# The Antioxidant Effects of Phospholipids on Perilla Oil

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**Antioxidant effect of phospholipids on the oxidation of refined perilla oil (PO; $\alpha$ – 18:3, 54.5%; 16:0, 7.2%; 18:0, 2.6%; 18:1, 18.6%; 18:2, 15.5%), tocopherol-free (POF) and tocopherol-enriched (POR) perilla oil were investigated by measuring weight-gains and by the oven test at 37°C. The oxidative stability of PO was especially increased by additions of phosphatidylethanolamine (PE) and phosphatidylserine (PS), but phosphatidylcholine (PC) scarcely showed an antioxidant effect. The oxidative stability of POF was markedly low, and none of the phospholipids (PC, PE, PS) showed an antioxidant effect on the oxidation of POF. The stability of POR was lower than that of PO regardless of its higher tocopherol contents. However, the oxidation of POR was significantly suppressed by additions of PE and PS, as was observed with PO. PC showed a small antioxidant effect on the oxidation of POR. Therefore, it seems that the antioxidant effects of phospholipids, especially of PE and PS, was due to the presence of tocopherols in the perilla oil.**

KEY WORDS: Antioxidant, oxidation, perilla oil, phospholipids, tocopherol.

It has been reported by many investigators that phospholipids show antioxidant effects on the autoxidation of fats and oils (1-8). At elevated temperatures some of the phospholipids, particularly phosphatidylethanolamine (PE), greatly enhanced the activity of primary antioxidants in edible oils. Phosphatidylcholine (PC) and phosphatidylserine (PS) are also reported to be effective (9,10). However, little information has been obtained concerning the synergistic antioxidant effects of phospholipids in fats and oils at ordinary temperatures. It seems important to know the antioxidant effects of phospholipids during the long-term storage of polyunsaturated fats at ordinary temperatures. Perilla oil contains $\alpha$-linolenic acid (n-3 fatty acid) at high levels and has been noted from the viewpoint of the importance of consuming fats containing n-3 fatty acids. However, perilla oil is sensitive to autoxidation because of its high $\alpha$-linolenic acid levels. In the present paper, antioxidant effects of phospholipids (purity above 98%) on the oxidation of refined perilla oil (PO), tocopherol-free (POF) and tocopherol-enriched (POR) perilla oil were investigated at 37°C.

## EXPERIMENTAL PROCEDURES

*Materials.* L-$\alpha$-phosphatidylethanolamine (PE) from soybean seeds L-$\alpha$-phosphatidyl-L-serine (PS) from bovine brain were obtained from Sigma Chemical Co. (St. Louis, MO). Soybean was the Fats product of Nippon Oil & Fats Co. (Tokyo, Japan). The purity of these phospholipids were above 98%. PO was obtained from Ohta Oil & Fats Co. (Aichi, Japan). Mixed tocopherols $\delta$-Toc = 95.7%) were obtained from Eisai Co. (Tokyo, Japan). POF was prepared by removing tocopherols through actived-alumina column chromatography (11).

*Analysis of perilla oil.* The fatty acid composition of perilla oil was determined by gas liquid chromatography (GLC); HP58A Gas Chromatography, Hewlett Packard Co., Palo Alto, CA) as follows: 16:0, 7.2%; 18:0, 2.6%; 18:1, 18.6%; 18:2, 15.5%; and 18:3, 54.5%. Tocopherols (Toc) in PO were determined by high performance liquid chromatography (HPLC) (12), and the contents were as follows: total Toc, 366 ppm; $\gamma$-Toc, 328 ppm; $\alpha$-Toc, 31 ppm; $\delta$-Toc, 7 ppm; and $\beta$-Toc, not detected. POF contained 15 ppm of $\gamma$-Toc. POR was prepared from PO by adding 500 ppm mixed tocopherols.

*Autoxidation studies.* The oven test was performed as follows: The oil samples (20 g) were kept in the dark at 37 ± 1°C. The oxidative stability was evaluated by the oven test as measured by peroxide values (PV) (13) and carbonyl values (CV) (14).

