Antioxidant Effects of Chlorophyll and Pheophytin on the Autoxidation of Oils in the Dark. I. Comparison of the Inhibitory Effects

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ABSTRACT

The effects of chlorophyll and pheophytin on the autoxidation of oils in the dark were investigated by oven tests. The results indicated that both chlorophyll and pheophytin show antioxidant activity when methyl linoleate is used as substrate. Furthermore, chlorophyll retarded the oxidative deterioration of triglycerides in rapeseed and soybean oils at 30 C. Among the four chlorophyll derivatives (chlorophylls a and b and pheophytins a and b), chlorophyll a showed the strongest antioxidant activity. The antioxidant effects of chlorophyll and pheophytin depended on the storage temperature and the kinds of oil used as substrate.

INTRODUCTION

Many vegetable oils contain chlorophyll (CHL) and carotenoids as natural pigments. CHL has been supposed to exert its prooxidative action on the deterioration of oils (1-5). CHL is known to act as a photosensitizer which accelerates the oxidation of oils when exposed to light. In particular, the oxidation products formed from several fatty acids by CHL-sensitized photooxidation have been studied by many workers (6-8). Moreover, photosensitizing activities have been reported for some CHL derivative compounds (9,10). In our previous papers, we reported the high photosensitizing activity of pheophytin (PHY), which is devoid of magnesium, in contrast to CHL, and the presence of considerable amounts of PHY in commercial vegetable oils (11-13). In fact, the effects of CHL on the photooxidation of oils have been investigated extensively, but the action of CHL and its decomposition products on the autoxidation and deterioration of oils in the dark remains unclarified. Matsushita et al. have studied the antioxidant effects of some porphyrins, including iron and copper chlorophyllins and PHY, by the determination of TBA value (14). But no data on CHL was reported.

To understand the effects of CHL and PHY on the autoxidation of commercial edible oils in the absence of light, oven tests of CHL and PHY dissolved in methyl linoleate (ML) and/or triglycerides as substrates were carried out.

MATERIALS AND METHODS

Materials

CHL, PHY and ML were prepared as previously reported (11).

Refined rapeseed and soybean oils without additives, obtained from oil manufacturers, were treated with activated carbon black-diatomaceous earth column chromatography to remove traces of CHL and PHY (7).

Methyl oleate was refined by silicic acid column chromatography and urea treatment of commercial methyl oleate followed by vacuum distillation. The refined methyl stearate was prepared by vacuum distillation of methyl stearate purchased from Tokyo Kasei Kogyo Co. Ltd. The purity of each methyl ester was greater than 98% in gas chromatographic analysis.

t-Butyl hydroxy toluene (BHT), a widely used synthetic antioxidant, was purchased from Tokyo Kasei Kogyo Co. Ltd.

Oven Test

ML (1.00 g) with and without CHL A and B, and PHY A and B (2.2×10^{-7}-10^{-9} mol) were each put in small glass beakers (ϕ 27 mm) and incubated at 30 C or 50 C in the dark. After fixed time intervals, the peroxide value (PV) and carbonyl value (CV) of the oxidized samples were measured according to the method described by AOCS (15) and Kumazawa and Oyama (16), respectively. (PV and CV of ML were 0.1 and 4.0 meq/kg, respectively, before the oxidation.) Residual amounts of CHL and PHY in ML were estimated by measuring the decrease in absorbance at a definite wavelength (CHL A:662, CHL B:646, PHY A:665, PHY B:653 nm) during autoxidation.

These experiments also were carried out using rapeseed (PV=1.5) and soybean oils (PV=1.3) as triglycerides, and methyl oleate (PV=0.3) and methyl stearate (PV=2.3), instead of ML.

