Effect of Phosphatidic Acid and Phosphatidylserine on Lipid Oxidation in Beef Homogenate During Storage and in Emulsified Sardine Oil

C.D. DACARANHE, J. TERAO

ABSTRACT: A study on the effect of exogenously added 2 anionic phospholipids, phosphatidylserine (PS), and phosphatidic acid (PA), against lipid oxidation in food systems was carried out using cooked beef homogenate and emulsified sardine oil. Measurements of thiobarbituric reactive-substances (TBARS) showed a pronounced inhibitory effect of egg yolk PA and dipalmitoyl PA on the oxidation of beef homogenate during storage at 4 EC for 6 d. The addition of dipalmitoyl PA to emulsions of fish oil effectively suppressed the hemoprotein-induced lipid oxidation. Meanwhile, the addition of dipalmitoyl PA and bovine brain PS to emulsions of fish oil suppressed the nonheme iron ion-induced lipid oxidation. Therefore, the unique protective effect of PA against the oxidation of beef homogenate could be partly explained by the suppression of the catalytic activity of nonheme iron and hemoprotein.

Abbreviations Used: BBPS, bovine brain phosphatidylserine; DPPS, dipalmitoyl phosphatidylserine; DPPA, dipalmitoyl phosphatidic acid; EYPA, egg yolk phosphatidic acid; EYPC, egg yolk phosphatidylcholine; PE, phosphatidylethanolamine; TBARS, thiobarbituric acid-reactive substances; Hepes, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; TLC, thin-layer chromatography.

Keywords: beef homogenate, phospholipids, lipid oxidation, hemoprotein, fish oil emulsions

Introduction

IT IS KNOWN THAT LIPID OXIDATION IS ONE OF THE CAUSES OF deterioration in lipid- containing foods during prolonged storage (Rhee and others 1987). Lipid oxidation products lower food quality, and their toxicity has been implicated in some pathological aspects such as mutagenesis and carcinogenesis (Holman and Elmer 1947; Labuza 1971; Porter 1980). In many foods, ionic iron exists in the form of nonheme or heme. For example, muscle tissue contains considerable hemoprotein, of which myoglobin is the most abundant (Love 1983). Food lipids such as those in milk and other emulsions usually exist in the form of small fat globules or droplets dispersed in an aqueous matrix that may contain a variety of water-soluble components including nonheme free iron ion (McClements 1998). This free ionic iron acts as one of the important initiators of lipid oxidation in foods because of its high reactivity and relatively high concentrations (Mei and others 1998). Free iron ion appears to catalyze lipid oxidation due to its capacity to generate reactive oxygen species and promoting breakdown of lipid hydroperoxides, which leads to an initiation of free-radical chain reactions. Heat-induced change in meat pigments, such as myoglobin and hemoglobin, which increases the exposure of the heme to the surrounding environment, should enhance lipid oxidation in meat, similarly to the action of free iron (Kristensen and Andersen 1997). The nature of iron complexes responsible for the promotion of lipid oxidation in cooked meat is still unclear, despite extensive studies (Liu and Watts 1970; Love and Pearson 1984; Maiorino and others 1994; Ahn and Kim 1998). Little is known about the antioxidants suitable for the protection of meat against the lipid oxidation during storage.

Phospholipids are widespread in foodstuffs and their antioxidant activity has been reported in different lipid oxidation model systems. Some reports have suggested either the individual antioxidant effect of phospholipids with nonionic polar head groups (King and others 1992) or their synergistic action with tocopherol (Hudson and Mahgoub 1980; Kashima and others 1991; Ohshima and others 1993) in bulk oil systems. The antioxidant activity of phospholipids with an anionic polar head group is supposed to be derived from the chelation or binding of positively charged prooxidant metal ions by their polar head group (Jacobson and Papahadjopoulos 1975; Viani and others 1990). Yoshida and others (1991) reported on the strong inhibitory effect of phosphatidylserine (PS) against the nonheme iron ion-induced lipid peroxidation of phosphatidylcholine (PC) liposomes. Meanwhile, because of their high unsaturation, endogenous phospholipids are supposed to be involved in the process of oxidation of meat (Igene and Pearson 1979; Roozen 1987; Pikul and Kummerow 1990). Consequently, it is of great interest to examine the protective role of exogenously added anionic phospholipids against lipid oxidation in foods during storage.

This study was conducted to examine or to determine the effect of 2 added phospholipids containing anionic polar head groups, phosphatidic acid (PA) and PS, against oxidation of cooked beef homogenate. In order to learn about the mechanism of their antioxidant action, their effect on nonheme and heme iron ion-induced lipid oxidation was also evaluated using sardine oil emulsions.

