil), supplemented with 0.02% BHT (butylated hydroxytoluene) or 2.5% or 5.0% WPL (white muscle phospholipid), and was incubated at 55°C.

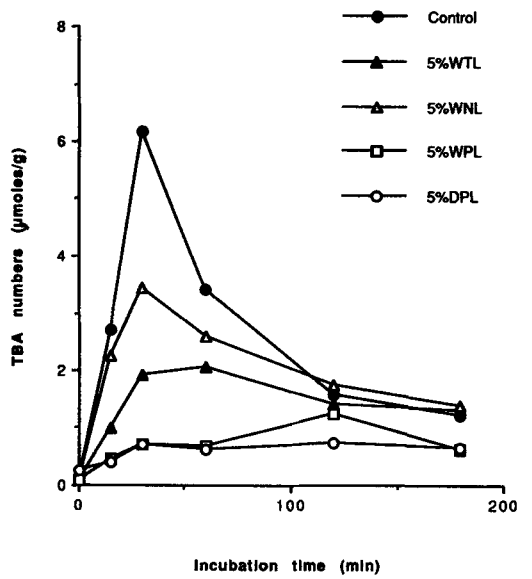


FIG. 3. Changes in TBA values (μmoles/g) of model system III containing control oil (salmon), supplemented with 5% WTL (white muscle total lipid), 5% WNL (white muscle neutral lipid), 5% WPL (white muscle phospholipid), or 5% DPL (dark muscle phospholipid), and was incubated at 180°C.

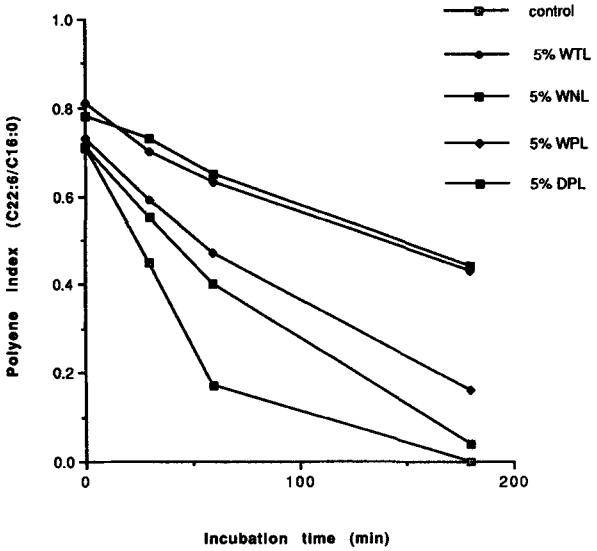


FIG. 4. Changes in polyene index (C22:6/C16:0) of model system III containing control oil (salmon oil), supplemented with 5% WTL (white muscle total lipid), 5% WNL (white muscle neutral lipid), 5% WPL (white muscle phospholipid), or 5% DPL (dark muscle phospholipid), and was incubated at 180°C.

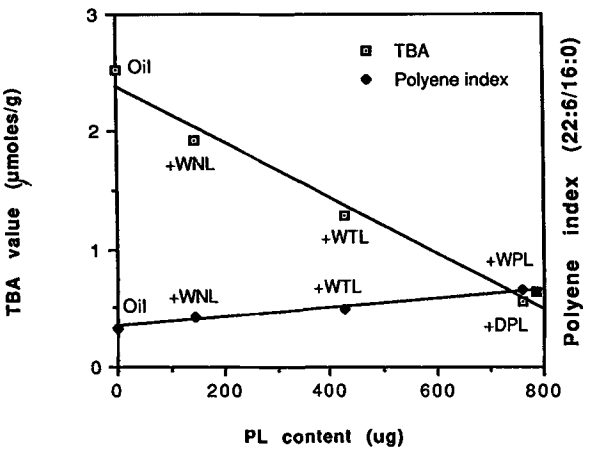


**FIG. 5. Correlation between mean values of TBA values and polyene index with content of PL in treatments of Model III during heating at 180°C (r = 0.99). Oil (control); WTL, white total lipid; WNL, white neutral lipid; WPL, white phospholipid; and DPL, dark phospholipid.**

period of heating. Examination of the chemical parameters (Fig. 5) used to measure oxidative stability of model III revealed excellent correlations (r = 0.99) between mean values for TBA numbers and polyene index with phosphorus content of extracted fractions added to the salmon oil model system.

Comparison of data from each of the three model systems in the present study indicates that PL-supplemented salmon oil exhibited greater stability to heating at 55°C or 180°C than controls (non-supplemented oil) or BHT-, TL-, or NL-supplemented oils, as noted by significantly lower TBA values and reduced PUFA loss. When compared to treatments containing added BHT and lower PUFA content, PL fractions from both white and dark muscle with skin were equally effective in improving oxidative stability of heated salmon oil. These findings are in agreement with several other *in vitro* model studies (12,13), which showed that the addition of small quantitites of PL to refined vegetable and animal fats improved their stability. These findings are significant in that PL fractions exhibited greater antioxidant activity, yet contained higher concentrations of PUFA and less tocopherol than found in TL, NL or BHT-supplemented salmon oil model systems. Since commercial fish oils are generally stripped of their PL content during processing, the re-addition of small quantities of PL could improve the overall stability of the oils while adding significantly to the PUFA content.

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