RESULTS AND DISCUSSION

ML samples containing CHL A and B and PHY A and B were incubated at 30 C in the dark. Changes in their PV and CV are illustrated in Figure 1. CHL and PHY retarded the production of both peroxides and carbonyl compounds from ML during incubation. The pattern in CV changes was similar to that in PV changes, because the inhibition of carbonyl production was attributable to the retardation of the formation of peroxides during the autoxidation. In comparing antioxidant activities of CHL and PHY, CHL retarded oxidative deterioration of ML more effectively than PHY did, at a concentration of 2.2×10^{-8} mol/g ML. In particular, CHL A had the strongest antioxidant activity among the four CHL derivatives, followed by BHT, CHL B, PHY A and B in this order.

Moreover, both CHL and PHY inhibited the production of conjugated diene during the autoxidation of ML at 30 C in the dark. Antioxidant activities also were observed in all CHL derivatives at the concentration of 2.2×10^{-7} mol/g ML in the same order as obtained at the 2.2×10^{-8} mol/g ML.

Figure 2 shows the time course of PV changes of ML with various concentrations of CHL and PHY during incubation at 30 C. The formation of peroxides from ML by autoxidation was greatly depressed as the addition levels of CHL and PHY were increased under this experimental condition. It is noted that trace amounts of CHL and PHY (2.2×10^{-9} mol) retarded the autoxidation of ML slightly. This result suggests that the presence of CHL derivatives in commercial vegetable oils also may contribute to the maintenance of oxidative stability when they are kept in the dark. Moreover, CHL A showed the strongest antioxidant activity among the four CHL derivatives in all concentrations used in this experiment.

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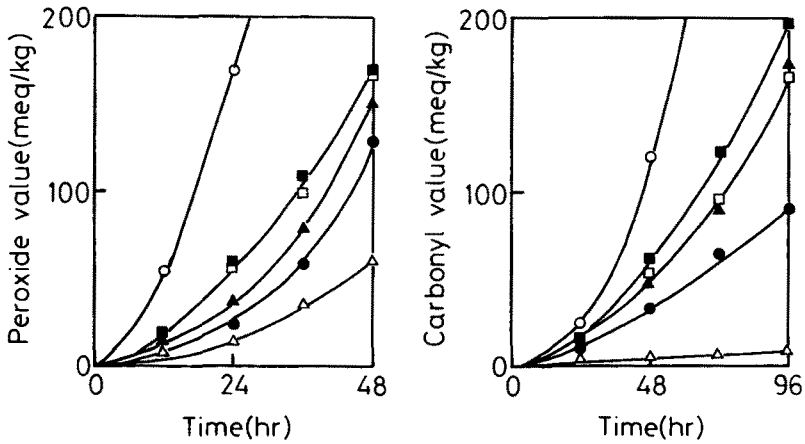

FIG. 1. Effects of chlorophylls and pheophytins on the autoxidation of methyl linoleate in the dark. 2.2×10^{-8} mol of CHL, PHY or BHT was added to 1 g of ML, and the sample was incubated at 30 C in the dark. ○, control; ●, BHT; △, CHL A; ▲, CHL B; □, PHY A, and ■, PHY B.