Experimental Procedure

Chemicals

Beef was obtained from a local market. Sardine oil (antioxidant-free oil), was kindly supplied by Tsukishima Food Co. (Tokyo, Japan). The fish oil was purified according to the method described by Hoshino and others (1997), in order to eliminate preformed hydroperoxides. Pure phospholipids (with more than 98 % purity), bovine brain PS (BBPS), dipalmitoyl PS (DPPS), dipalmitoyl PA (DPPA), egg yolk PA (EYPA), egg yolk PC (EYPC), bovine methemoglobin, and vitamin E (*dl*-α-tocopherol) were purchased from Sigma Chemical Co (St. Louis, Mo., U.S.A.). Triton X-100, 2-thiobarbituric acid (TBA), iron (III) nitrate and metmyoglobin from horse muscle were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other chemicals used were of reagent grade.

Separation and quantification of lipids from beef

Total lipids were extracted and quantified according to the method of Bligh and Dyer (1959). The phospholipids and neutral lipids in the total lipids were separated by column chromatography as described by Choudhury and others (1960). The fractions of phospholipids and neutral lipids were identified by thin-layer chromatography (TLC) and the phospholipids were quantified by phosphorus assay (Bartlett 1959). Each phospholipid was identified by the comparison of its Rf value to those of respective standard phospholipids on TLC plates (20 × 20 cm precoated silica gel 60 F254; Merck, Darmstadt, Germany). The separation of phospholipids into their classes was accomplished by a solvent of CHCl3/MeOH/H2O (65/35/4, v/v/v). Spots of phospholipids were scraped and extracted with the solvent CHCl3/MeOH (1/2, v/v) followed by centrifugation at 3,000 rpm for 5 min. Neutral lipids were recovered using the same procedure with extracting solvent CHCl3/MeOH (2/1, v/v).

Fatty acid composition

For the analysis of fatty acid composition of each lipid class, phospholipids and neutral lipids were esterified using 5% HCl-MeOH. The analysis of fatty acid methyl esters was performed using a Shimadzu gas chromatograph (GC-18A; Shimadzu, Kyoto, Japan). The column used was SP2330 (30 m × 0.25 mm × 0.2 μm; Supelco, Bellefonte, Pa., U.S.A.). The column temperature was set at 190 °C and the detector and injector at 220 °C. The carrier gas was helium and the flow rate was maintained at 0.7 mL/min. Each fatty acid was identified by the comparison of its retention time to those of respective standard fatty acids.

Preparation and storage of meat homogenate

Ground beef (about 20% fat) was homogenized (approximately 70% w/v) in 50 mM Hepes buffer using a Polytron homogenizer (PT-2000; Kinematica AG, Littau, Switzerland) for 10 min. The homogenate (10 g) was mixed with phospholipids or vitamin E (1000 ppm) dispersed in 3 mL of 50 mM of Hepes buffer (in the control case, 3 mL of 50 mM of Hepes buffer was added). Then the mixture was cooked in a boiling water bath for 15 min with an occasional stirring, followed by cooling to room temperature in tap water, then the mixture was stored in a refrigerator at 4 °C.

Preparation of a mixture of sardine oil and phospholipids in emulsion

A hexane solution of sardine oil (23 Fmol) and a chloroform/methanol (95:5, v/v) solution of phospholipids (20 Fmol) were mixed by vortex and the solvent was removed completely by a stream of nitrogen and a vacuum pump. The residue was dispersed in 2.0 mL Tris-HCl buffer (10 mM, pH 7.4) containing Triton X-100 at 10 mM, then mixed by vortex for 30 s, followed by ultrasonic irradiation with an Astrason Sonicator W-380 (20 kHz; Heat System Ultrasonics, Inc., Plainview, N.Y, U.S.A.) for 5 min in a water bath.

Oxidation of fish oil emulsion containing phospholipids

Emulsions of fish oil containing phospholipids were stored in the refrigerator at 4 °C without the addition of ferric nitrate/ascorbic acid or hemoprotein. In a separate experiment, to the emulsions (0.8 mL) were added 0.2 mL of ferric nitrate/ascorbic acid or hemoprotein dissolved in Tris-HCl buffer (10 mM, pH 7.4). The final concentration of each component in the reaction mixture was as follows: fish oil, 9.2 mM; phospholipids, 10 mM; ferric nitrate/ascorbic acid, 100 FM and 1.0 mM, respectively; metmyoglobin and hemoglobin, 500 FM. The reaction was carried out at 37 °C with continuous shaking.

Measurement of TBARS

The thiobarbituric acid assay was carried out following the method of Buege and Aust (1978) for beef homogenate samples and the method of Uchiyama and Mihara (1978) in the case of sardine oil emulsion. The amount of TBARS was expressed as FM malonaldehyde using an equation obtained from a standard curve of tetraethoxypropane at appropriate concentrations.

Statistical analysis

Assays were carried out using triplicate samples. Statistical analysis was performed using analysis of variance. Mean separations were achieved using the Student's t-test.