The weight-gain method was performed as follows: oil samples (1 ± 0.005 g) were placed in a petri dish and kept in the dark at 37 ± 1°C. The induction period was gravimetrically estimated by the weight-gain method and defined as days required to increase the weight of substrate oil by 0.5%. The residual amounts of >-Toc in the sample oil during the oxidation experiments were determined by HPLC (12).

## RESULTS AND DISCUSSION

Figures 1 and 2 show the antioxidant effects of phospholipids on perilla oil containing endogenous tocopherols of 366 ppm. The oxidative stability of the oil was evaluated by both the oven test (PV and CV) and weight-gain method at 37°C. In the case of the oven test (Fig. 1), control and PC-added oil accumulates large amounts of hydroperoxides from the initial days of the storage. The additions of PE and PS reduced the induction period of about three weeks. In the case of the weight-gain method (Fig. 2), the induction period was 17 days for control and 19 days for PC-added oil but the induction period did not appear in the presence of PE or PS during 25 days storage at 37°C. Figure 3 shows the antioxidant effects of phospholipids on POF by the oven test at 37°C. None of the phospholipids (PC, PE, PS) showed an antioxidant effect on POF. The additions of PE or PS delayed the oxidation of the oil by acting synergistically with the endogenous tocopherols in the oil. Figures 4 and 5 show the antioxidant effects of phospholipids on POR prepared by adding exogenous tocopherols of 500 ppm to the refined perilla oil. As shown by the oven test (Fig. 4), regardless of its higher tocopherol contents, the oxidation stability of POR was lower than that of PO after 15 days storage. However, PE and PS remarkably suppressed the oxidation of both POR and PO. PC scarcely showed an antioxidant effect on the oxidation of POR.

As shown by the weight-gain methods (Fig. 5), PE and PS remarkably suppressed the oxidation of POR, but PC scarcely showed an autoxidant effect as also observed by

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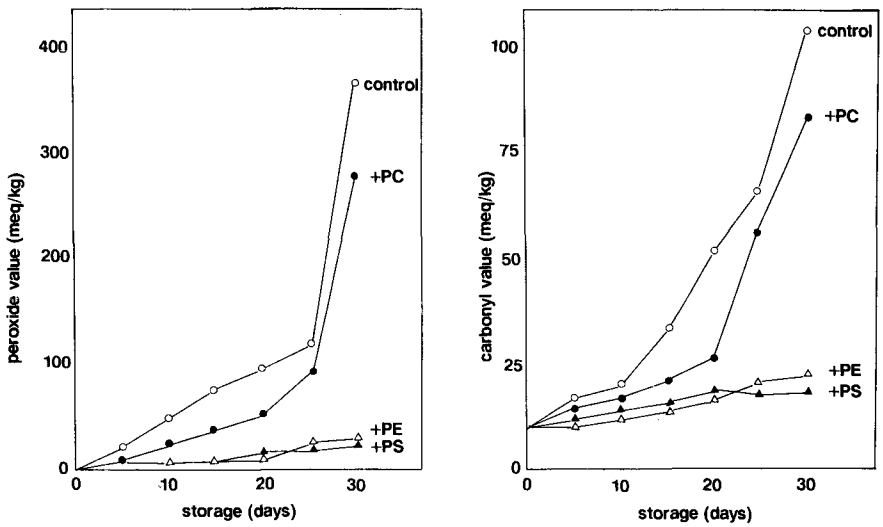


**FIG. 1. Effects of phospholipids on autoxidation of refined perilla oil (PO) stored for 30 days at 37°C in the dark. Results were determined by the oven test (PV and VC). Levels of phospholipids were 500 ppm. ○, control, without phospholipids; •, PC; △, PE; and ▲, PS.**

