Antioxidative Activity of Egg Yolk Phospholipids

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The antioxidative activities of 0.1% additional egg yolk lipid (EYL) fractions (egg yolk neutral lipids, EYL-N; egg yolk lipids containing 31.3% phospholipids, EYL-30; egg yolk lipids containing 68.0% phospholipids, EYL-65; and egg yolk lipids containing 96.8% phospholipids, EYL-95) in rich docosahexaenoic acid (DHA) oil were investigated. The antioxidative activity of EYL was in the order EYL-95 > EYL-65 > EYL-30 ≫ EYL-N and proportional to phospholipid concentration. The antioxidative capability of purified egg yolk phospholipids (egg yolk phosphatidylcholine, EY-PC; egg yolk phosphatidylethanolamine, EY-PE; hydrogenated fatty acid chain-containing phosphatidylcholine, HFA-PC; and hydrogenated fatty acid chain-containing phosphatidylethanolamine, HFA-PE) in rich DHA oil was also examined. The result indicated that the antioxidative activity of phospholipids was in the order EY-PE > EY-PC ≫ HFA-PE > HFA-PC and decreased with an increase in the degree of saturation of fatty acid chains within the phospholipids. The antioxidative effect of EYL-30 in squalene was also examined, and the result indicated that the effect of the antioxidant is the concentration dependent.

Keywords: *Phospholipid; lecithin; antioxidant; DHA oil; squalene*

INTRODUCTION

Long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) and arachidonic acid, are essential fats in the diet for growth, brain function, and visual acuity, especially for infants (Neuringer et al., 1984; Yamamoto et al., 1987). Many products, e.g., milk, infant formulations, yogurt, bread, etc., containing DHA as a functional food ingredient have been launched in the market because of their increased health benefits (Zwier and Newton, 1995). The degree of unsaturation of highly unsaturated fatty acids, such as DHA with six pairs of double bonds, makes it extremely sensitive to oxidation, resulting in lipid peroxide and subsequent development of off flavors, odors, and dark color, which decrease the nutritive value of polyunsaturated oils and related food. DHA is extremely susceptible to free radical mediated oxidation. Squalene is found in the liver oil of shark in large quantities and is known as the precursor of cholesterol (Cook, 1958; Langdon and Bloch, 1953; Ritter and Dempsey, 1971). Squalene has been used as a functional food but is susceptible to oxidation because of its double bonds (Imaeda et al., 1983; Kohono et al., 1993). The antioxidation of DHA and other similar fatty acids has attracted renewed research interest. The control of oxidation of polyunsaturated fatty acids in foods is desirable.

Antioxidative properties of soy phospholipids have been demonstrated in fish oil, vegetable oil, and animal fat (Dziedzic and Hudson, 1984; Hara et al., 1992; Hilderbrand et al., 1984; King et al., 1992; Segawa et al., 1994, 1995) . Lecithin has been used as an antioxidant synergist (Hara et al., 1992; Kashima et al., 1991; Ohshima et al., 1993). Significant studies on the use of egg yolk lipids (EYL) as an antioxidant (Husain et al., 1986) has been performed only to a limited extent. In the present investigation, the antioxidative activities of EYL with various lipids contents in rich DHA oil and squalene were studied.

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MATERIALS AND METHODS

Materials. Rich DHA oil (DHA-27) containing 0.3% natural tocopherols was purchased from Tamaseikagaku Co., Ltd. (Tokyo, Japan). Squalene was obtained from Kira Cosmetics Co., Ltd. (Tokyo, Japan). Palladium carbon (5%) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Ethanol of special reagent grade was used for the phospholipid purification.

Preparation of Various Egg Yolk Lipids. EYL-30 was extracted from egg yolk powder (1 ton) twice with 14-fold by volume ethanol at 40 °C. EYL-65 and EYL-95 were further purified from EYL-30 by acetone as reported previously (Juneja et al., 1994). The characterization of EYL-N, EYL-30, EYL-65, and EYL-95, including phospholipid content, acid value, iodine value, and peroxide value (The Japan Oil Chemists Society, 1966) was listed in Table 1. EYL-95 (containing more than 95% phospholipids) was applied on a silica gel column (15−30 μm; Soken Kagaku Co., Ltd., Tokyo, Japan), and egg yolk phosphatidylcholine (EY-PC) and egg yolk phosphatidylethanolamine (EY-PE) were purified from the EYL-95 (200 g) with methanol/water (98:2; v/v) as an eluent at the flow rate of 200 mL/min (Juneja et al., 1994). 81.1 g of EY-PC (>99% purity) and 22.5 g of EY-PE (>99% purity) were yielded and used in the present study.