Results

Distribution of individual phospholipids and their fatty acid composition in tested beef

The TLC results indicated PC and PE were the main phospholipids in the meat used in this study. Their amounts in a fraction of total lipids were as follow: PC ranged from 39.45 % to 42.03 %, PE from 47.80 % to 53.37 %. Relatively lower amounts of lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) were detected in the remaining portion. Neither PA nor PS was detected in the phospholipid fraction.

Fatty acid composition of neutral lipid and phospholipid fractions are presented in Table 1. For PC and PE, palmitic acid (C16:0) was the predominant fatty acid followed by stearic acid (C18:0). Total phospholipids were composed mainly of stearic acid and palmitic acid. Arachidonic acid (C20:4) was the main polyunsaturated fatty acid present in PC, PE, and total lipids. The percentages of polyunsaturated fatty acids (PUFAs) were: 12.1 % for PC, 26.6 % for PE and 26.5 % for total lipids. The neutral lipids were mainly composed of triacylglycerols and free fatty acids. The mixture of triacylglycerols and free fatty acids contained 5 % PUFAs.

Effect of PS and PA on oxidation of meat homogenate during storage

The effect of added phospholipids at 1000 ppm against oxidation of meat homogenate during the storage at 4 °C was

assessed by measuring the accumulation of TBARS during 6 d. DPPA and EYPA retarded the rate of accumulation of TBARS, indicating that these phospholipids exerted a strong inhibitory effect (Figure 1). The inhibitory effect of PA was even stronger than that of vitamin E. During the 6 d of storage, the effect of DPPA was comparable to that of EYPA. In all cases, the amounts of TBARS increased continuously throughout the 6 d of storage. In the control case, the initial amount TBARS (at start of storage) increased about 3.3-fold after d 3 and 5.7-fold after d 6 of storage. Meanwhile, as compared to the control case, the incorporation of DPPA and EYPA into the meat homogenate decreased the formation of TBARS by 51-53 % after d 3 of storage, and in 56-58 % after d 6 of storage, while the incorporation of vitamin E decreased the amounts of TBARS by about 13 % after d 3 and by about 27 % after d 6 of storage. The effectiveness of DPPA was found to increase by elevating the concentration from 250 ppm to 1000 ppm (Figure 2). During the 6 d of storage, no significant difference ($p > 0.05$) was observed between the control sample and samples with DPPA added to meat homogenate at 250 ppm. Throughout the storage, for the con-

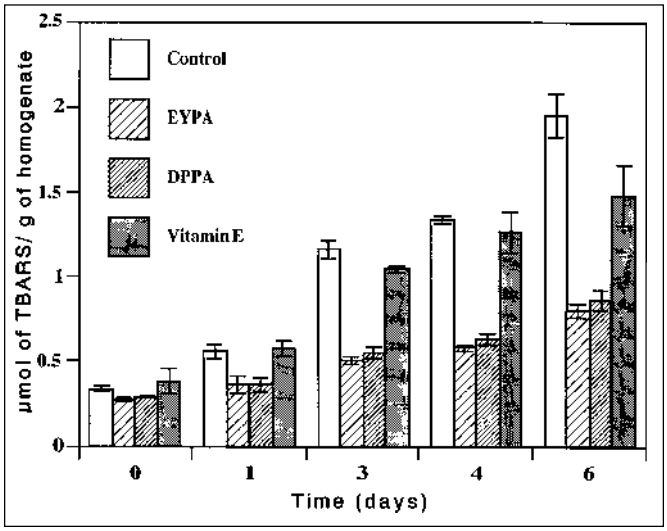

Figure 1—Effect of added phosphatidic acid (EYPA and DPPA) and vitamin E on oxidation of cooked beef homogenate. Ground beef (about 20 % fat) was homogenized (approximately 70 % w/v) in 50 mL of Hepes buffer, pH 7.4 and mixed with phospholipids and vitamin E (each at 1000 ppm) dispersed in 50 mM of Hepes buffer, pH 7.4. The samples were cooked in a boiling water bath for 15 min, with a regular stirring. The cooked samples were stored at 4 EC. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of the control after the same storage period (p< 0.05).

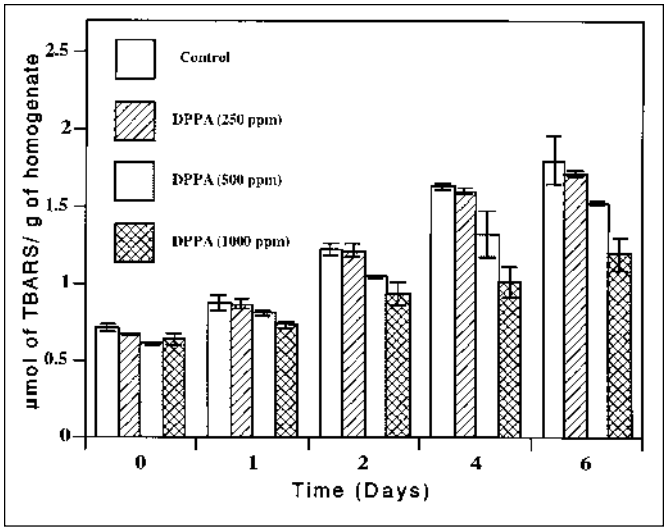

Figure 2—Effect of DPPA on oxidation of cooked beef homogenate at different concentrations (250-1000 ppm). Preparation and storage of the samples were the same as described in Fig. 1. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of the control after the same storage period (p< 0.05).

