Effect of Browning Reaction Products of Phospholipids on Autoxidation of Methyl Linoleate

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Methyl linoleate was allowed to autoxidize in bulk phase at 50 C in the presence of either synthetic phospholipids consisting of saturated fatty acids or egg yolk phospholipids to estimate the effect of base group and fatty acid moiety of phospholipids on oil autoxidation. Dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylethanolamine (DPPE) exhibited poor antioxidant activity at 50 C and showed no synergistic effect with α-tocopherol. The addition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of egg yolk accelerated the oxidation of methyl linoleate. However, egg yolk PC and PE collected at various stages of heating at 180 C inhibited hydroperoxide formation at the initial stage of oxidation. This effect could be attributed to the browning products formed during heating reaction. Thus, browning color products formed from unsaturated phospholipids at high temperatures may influence oil stability, although the base group of phospholipids did not exert any significant effect.

Polyunsaturated fatty acids in foods are readily susceptible to autoxidation, and in turn produce the odorous compounds which cause rancid flavor. To overcome this problem, extensive research has been carried out to search for substances which can give foods better stability against autoxidation.

Phospholipids are widespread in foods (1), and their effect on oil stability has been studied comprehensively using various oxidation conditions. Tsai and Smith (2) studied pro- and antioxidant effects of bases and phosphoryl bases of phospholipid classes. Bhatia et al. (3) also evaluated the antioxidant activity of phospholipids from various plant seeds against ghee (clarified butter fat) and observed that phosphatidylethanolamine (PE) was the most effective antioxidant independent of the fatty acid portion of their molecules. However, Olcott and Vander Veen (4) noted that synthetic phosphatidylcholine (PC) and PE are inactive as antioxidant for menhaden oil, but show appreciable synergistic activity when used with ethoxyquin. Synergism of phospholipids with tocopherols has been evaluated in methyl linoleate (5) and soybean oil (6,7). Recently, Dziedzic and Hudson (8) demonstrated that dipalmitoyl phosphatidylethanolamine (DPPE) is a potent synergist for a wide range of primary antioxidants in edible oil at elevated temperature. It also has been suggested that egg yolk PC and PE act as synergists with α-tocopherol in the autoxidation of lard (9).

On the other hand, phospholipids containing unsaturated fatty acids in their molecule, like egg yolk phospholipids, are known to undergo browning color reaction (10,11). In our previous work, we suggested that conjugated carbonyls are the oxidized products chiefly responsible for the coloring reaction (12) similar to the Maillard reaction between sugar carbonyl and amino acids. The antioxidant activity of Maillard reaction products has attracted much attention (13-20). However, there is little knowledge of the effect of browning color products of phospholipids. The behavior of phospholipids in oil autoxidation seems to be of much importance, because they usually are present in crude oil and are removed during refining.

The purpose of this work is to clarify the effect of the fatty acid moiety and the base group of phospholipids on oil stability. The effectiveness of phospholipids containing polyunsaturated fatty acids and of their browning color products on autoxidation of edible oil are discussed by comparing the effect of saturated phospholipids and egg yolk phospholipids with and without heating.

MATERIALS AND METHODS

Methyl linoleate (99%), DPPC (99%) and DPPE (99%) were obtained from Sigma Chemical Co., St. Louis, Missouri. Prior to each experiment, methyl linoleate was purified by column chromatography with Florisil (100-200 mesh) to remove any peroxides (21). dl-α-Tocopherol was purchased from Nakarai Chemical Co., Kyoto, Japan, and was used without further purification. PC and PE of egg yolk were prepared as follows. Total lipids were extracted by the method of Bligh and Dyer (22), and the phospholipid fraction was obtained by the acetone precipitation of total lipids according to the procedure of Kates (23). Pure PC and PE were isolated from the phospholipid fraction by column chromatography as described in our previous paper (24). The fatty acid compositions of egg yolk PC and PE were determined by GLC (24) as follows. PC: 16:0, 36.6; 16:1, 2.1; 18:0, 9.6; 18:1, 33.8; 18:2, 14.1; 20:4, 1.8; and 22:6, 2.0%. PE: 16:0, 25.5; 16:1, 1.6; 18:0, 25.9; 18:1, 26.3; 18:2, 7.4; 20:4, 6.3, and 22:6, 7.0%. The solvents used for chromatography were of HPLC grade.

Heating of egg yolk phospholipids and preparation of their browning color products. Egg yolk PC or PE (100 mg each) were heated at 180 C, and aliquots were collected at hourly intervals. The color intensity was determined by the measurement of absorbance at 430 nm on a Shimadzu Double Beam UV-200 spectrophotometer.