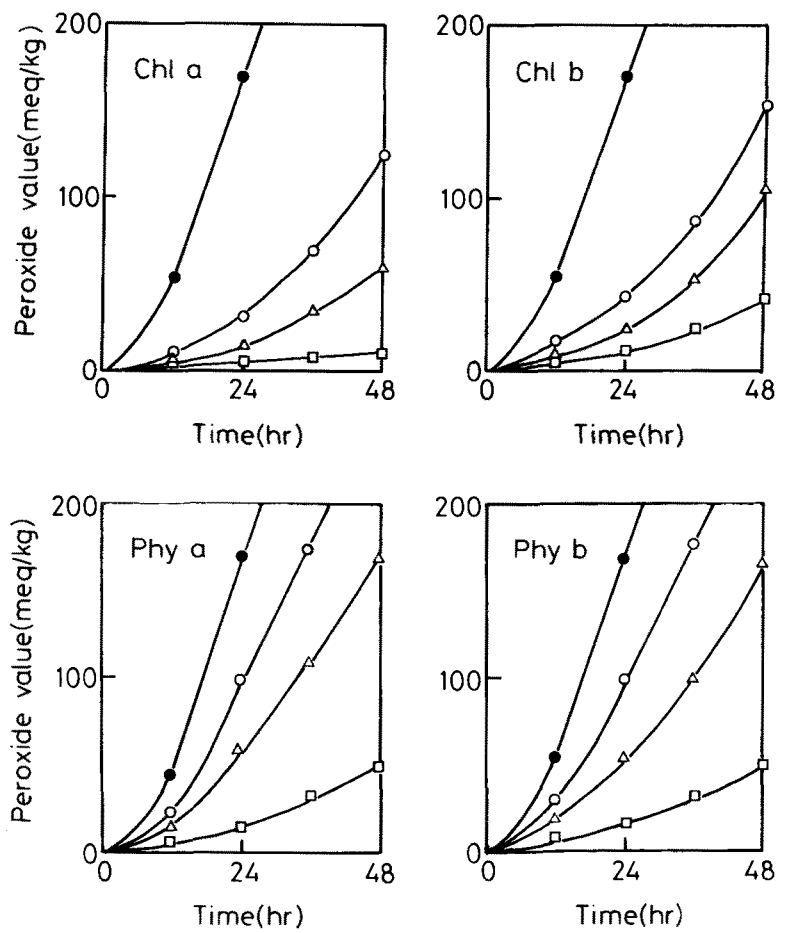

FIG. 2. Autoxidation of methyl linoleate with different amounts of chlorophylls and pheophytins. ML with CHL A and B (Fig. 2a), and with PHY A and B (Fig. 2b) was oxidized at 30 C in the dark. Additional levels of CHL and PHY were from 2.2×10^{-7} to 2.2×10^{-9} mol/g ML. ●, control; ○, 2.2×10^{-9}; △, 2.2×10^{-8}, and □, 2.2×10^{-7} (mol/g).

To investigate the effect of incubation temperature on the antioxidant activity of CHL and PHY, ML added CHL or PHY were autoxidized at 50 C. As shown in Figure 3, a pattern similar to that observed at 30 C was noticed in the changes of PV of ML during autoxidation in the dark. Both CHL and PHY also retarded the oxidative deterioration of ML, but their antioxidant activities at 50 C were lower than those at 30 C.

In order to clarify the decrease in antioxidant activities of CHL and PHY at high temperatures, oxidative stabilities of CHL and PHY in ML were estimated in the dark by measuring their absorbance at visible absorption maxima during

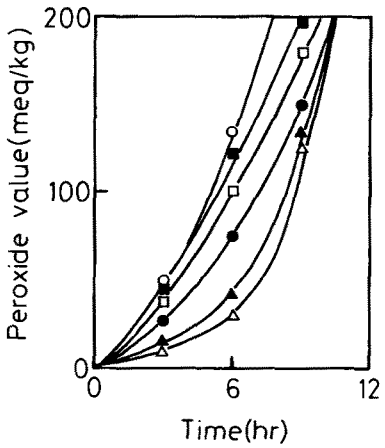

FIG. 3. Autoxidation of methyl linoleate with chlorophylls and pheophytins at 50 C in the dark. Same experimental conditions as Fig. 1 were used except the incubation temperature. ○, Control; ●, BHT; △, CHL A; ▲, CHL B; □ PHY A, and ■, PHY B.

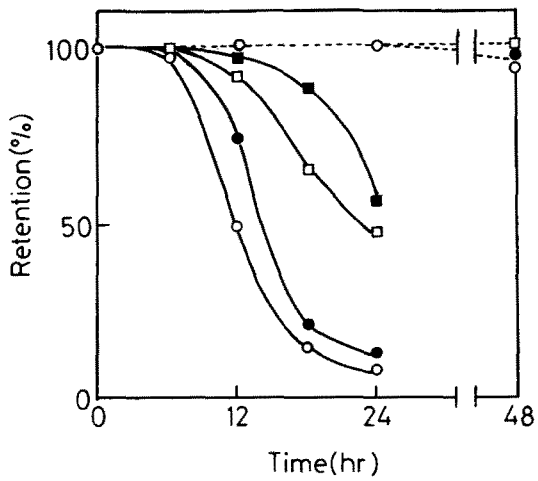

FIG. 4. Degradation of chlorophylls and pheophytins in methyl linoleate during autoxidation. 2.2 × 10^{-8} mol CHL or PHY was added to 1 g of ML and was incubated at 30 C (- - -) and 50 C (—). Measurement of residual CHL and PHY was described in this text. ○, CHL A; ●, CHL B; □, PHY A, and ■, PHY B.