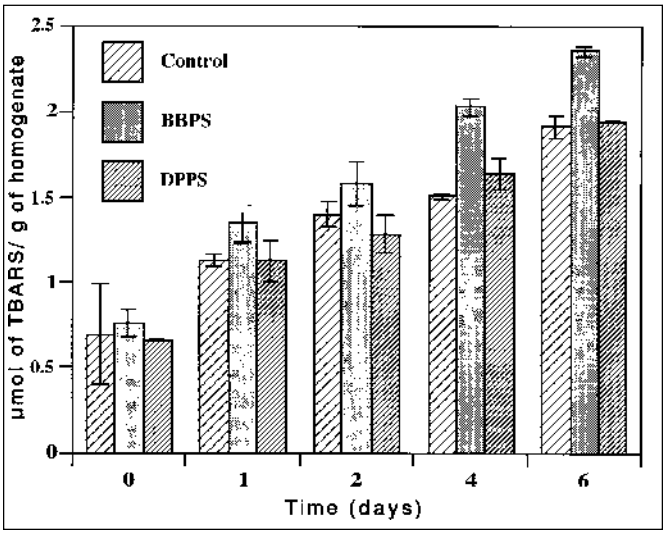

Figure 3—Effect of phosphatidylserine (BBPS and DPPS) on oxidation of cooked beef homogenate. Preparation and storage of the samples were the same as described in Fig. 1. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of the control after the same storage period (p< 0.05).

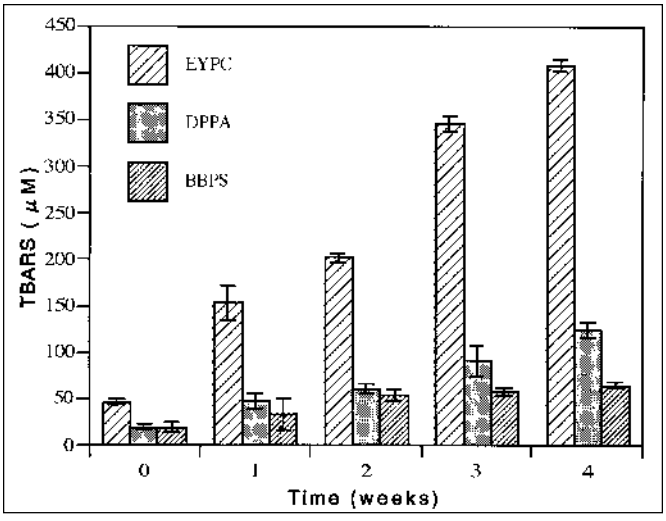

Figure 4—Effect of presence of DPPA, BBPS and EYPC on oxidation of fish oil in emulsion during 4 weeks of storage at 4 EC. The final concentrations of components in the reaction mixture were: fish oil, 9.2 mM; phospholipids, 10 mM. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of emulsions of fish oil containing EYPC at the same storage period (p< 0.05).

trol case, the initial amounts of TBARS increased about 2.2-fold after d 4 and about 2.4-fold after d 6. As compared to the amounts of TBARS formed in the control case, the addition of PA to meat homogenate at 500 ppm decreased the formation of TBARS by about 12 % after d 4 and by about 10 % after d 6, while the addition of PA at 1000 ppm decreased TBAR formation by about 48 % after d 4 and by about 31% after d 6 of storage. Thus, lipid oxidation in beef homogenate was suppressed by DPPA in a concentration-dependent manner. On the other hand, neither BBPS nor DPPS at 1000 ppm enhanced the oxidative stability of meat homogenate (Figure 3). Throughout the 6 d of storage, no significant difference between the control and the meat with added DPPS was verified. BBPS showed a slight prooxidant effect.