Autoxidation procedure. Methyl linoleate (100 mg) either with or without additive was placed in a glass vial (10 mm i.d.) and allowed to oxidize in the dark at 50 C.To ensure that methyl linoleate and additives would be mixed completely, they were dissolved in a small volume of hexane and evaporated under the stream of nitrogen gas. Aliquots of samples were taken out at regular intervals for HPLC analysis.

HPLC was carried out as described in our previous

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paper (12). Hexane solution of the sample was injected directly into the HPLC apparatus. The elution pattern observed for the isomeric hydroperoxides produced from methyl linoleate was almost the same as that shown by Chan et al. (25). The weight percentage of total hydroperoxides was calculated from the sum of their peak areas by using a standard curve of purified methyl linoleate hydroperoxides (26).

RESULTS

Effects of saturated phospholipids on autoxidation of methyl linoleate. The effects of DPPC and DPPE on methyl linoleate autoxidation are shown in Figure 1. The autoxidation curve of methyl linoleate with no additive showed two phases in hydroperoxide formation, i.e. induction and propagation. The formation of hydroperoxides was very slow up to 24 hr and then increased significantly (Fig. 1A). The addition of DPPC and DPPE could not suppress the steep increase of hydroperoxide level after 24 hr incubation, and there was no significant difference between the level of hydroperoxides produced by methyl linoleate with no additive and with either phospholipids. However, the level of hydroperoxides was observed lower in the initial stage (Fig. 1B) when DPPE (0.1%) was added to methyl linoleate; this effect could not be continued after this stage. Thus, DPPE was found to exert only little antioxidant effect in the induction period. DPPC also was not a very effective inhibitor of the formation of hydroperoxides. The level of hydroperoxides produced from methyl linoleate containing DPPC was lower than those from that containing 0.1% α-tocopherol during the first 24 hr (Fig. 1B) and then increased significantly. A higher amount of hydroperoxides accumulated in methyl linoleate containing 0.1% α-tocopherol than with no additive as shown in Fig. 1B.

Synergistic effect of saturated phospholipids with α-tocopherol. The effects of coexistence of DPPC or DPPE (1.0%) with α-tocopherol (0.1 and 1.0%) were estimated by determining the hydroperoxide formation from methyl linoleate at 50 C. The results shown in Table 1 indicate that neither DPPC nor DPPE exhibited any synergistic effects with α-tocopherol. On the contrary, both phospholipids caused the increase in the hydroperoxide level at 50 C.

Relationship between effects of egg yolk phospholipids on autoxidation of methyl linoleate and their browning color reaction. Figure 2 shows the generation of browning color by heating of egg yolk PC and PE at 180 C. The brown color intensity of heated PC and PE increased with the lapse of heating time. PE gave higher color intensity than PC. Autoxidation of methyl linoleate in the presence of egg yolk PC and PE before and after heating are depicted in Table 2. Both PC and PE before heating increased hydroperoxide level significantly, and appeared to act as prooxidant. However, both phospholipids collected at various stages of heating decreased the level of hydroperoxides to less than half of the control at 24 hr. After 48 hr incubation time, every sample containing phospholipid increased the hydroperoxide formation and no significant difference was noted in any sample except with 1.0% PE.

DISCUSSION

We dealt with the oxidation of methyl linoleate in bulk phase at 50 C without the addition of any free radical initiator, and the level of hydroperoxides was determined directly by measuring hydroperoxides by HPLC. Although the oxidation rate might depend upon the initial level of hydroperoxides before incubation, the system consisting of methyl linoleate and additives was designed as a model for edible oils in storage and cooking.