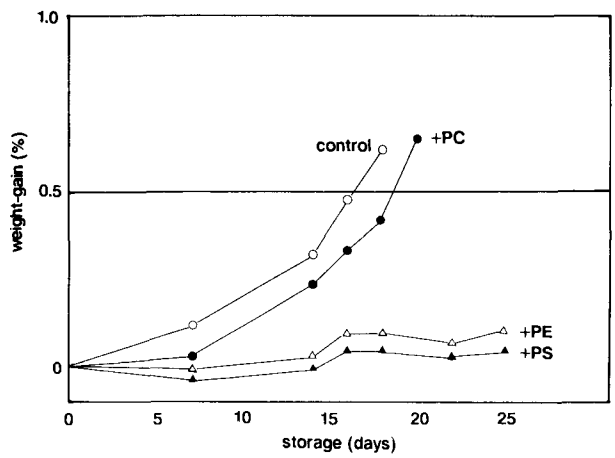


**FIG. 2. Effects of phospholipids on autoxidation of perilla oil (PO) stored for 25 days at 37°C in the dark. Results were determined by the weight-gain method. Levels of Phospholipids and the symbols used are the same as those in Figure 1.**

the oven test (Fig. 4). From the above results, it is thought that PE and PS suppress the prooxidant effect of overabundant tocopherols on the oil. Figure 6 shows the effect of phospholipids on the residual rate of endogenous $\gamma$-Toc in PO with or without phospholipids during the autoxidation test. Both PE and PS remarkably suppressed the oxidative decomposition of $\gamma$-Toc, but PC did not. The antioxidant synergism between tocopherols and phospholipids are seen to be closely associated with the effects of phospholipids on suppressing the oxidative decomposition of tocopherols. From the viewpoint of suppressing the oxidative decomposition of tocopherols, we are now studying the antioxidant synergistic interactions between tocopherols and phospholipids on long-term deterioration processes during storage at ordinary temperature.

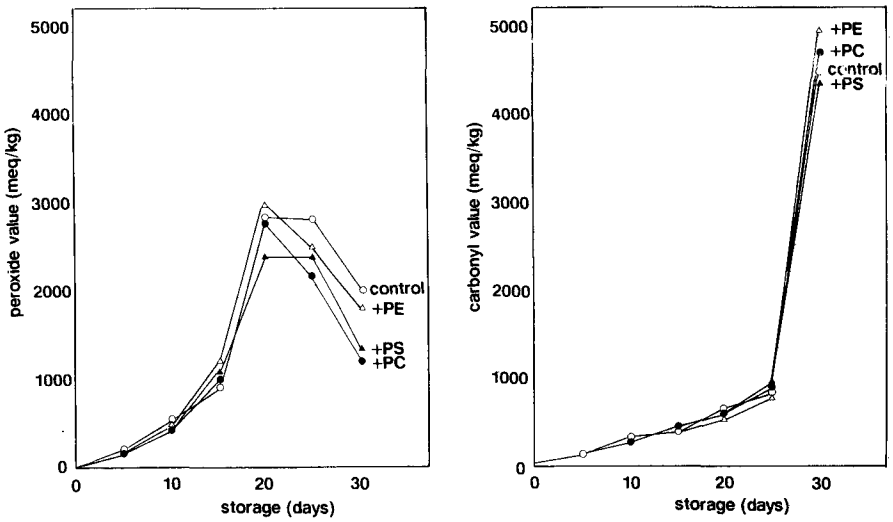


FIG. 3. Effects of phospholipids on autoxidation of tocopherol-free perilla oil (POF) stored for 30 days at 37°C in the dark. Results were determined by the oven test. Levels of phospholipids and the symbols used are the same as those in Figure 1.