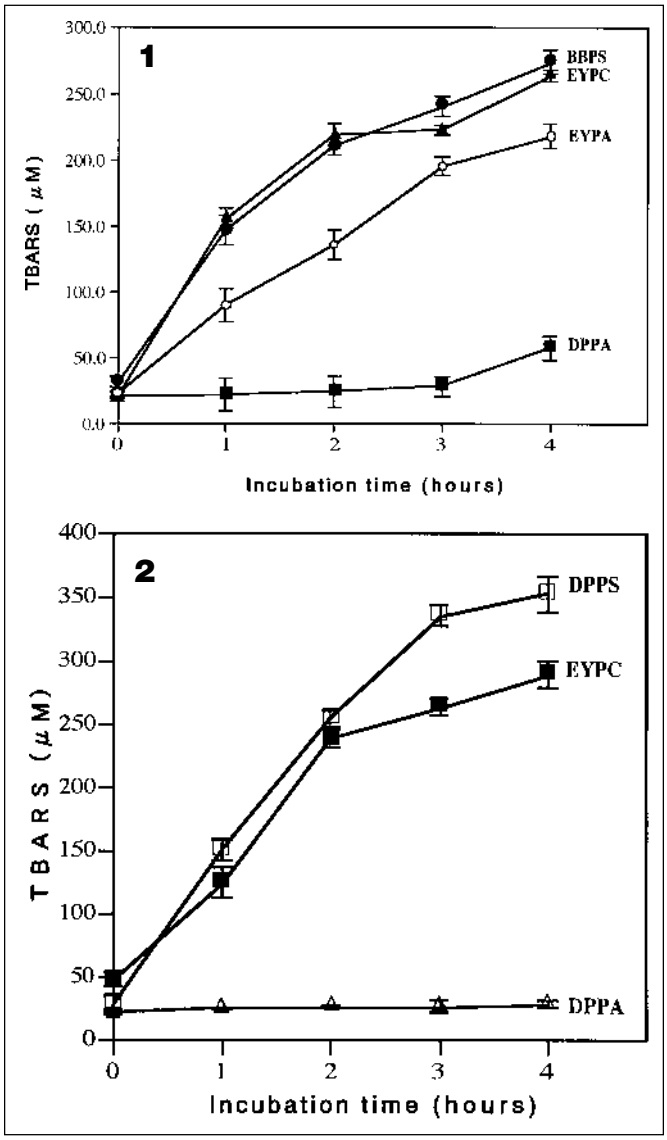

Figure 5—Effect of the presence of phospholipids against the hemoprotein-induced oxidation of fish oil in emulsion: (1) metmyoglobin, (2) methemoglobin. The final concentrations of components in the reaction mixtures were: fish oil, 9.2 mM; phospholipids, 10 mM; hemoprotein, 500 FM. The reaction was carried out at 37 EC with continuous shaking. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of emulsions of fish oil containing EYPC (p< 0.05).

Effect of the addition of phospholipids on lipid oxidation in sardine oil emulsions

During the storage of sardine oil emulsions together with EYPC for 4 wk, the amount of TBARS increased with time of storage (Figure 4). No significant difference was observed between data of emulsions of fish oil with added DPPA and BBPS during the first 2 wk of storage. However, as compared to the amounts of TBARS in emulsions of fish oil with added EYPC, after 4 wk of storage, the incorporation of BBPS and DPPA in emulsions of fish oil lowered the amount of formed TBARS in about 87 % and 72%, respectively.

For the metmyoglobin-induced lipid oxidation, during 4 h of incubation the fish oil emulsions with added DPPA had minimally elevated amounts of TBARS, followed by far by EYPA, which had a little inhibitory effect (Figure 5-1). No significant difference was observed for emulsions of fish oil with added EYPC and BBPS. Thus the addition of BBPS to fish oil emulsions did not affect significantly the oxidation of fish oil emulsions.

Regarding the methemoglobin-induced oxidation of emulsions of fish oil, the addition of DPPA provided better protection for the emulsions than the addition of EYPA and DPPS. DPPS was found to possess a little prooxidant effect (Figure 5-2). Compared to the emulsions with added EYPC, the addition of DPPS increased the formation of TBARS by about 7 % after 2 h of incubation and by about 22 % after 4 h incubation, while fish oil with added DPPA lowered the TBARS by about 90 % after 2 h incubation, and 91 % after 4 h.

Regarding nonheme iron ion induced-lipid peroxidation of fish oil emulsions, the amounts of formed TBARS was lowered by the presence of phospholipids in the order of EYPC < DPPA < BBPS (Figure 6). Compared to emulsions of fish oil with added EYPC, the addition of DPPA to fish oil