Phospholipids are considered to affect oil stability

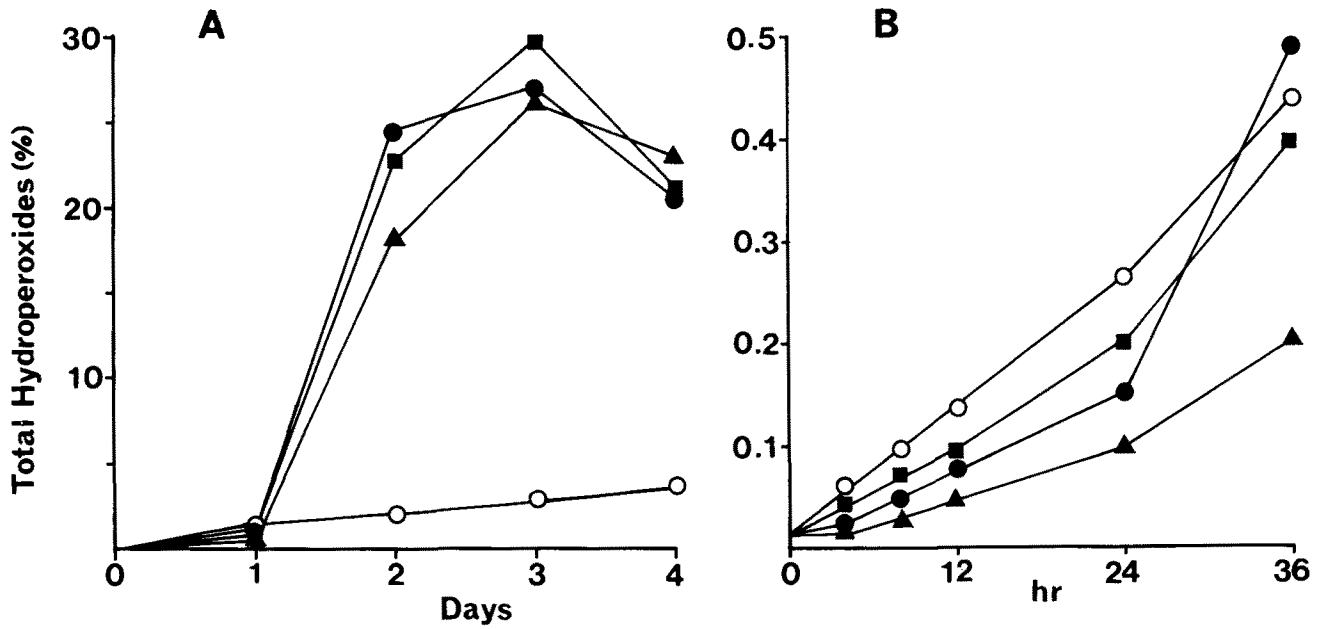

FIG. 1. Autoxidation of methyl linoleate at 50 C for 4 days (A) and for initial stage (B) with no additive (•), 0.1% DPPC (■), 0.1% DPPE (▲), and 0.1% α-tocopherol (O). Level of hydroperoxide before incubation was estimated to be 0.008% of methyl linoleate.

TABLE 1

Synergistic Effect of Saturated Phospholipids with α-Tocopherol at 50 C.

Additive[b]	Hydroperoxide produced (%)[a] (relative ratio to the control) 12 hr	24 hr
0.1% α-tocopherol (control)	0.14 (100)	0.26 (100)
+DPPC	0.18 (130)	0.47 (181)
+DPPE	0.15 (114)	0.35 (136)
1.0% α-tocopherol (control)	0.99 (100)	1.85 (100)
+DPPC	1.65 (167)	3.35 (181)
+DPPE	1.11 (122)	2.33 (126)

[a]The level of hydroperoxides before incubation was estimated to be 0.029% of methyl linoleate.
[b]1.0% of DPPE and DPPC was added to methyl linoleate in each experiment.

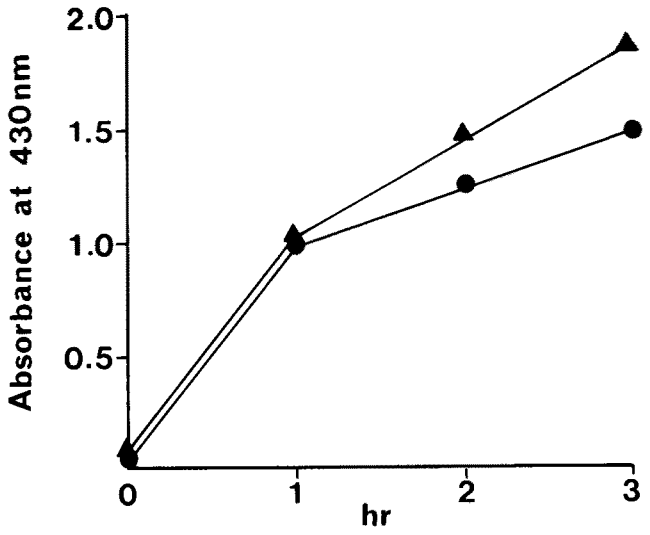

FIG. 2. Increase in the color intensity during the heating of egg yolk PC (•) and PE (▲) at 180 C (10 mg of aliquot was dissolved in 3 ml chloroform to determine the absorbance).

when present in oil. There have been contradictory reports on the effect of phospholipids on oil autoxidation (27,28). This confusion may be due to the structural complexity of phospholipids containing different base groups and various fatty acid compositions. For a better understanding of the role of base group, we first estimated the effect of saturated phospholipids on autoxidation. Both saturated PC and PE showed little effect on the autoxidation of methyl linoleate (Fig. 1). The prooxidant effect of α-tocopherol in the initial stage of autoxidation is consistent with earlier reports (25,29). However, DPPC and DPPE do not show any prooxidant effect as does α-tocopherol in the initial stage. Thus, it may be concluded that the base group of phospholipids does not appear to influence oil stability significantly. Moreover, neither phospholipid exhibited synergism with α-tocopherol at low temperature (50 C), indicating that perhaps unsaturated moiety is necessary for a synergistic effect.