Preparation of Hydrogenated Fatty Acid Chain-Containing Phospholipids. EY-PE and EY-PC were purified from EYL-95 as described above, and hydrogenated fatty acid chain-containing -PC (HFA-PC) and -PE (HFA-PE) were prepared as the following procedure: 1 g of EY-PC or EY-PE was dissolved in 10 mL of methanol, 100 mg of palladium carbon as a catalyst was added to the mixture, and the reaction was carried out at a pressure of 80 kgf/cm^2 under a hydrogen atmosphere at 100 °C for 3 h. The reaction mixture was filtered and evaporated at 45 °C under reduced pressure. HFA-PC (882 mg) and 920 mg of HFA-PE were yielded.

Analysis of Lipids Composition. The purity of EY-PE and EY-PC was detected by thin layer chromatography coupled to a flame ionization detector (TLC/FID) (Iatoroscan TH-10

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Table 1. Characterization of Egg Yolk Lipids

	EYL-N	EYL-30	EYL-65	EYL-95
phospholipids (%)	*a*	31.3	68.0	96.8
acid value[b]	1.4	6.3	16.0	19.3
iodine value[c]	82.0	75.2	68.7	62.1
peroxide value[d]	2.0	<0.1	<0.1	<0.1

[a] Below the detection limit analyzed by TLC/FID. [b] Acid value generally indicates the content of free fatty acids in the lipid but in this case reflects the content of polar-heads of phospholipids. [c] Iodine value indicates the degree of unsaturation of fatty acyl chain-containing lipids. [d] Peroxide value indicates the content of peroxidation products.

TLC/FID analyzer, Iatron Laboratories Inc., Tokyo, Japan) attached with an integrator (Iatrocorder TC-11, Iatron Laboratories Inc.) (Tanaka et al., 1977). The lipid sample was spotted on the chromarod SII quartz rods coated with silica gel (Iatron Laboratories Inc.). The spotted rods were developed with a mixture of chloroform:methanol:25% ammonium (65:25:4; v/v/v) (Creer et al., 1985). The developed rods were analyzed by FID detection.

Analysis of Fatty acid of Lipids. Fatty acid methyl esters derived from the phospholipids were analyzed on a gas chromatography (HP-5890 GLC Hewlet-Packard Co., CA) equipped with a capillary column DB-WAX (0.25 mm i.d. × 30 m, J&W Scientific, Folsom, CA). The column temperature was maintained at 140 °C, was increased at a rate of 2.0 °C/min to 240 °C, and then kept for 10 min. Carrier gas flow rate was 1.0 mL/min. Helium gas was used as the carrier gas. Injector and detector temperatures were maintained at 250 and 300 °C, respectively (Schlenk and Gellerman, 1960). Identification of fatty acid methyl esters was based on comparisons of retention times of unknown peaks to authentic fatty acid methyl esters (Wako Pure Chemicals Co., Ltd., Osaka, Japan). Fatty acid composition was expressed as weight percent of total fatty acid methyl esters.

Measurement of Antioxidative Activity. Antioxidative property of EYL in rich DHA oil was performed by an active oxygen method (AOM, Kuramochi Kagaku Co., Ltd., Tokyo, Japan). EYL-30 (0.1%, 0.5%, 1.0%, and 5.0%) was added into 20 mL of rich DHA oil, respectively. EYL-N, EYL-30, EYL-65, EYL-95, EY-PC, EY-PE, HFA-PC, and HFA-PE (0.1% each) were added into it, respectively. Air flow in the AOM was at the rate of 1.23 mL/min incubated at 98 °C. Oxidative stability of oil was evaluated by peroxide value (PV) (AOCS, 1960). Antioxidative examination on a squalene was performed by oven test (Kashima et al., 1991). EYL-30 (0.5%, 1.0% and 2.0%) was added into 3 g of squalene, respectively. The squalene mixture was incubated at 60 °C, and the oxidative stability was evaluated by PV.

RESULTS AND DISCUSSION

Antioxidative Activity of EYL in Rich DHA Oil. EYL-30 present in rich DHA oil containing 0.3% tocopherols showed stronger antioxidative activity as compared with that of the control. The effect of antioxidant was dose-dependent (Figure 1). The PV of rich DHA oil was below 41 mequiv/kg at 5 h at the presence of 0.5% EYL-30.