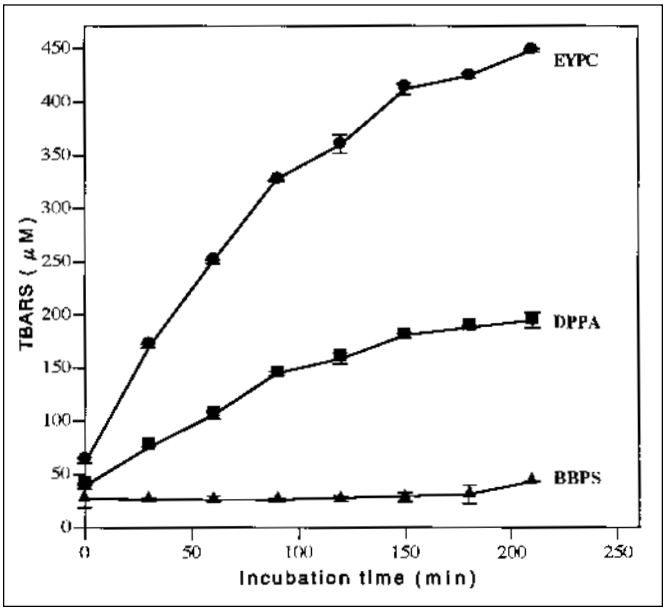

Figure 6—Effect of presence of EYPC, DPPA and BBPS on nonheme iron ion-induced lipid oxidation of fish oil emulsion. The final concentrations of the components in the reaction mixture were: fish oil, 9.2 mM; phospholipids, 10 mM; ferric nitrate/ascorbic acid, 0.1mM/ 1mM, respectively. The reaction mixture was incubated as described in Fig. 5. Results are means " SD of 3 independent measurements. * - Values are significantly different from those of emulsions of fish oil containing EYPC (p< 0.05).

Table 1—Fatty acid composition of phospholipids and neutral lipids in the beef homogenate

	PC (%)	PE (%)	Tpho (%)*	NL (%)
14:0	3.2 ± 0.1	4.7 ± 0.9	5.5 ± 0.1	2.8 ± 0.4
14:1	1.5 ± 0.7	2.9 ± 0.8	3.9 ± 0.1	1.6 ± 0.2
16:0	36.1 ± 0.3	28.9 ± 2.5	18.1 ± 3.9	23.5 ± 1.1
16:1	1.7 ± 0.3	1.3 ± 0.4	4.1 ± 0.9	6.1 ± 0.3
18:0	29.9 ± 1.9	21.4 ± 1.6	20.9 ± 4.2	13.6 ± 0.5
18:1	11.7 ± 3.1	12.6 ± 1.0	14.0 ± 2.6	45.7 ± 1.3
18:2	0.5 ± 0.5	3.8 ± 1.9	5.1 ± 1.0	1.6 ± 0.1
18:3	0.3 ± 0.1	1.5 ± 2.1	1.1 ± 0.9	0.2 ± 0.1
20:0	1.5 ± 1.1	0.3 ± 0.4	0.5 ± 0.4	0.7 ± 0.2
20:1	1.1 ± 0.5	(ND)	0.7 ± 0.1	0.3 ± 0.1
20:2	1.1 ± 0.6	1.3 ± 0.4	1.5 ± 0.6	0.5 ± 0.1
20:3	0.7 ± 0.2	2.2 ± 0.3	3.7 ± 0.2	0.2 ± 0.1
20:4	7.4 ± 0.4	15.7 ± 1.8	10.9 ± 1.1	3.1 ± 0.1
20:5	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.2 ± 0.1
22:0	0.7 ± 0.3	0.5 ± 0.2	2.8 ± 0.1	0.2 ± 0.1
22:1	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
unknown	0.4 ± 0.1	0.4 ± 0.1	2.8 ± 0.1	(ND)
22:6	0.9 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
24:0	0.7 ± 0.1	(ND)	1.9 ± 0.1	(ND)
24:1	0.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.0	(ND)
	PC (%)	**PE (%)**	**Tpho (%)**	**NL (%)**
Sat	71.6±3.6	55.7±4.1	49.7±2.2	40.6±4.9
Mono	16.4±4.9	17.7±2.3	23.9±5.1	53.7±0.9
PUFA	12.1±1.5	26.5±6.3	26.5±1.9	5.7±0.1

*Tpho: total phospholipids
Mono: monounsaturated fatty acid
Sat: saturated fatty acid
PUFA: polyunsaturated fatty acid
NL: neutral lipids
ND: not detected

emulsions lowered the TBARS by about 33 % after 2 h of incubation and by about 21.5% after 3.5 h incubation, while the addition of BBPS to fish oil emulsions reduced TBAR formation by 89 % after 2 h of incubation and 84 % after 3.5 h incubation.

Discussion

STUDY RESULTS INDICATE THAT PC AND PE ARE THE MAIN endogenous phospholipids in tested meat. The effect of PA and PS in this beef homogenate system obviously originated from the added sources, because neither PA nor PS was detected in the tested meat. The fatty acid composition in Table 1 showed that the amount of PUFAs in the phospholipids was higher than that in neutral lipids.

This study showed that added PA, but not added PS, possesses a protective effect in oxidation of cooked beef homogenate. Its effect is independent of its acyl chain, because both DPPA and EYPA increased the oxidative stability of meat homogenate at similar levels (Fig. 1). It is likely that the nature of the PA acyl chain does not interfere with its inhibitory effect and that its effectiveness is derived from the anionic polar head group. On the other hand, DPPS and BBPS, despite their anionic structure, were found to have little effect (Fig. 3). The structure of the polar head group of DPPA differs from the DPPS on serine base. This structural difference might be responsible for the observed different antioxidant activities of the 2 phospholipids.