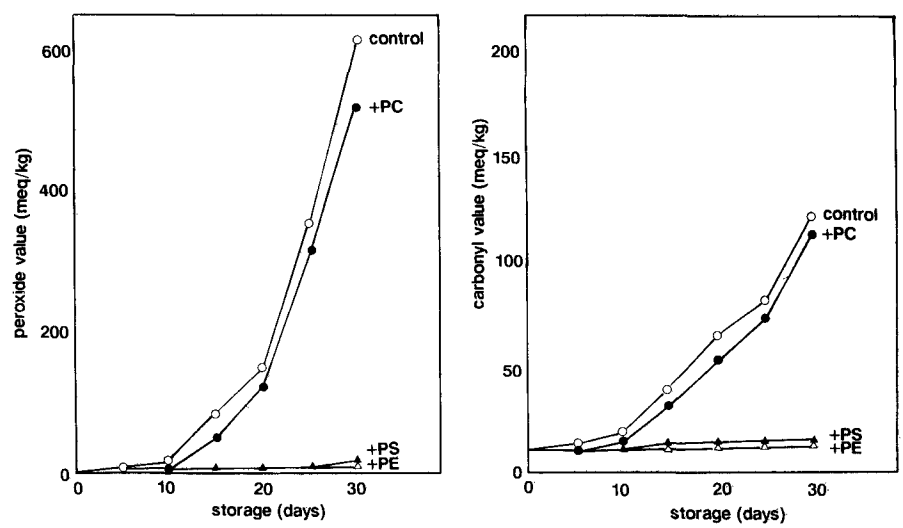


FIG. 4. Effects of phosopholipids on autoxidation of tocopherol-enriched perilla oil (POR) stored for 30 days at 37°C in the dark. Results were determined by the oven test. Levels of phospholipids and the symbols used are the same as those in Figure 1.

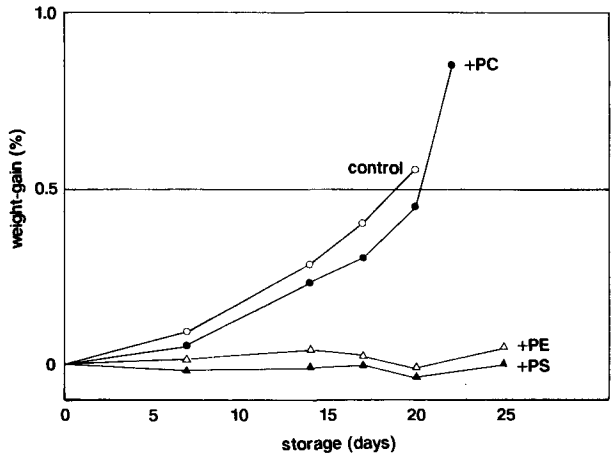


**FIG. 5. Effects of phospholipids on autoxidation of tocopherol-enriched perilla oil (POR) stored for 25 days at 37°C in the dark. Results were determined by the weight-gain method. Levels of phospholipids and the symbols used are the same as those in Figure 1.**

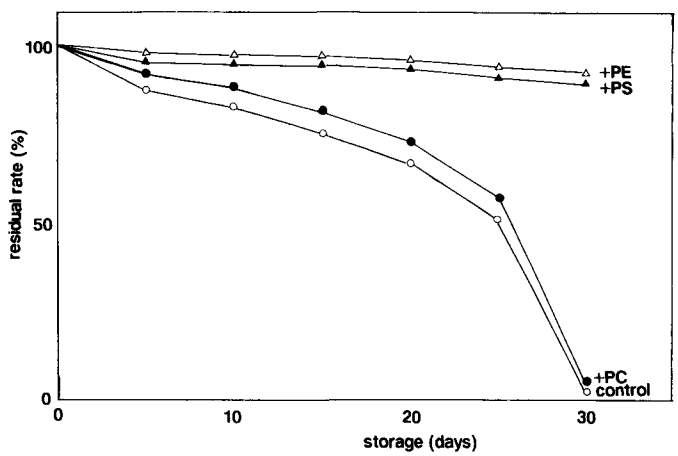


**FIG. 6. Effects of phospholipids on the residual rate of endogenous γ-Toc in refined perilla oil stored for 30 days at 37°C in the dark. Levels of phospholipids and the symbols used are the same as those in Figure 1.**

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