Egg yolk phospholipids with a high degree of unsaturation showed clear prooxidant activity during the initial stage of autoxidation, as shown in Table 2. Unsaturated phospholipids are themselves known to undergo autoxidation faster than triglyceride fractions (30). The prooxidant effect of egg yolk may be due to the unstable polyunsaturated fatty acids such as arachidonic and docosahexaenoic acids. They might produce free radicals responsible for autocatalytic oxidation of methyl linoleate. Therefore, the influence of unsaturation apparently overwhelmed the effect of the base group on egg yolk phospholipids.

On the other hand, the egg yolk phospholipids retarded the hydroperoxide formation by heating them at 180 C (Table 2). This activity may be generated by the loss of polyunsaturated acids during heating reaction.

Moreover, the results shown in our previous work (12) suggest a more probable factor that highly polar browning color products of heated phospholipids chelate

TABLE 2

Effects of Browning Color Reaction on the Behavior of Egg Yolk Phospholipids in Hydroperoxide Formation from Methyl Linoleate at 50 C

Additive	Heating time (hr)	Hydroperoxides Produced (%)[a] Incubation time 24 hr	48 hr
None		2.4	24.5
+ 0.1% PC	0	7.1	26.6
	1	1.1	25.0
	2	1.0	23.7
	3	1.0	22.6
+ 1.0% PC	0	11.0	30.0
	1	2.8	25.4
	2	1.4	23.7
	3	1.2	19.3
+ 0.1% PE	0	4..1	23.6
	1	0.9	23.8
	2	1.1	24.1
	3	0.9	23.6
+ 1.0% PE	0	12.2	24.5
	1	0.9	14.9
	2	1.0	16.4
	3	0.9	17.7

[a]The level of hydroperoxides before incubation was estimated to be 0.032% of methyl linoleate.

with metal ions to show antioxidative properties in the initial stage. The results shown in Table 2 indicate that the antioxidant activity of heated PC and PE other than 1.0% PC were almost independent of heating time. They suggest that a considerable amount of browning color products responsible for inhibitory effect are produced

during the early stage of heating. Further studies are necessary to ascertain the structure of products responsible for the antioxidative properties of heated phospholipids.

From the foregoing discussion, it can be concluded that polar browning reaction products affect oil stability when oil is heated in the presence of phospholipids.

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High Oil- and Polyphenol-Producing Species of the Northwest

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The examination of plant species for their potential as renewable sources of industrial raw materials, conducted at the Northern Regional Research Center, has been extended to include 110 species from North Dakota (ND), Colorado (CO), and Oregon (OR), U.S.A. Plant samples were collected and analyzed for yields of "oil," "polyphenol," "hydrocarbon" and crude protein as well as for botanical characteristics. Data are presented only for the relatively high-yielding species. Oil and hydrocarbon extracts of plants that yielded at least 3.0% oil (dry, ash-free, plant sample basis) and/or at least 0.4% hydrocarbon were analyzed for classes of constituents. Oils of such species were saponified to determine yields of fatty acids and unsaponifiable matter. Hydrocarbon was examined for the presence of rubber, gutta and/or waxes. Polyisoprenes were analyzed for average molecular weight and molecular weight distribution. Even when compared to about 1000 species previously analyzed in this program, seven of the species yielded high amounts of oil (7.1-11.1%) plus substantial amounts of polyphenol (10.0-19.7%). Of these, six are evergreen trees or shrubs and one is a nonwoody perennial. Another seven species yielded substantial amounts of oil (5.4-6.6%), of which five gave 17.1-24.7% polyphenol. The most notable oil-producing species were *Juniperus scopulorum* (11.1%), *Pinus albicaulis* (10.1%), *Pinus flexilis* (9.3%), *Pinus mugo* (8.4%), *Liatris punctata* (8.0%) and *Juniperus communis* (7.8%). Crude protein contents for all 22 species were low (4.2%) to moderate (10.4%). Maximum hydrocarbon content for the 22 selected species reported was only 0.5%. The highest total amount of oil, polyphenol, hydrocarbon and crude protein was 38.9% for *Acer ginnala*. Data obtained in this study are discussed with respect to those from species previously analyzed in this program.

Development of new crops for production on underused land could stimulate industrial and economic growth without competing with established crops (1-4). Currently, U.S. food crops are much in excess of domestic needs. New nonfood crop developments could reduce our nation's dependency upon foreign sources of essential and strategic materials as well as have numerous other benefits (1,4).

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