The preparation of meat homogenate involves cooking; which causes denaturation of hemoproteins accompanied with release of heme iron (Han and others 1993). The relative importance of nonheme iron versus heme iron in the oxidation of cooked meat is not clear. Liu and Watts (1970) suggested that both heme and nonheme iron could function as prooxidants in cooked meat. Taking into account the cationic nature of nonheme iron ion, we expected the protective role of anionic phospholipids, PA and PS, against the catalysts of nonheme iron ions through chelation or binding of iron ions by anionic structures. However, little is known about the interaction between phospholipids and hemoproteins. It is still unclear whether anionic phospholipids can inhibit hemoprotein-induced lipid oxidation.

To better understand the effect of PA and PS on lipid oxidation in muscle foods, we evaluated the role of their addition on the oxidation of sardine oil emulsions. Emulsions of fish oil were chosen for this study, because the highly unsaturated nature of its PUFAs easily allows the assessment of lipid oxidation by TBARS measurements. Triton X-100 (Nacalai Tesque, Inc., Kyoto, Japan) is the nonionic surfactant that was used to increase the stability of emulsions. The differences and changes in oxidative rate of emulsions of fish oil-stabilized by Triton X-100 with those prepared without Trition X-100 were small (less than 5 %) (data not shown). It has been claimed (Fukuzawa and others 1995; Mancuso and others 1999) that the effect of nonionic surfactant on iron-induced lipid oxidation is less than the effect of anionic surfactants (Fukuzawa and others 1995; Mancuso and others 1999). In both metmyoglobin-induced and methemoglobin-induced lipid oxidation of the emulsions of sardine oil, DPPA was found to possess considerable inhibitory effect. Nevertheless, neither BBPS nor DPPS provided an inhibitory effect in hemoprotein-induced lipid peroxidation. As compared to EYPC, the incorporation of both DPPA and BBPS greatly protected the fish oil emulsions against the oxidation. Similar results were observed in the case of nonheme iron ion- induced lipid oxidation of fish oil emulsion, with BBPS being more efficient than DPPA. Interestingly, the unique protection by PA found in hemoprotein-induced lipid oxidation was also observed in the case of meat homogenate oxidation. The inhibition of nonheme iron ion-induced lipid oxidation of emulsions with phospholipids may be related to the ability of polar head groups to interact with iron ion as described above. Studies on emulsified salmon oil (Mei and others 1998; Mancuso and others 1999) and menhaden oil (Donnelly and others 1998) showed that iron-promoted lipid oxidation was affected by the electrostatic repulsion and attraction of iron to the surface/interface of lipid droplets. It is therefore likely that free iron ion is trapped by an acidic polar group of BBPS and DPPA, and bound iron does not promote lipid oxidation.

Our results demonstrated that PA is an effective inhibitor of the catalytic action of both nonheme iron?induced and hemoprotein-induced lipid oxidation. In hemoprotein-induced lipid oxidation, the antioxidant mechanism of PA may differ from that of the nonheme iron ion-induced lipid oxidation, because PS did not provide significant inhibitory effect. The effectiveness of PA against the oxidation of meat homogenate seems to be due to its effectiveness against both nonheme-induced and hemoprotein-induced lipid oxidation. PS was lack of inhibitory effect on hemoprotein-induced lipid oxidation. This fact may explain why PA, rather than PS, is an efficient phospholipid for the enhancement of oxidative stability in meat homogenate, since both nonheme and heme iron seem to be responsible for meat homogenate oxidation.

Conclusion

PHOSPHATIDIC ACID POSSESSES A PROTECTIVE ROLE IN THE oxidation of cooked meat homogenate during a short-term storage. This effect could be partly explained by the suppres-

sion of the catalytic activity of nonheme iron and hemoprotein.

References

Ahn DU, Kim SM. 1998. Prooxidant effects of ferrous iron, hemoglobin, and ferritin in oil emulsions and cooked-meat homogenates are different from those in raw-meat homogenates. Poult Sci 77 (2):348-355.

Bartlett GR. 1959. Phosphorus assay in column chromatography. J Biol Chem 234 (2): 466-468.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. In: Fleischer S, Packer L editors. Methods in enzymology, vol. 52. New York: Academic Press. p. 302-310.

Choudhury RB, Roy B, Arnold LK. 1960. The determination of the neutral oil content of crude vegetable oil. J Am Oil Chem 37 (1): 87-92.

Donnelly JL, Decker EA, McClements DJ. 1998. Ability of emulsifiers to influence iron-catalyzed oxidation of emulsified menhaden oil. J Food Sci 63 (5): 997-1000.

Fukuzawa K, Soumi K, Iemura M, Goto S, Tokumura A. 1995. Dynamics of xanthine oxidase- and Fe3+- ADP-dependent lipid oxidation in negatively charged phospholipids. Arch Biochim Biophys 316(1):83-91.