To investigate the relationship between antioxidative effects and phospholipid contents, the antioxidative effects of 0.1% additional EYL-N, EYL-30, EYL-65, and EYL-95 in rich DHA oil were examined. EYL-N had no antioxidative activity, whereas EYL-95 showed the best effect (Figure 2). The order of effectiveness of various EYL fractions in inhibiting oxidation of rich DHA oil was EYL-95 > EYL-65 > EYL-30 ≫ EYL-N. The PVs of EYL-95, EYL-65, and EYL-30 were 8, 13, and 42 mequiv/kg at 3 h, respectively. The PV of EYL-N (127 mequiv/kg) was almost same as that of the control (132 mequiv/kg) at 3 h after the start of the test. The contents of phospholipids of EYL-N, EYL-30, EYL-65, and EYL-95 were 0%, 31.3%, 68.0%, and 96.8%, respectively (Table 1). The phospholipids of EYL-30 consisted of 25.5% PC and 4.7% PE, those of EYL-65 were 48.7% PC and 10.2% PE, and those of EYL-95 were 80.8% PC and 11.7% PE, respectively. Therefore the antioxidative activity of EYL is associated with the proportion of phospholipids present in egg yolk lipids.

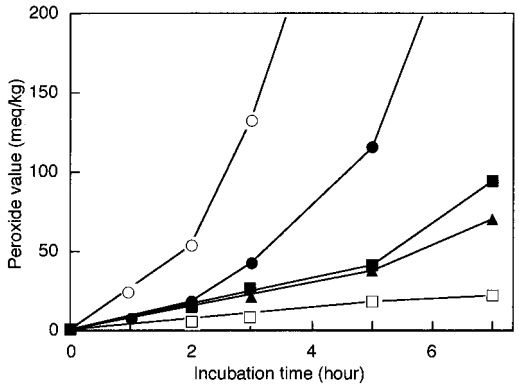

Figure 1. Effect of EYL-30 on autoxidation of rich DHA oil containing 0.3% tocopherols at 98 °C: control (○), 0.1% EYL-30 (●), 0.5% EYL-30 (■), 1.0% EYL-30 (▲), and 5.0% EYL-30 (□).

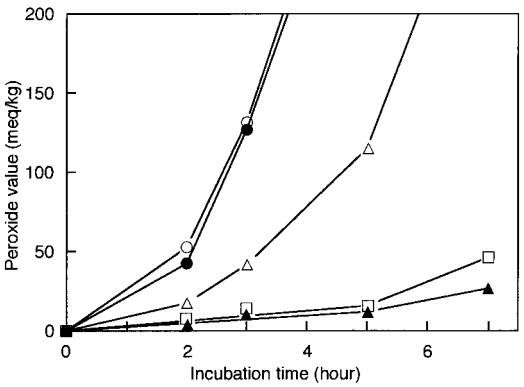

Figure 2. Effect of addition of 0.1% each EYL on autoxidation of rich DHA oil containing 0.3% tocopherols at 98 °C: control (○), EYL-N (●), EYL-30 (△), EYL-65 (□), and EYL-95 (▲).

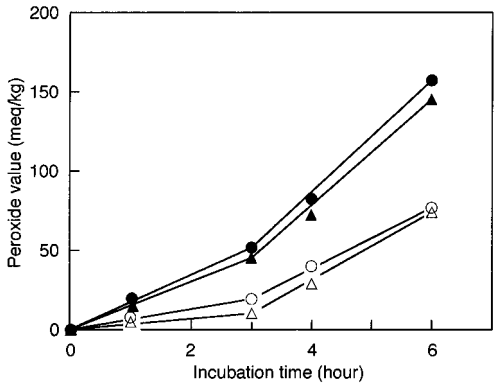

Figure 3. Effect of addition of 0.1% of purified egg yolk phospholipids on autoxidation of rich DHA oil containing 0.3% tocopherols at 98 °C: EY-PC, egg yolk phosphatidylcholine (○); HFA-PC, hydrogenated fatty acid chain-containing phosphatidylcholine (●); EY-PE, egg yolk phosphatidylethanolamine (△); HFA-PE, hydrogenated fatty acid chain-containing phosphatidylethanolamine (▲).