Han D, McMillin KW, Godber JS, Bidner TD, Younathan MT, Marshall DL, Hart L. 1993. Iron distribution in heated beef and chicken muscles. J Food Sci_58 (4): 697-700.

Holman RT, Elmer OC. 1947. The rates of oxidation of unsaturated fatty acids and esters. J Am Oil Chem Soc 24 (1): 127-129.

Hoshino C, Tagawa Y, Wada S, Oh J-H, Park D-K, Nagao A, Terao J. 1997. Antioxidant activity of quercetin against metmyoglobin-induced oxidation of fish oil bile salt emulsion. Biosci Biotech Biochem 61 (10): 1634-1640 (1997)

Hudson BJF, Mahgoub SEO. 1980. Synergism between phospholipids and naturally occurring antioxidants in leaf lipids. J Sci Food Agric 32 (2):208-211.

Igene JO, Pearson AM. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. J Agric Food Chem 27 (2): 838-843.

Jacobson K, Papahadjopoulos D. 1975. Phase transition and phase separations in phospholipid membranes induced changes in temperature, pH and concentration of bivalent cations. Biochemistry 14 (1): 152-161.

Kashima M, Cha GS, Isoda Y, Hirano J, Miyazawa T. 1991. The antioxidant effects of phospholipids on perilla oil. J Am Oil Chem Soc 68 (2): 119-122.

King MF, Boyd LC, Sheldon BW. 1992. Antioxidant properties of individual phospholipids in a salmon oil model system. J Am Oil Chem Soc 69 (3): 545-551.

Kristensen L, Andersen H. 1997. Effect of heat denaturation on the pro-oxidative activity of metmyoglobin in linoleic acid emulsions. J Agric Food Chem 45 (1): 7-13.

Labuza, T.P. (1971). Kinetics of lipid oxidation in foods. CRC Critical Rev Food Technol 2:355-405

Liu H, Watts BM. 1970. Catalysts of lipid peroxidation in meat: catalysts of oxidative rancidity in meat. J Food Sci 35: 596-598.

Love JD. 1983. The role of heme iron in the oxidation of lipids in red meats. Food Technol 37 (7): 117-120, 129.

Love JD, Pearson AM. 1974. Metmyoglobin and nonheme iron as proooxidants in cooked meat. J Agric Chem 22 (6): 1032-1034.

Maiorino M, Ursini F, Cadenas E. 1994. Reactivity of metmyoglobin towards phospholipids hydroperoxides. Free Radic Biol Med 16 (5): 661-667.

Mancuso JR, McClements DJ, Decker EA. 1999. The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. J Agric Food Chem 47 (10): 4112-4116.

McClements DJ. 1998. Context and background. In: Food Emulsions: Principles, practice and techniques. CRC Press. FL: Boca Raton. p. 1-16.

Mei L, Decker EA, McClements DJ. 1998. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. J Agric Food Chem 46 (12): 5072-5077.

Ohshima T, Fujita Y, Koizumi CJ. 1993. Oxidative stability of sardine and mackerel lipids with reference to synergism between phospholipids and á- tocopherol. J Am Oil Chem Soc 70 (3): 269-276.

Pikul J, Kummerow FA. 1990. Relative role of individual phospholipids on thiobarbituric acid reactive substances formation in chicken meat, skin and swine aorta. J Food Sci 55 (5): 1243-1248.

Porter WL. 1980. In Simic MG, Karel M editors. Recent trends in food applications for antioxidants. New York: Plenum Press. p. 1-23.

Rhee KS, Ziprin YA, Ordonez G. 1987. Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-H202, nonheme iron and enzyme systems. J Agric Food Chem 35 (6): 1013-1017.

Roozen JP. 1987. Effects of type I, II and III antioxidants on phospholipids oxidation in meat model for warmed-over flavor. Food Chem 24 (1): 167-185.

Spanier AM, St. Angelo AJ, Shaffer GP. 1992. Response of beef flavor to oxygen depletion and an antioxidant chelator mixture. J Agric Food Chem 40 (9): 1656-1662.

Uchiyama M, Mihara M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric test. Anal Biochem 86: 271-278.

Viani P, Cervato G, Florilli A, Rigamonti E, Cestaro B. 1990. Studies on peroxidation of model membranes and synaptosomes: role of phosphatidic acid. Chem Phys Lipids 52 (1): 49-55.

Yoshida K, Terao J, Suzuki T, Takama K. 1991. Inhibitory effect of phosphatidylserine on iron-dependent lipid peroxidation. Biochem Biophys Res Commun 179 (2): 1077-1081.

MS 20000919

Authors are affiliated with the Department of Nutrition, School of Medicine, The University of Tokushima, Japan. Please address inquiries to author Terao, e-mail: terao@nutr.med.tokushima-u.ac.jp