Antioxidative Activity of EY-PC and EY-PE in Rich DHA Oil. Additional 0.1% EY-PC and EY-PE in rich DHA oil showed stronger antioxidative activities, respectively, compared with HFA-PC and HFA-PE (Figure 3). The antioxidative activities of both phospholipids in rich DHA oil was decreased by the hydro-

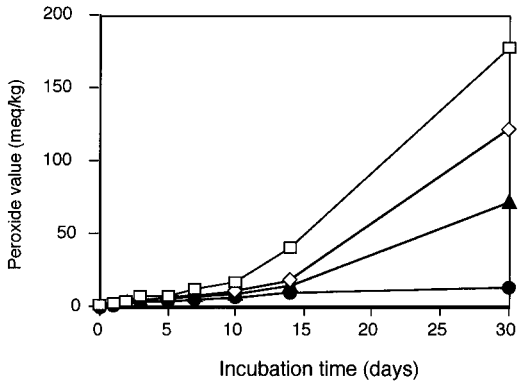

Figure 4. Effects of EYL-30 on autoxidation of squalene at 60 °C: control (□), 0.5% EYL-30 (◆), 1.0% EYL-30 (▲), and 2.0% EYL-30 (●).

Table 2. Fatty Acid Composition of Phospholipids Tested as Antioxidants[a]

	phospholipids[b]			
fatty acids	EY-PC	HFA-PC	EY-PE	HFA-PE
C16-0	29.69	25.6	12.64	16.41
C16-1	0.11	0	0.21	0
C17-0	0.3	0.35	0.36	0
C17-1	0	0	1.54	0
C18-0	18.12	65.71	35.53	47.98
C18-1	40.08	0	21.73	4.09
C18-2 (n-6)	8.16	0	8.6	0
C18-3 (n-6)	0.08	0	0.11	0
C18-3 (n-3)	0.05	0	0.09	0
C20-0	0	4.05	0	13.44
C20-4 (n-6)	1.1	0	9.42	0
C22-0	0	2.63	0	10.81
C22-4 (n-6)	0.3	0	1.27	0
C22-6 (n-3)	0.13	0	4.77	0
others	1.88	1.66	3.73	7.27
saturates	48.11	98.34	48.53	88.64
unsaturates[c]	50.01	0	47.74	4.09

[a] Fatty acid concentration expressed as wt % of individual fatty acid methyl esters in the total fatty acid methyl esters. [b] EYPC, egg yolk phosphatidylcholine; HFA-PC, hydrogenated fatty acid chain-containing phosphatidylcholine; EY-PE, egg yolk phosphatidylethanolamine; HFA-PE, hydrogenated fatty acid chain-containing phosphatidylethanolamine. [c] The unsaturation ratio of fatty acid was calculated on the basis of the analytical data of the gas chromatography:

$$\text{unsaturation ratio (\%)} = \frac{\text{unsaturated fatty acids}}{\text{total fatty acids}} \times 100$$

genation of the double bonds within the fatty acid chains of the phospholipids (Table 2).

The antioxidative (Kashima et al., 1991; King et al., 1992) and synergistic (Husain et al., 1986) activities of phospholipids have been reported to relate to the structural diversity of polar-heads and fatty acid compositions within phospholipids. Our results suggest that the degree of unsaturation of fatty acyl chain-containing phospholipids is closely associated with their antioxidant capability.

Antioxidative Activity of EYL-30 in Squalene. EYL-30 showed stronger antioxidative activity in squalene as compared to control. The antioxidant effect was dependent on the concentration of EYL-30 (Figure 4). After 30 days, the PV of squalene at the presence of additional 0.5%, 1%, and 2% EYL-30 were 123, 71, and 13 mequiv/kg, respectively, compared with 178 mequiv/kg from the control. The antioxidative activities of *dl*-α-tocopherol and vitamin D_3 have been reported against squalene (Imaeda et al., 1983). To our knowledge no work has been reported on yolk phospholipids as an antioxidant against squalene. Therefore, our results suggest that EYL might be useful as a natural antioxidant against squalene.

CONCLUSION

We have demonstrated the antioxidative activity of EYL in rich DHA oil and squalene. The degree of unsaturation of fatty acyl chain-containing phospholipids is closely associated with the antioxidative capability. The use of EYL-30 as an antioxidant safely could increase the oxidative stability of squalene which is used as functional food. Our results suggest that EYL could be a suitable natural antioxidant in preventing oxidation of polyunsaturated oils, squalene, and related food ingredients, which are susceptible to oxidation.

ABBREVIATIONS USED

EYL, egg yolk lipids; DHA, docosahexanoic acid; EY-PC, egg yolk phosphatidylcholine; EY-PE, egg yolk phosphatidylethanolamine; HFA-PC, hydrogenated fatty acid chain-containing phosphatidylcholine; HFA-PE, hydrogenated fatty acid chain-containing phosphatidylethanolamine; PV, peroxide value.

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AOCS. Peroxide value. *AOCS Official Method* **1960**, 8−53.

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