the incubation at 30 C and 50 C (Fig. 4). The absorption maxima of CHL A and B gradually disappeared, while those of PHY A and B were unchanged during incubation at 30 C. This also was observed in the bleaching of CHL and PHY in ML by light irradiation (17). However, PHY is unstable against heat and is also bleached during autoxidation at 50 C, as is CHL. The decomposition rates of CHL and PHY in ML during autoxidation at 50 C were greater than those at 30 C. It is reported that CHL is bleached not only by hydroperoxides produced during the oxidation of oils (18), but also by the heat (19). In any case, the decreasing antioxidant effects of CHL and PHY at high temperature might be due to their poor thermo-stabilities.

Using photosensitizer-free rapeseed and soybeans oils as substrate, antioxidant activities of CHL and PHY on the autoxidation of oils at 30 C were evaluated (Table I). CHL A and B inhibited the oxidative deterioration of both oils in the dark and showed antioxidant activities comparable to those of BHT in rapeseed oil, but there was no difference between the antioxidant activities of CHL A and B. CHL A and B reduced the oxidation rate of rapeseed and soybean oils by one quarter and one half, respectively, but CHL showed lower antioxidant activity in rapeseed oil than in soybean oil. On the other hand, PHY A and B did not exhibit antioxidant effects in the autoxidation of rapeseed and soybean oils at a concentration of 2.2 × 10^{-8} mol/g oil. This result is definitely in disagreement with that using ML as substrate. Lipophilic antioxidants such as BHA, BHT, N, N′-diphenyl-p-phenylenediamine and ethoxyquin often have very low activity in vegetable oils (20,21). Then, one reason for the lack of antioxidant activity of PHY on the autoxidation of vegetable oils may be the difference in the component fatty acids of the triglycerides. Another possible reason may be the antioxidant effects of tocopherols essentially contained in vegetable oils.

Frankel et al. (22) reported that appearance of antioxidant activity depended on the content of highly unsaturated fatty acid composition of oils. Then, the effects of the differences of fatty acids on the antioxidant activities of CHL and PHY were investigated by using methyl stearate and methyl oleate as substrate instead of ML. PV changes in the stearate and oleate with or without CHL A and PHY A at 30 C are shown in Table II. CHL A retarded the increase in the amounts of peroxides formed by autoxidation of stearate and oleate in the dark. PHY A, however, had very little effect on either ester. This result suggests that the variety of constituent fatty acids of the substrate may affect the

TABLE I

Effects of Chlorophyll and Pheophytin on the Autoxidation of Soybean and Rapeseed Oils at 30 C in the Dark

	Induction period (day)[a]	
Additives[b]	Soybean[c]	Rapeseed[c]
None	6	13
CHL A	12	17
CHL B	12	17
PHY A	5	13
PHY B	6	13
BHT	8	17

[a]The time in days to reach 20 meq/kg in PV.
[b]Addition level is 2.2 × 10^{-8} mol/g oil.
[c]Initial PV of soybean and rapeseed oils are 1.3 and 1.5 meq/kg, respectively.

TABLE II

Effects of Chlorophyll and Pheophytin on the Autoxidation of Methyl Stearate and Methyl Oleate at 30 C in the Dark

	Peroxide value (meq/kg)		
	Oxidation time (day)		
Sample[a]	30	60	90
Methyl stearate[b]			
None	6.1	7.4	8.5
CHL A	2.9	5.1	6.5
PHY A	6.5	7.3	7.8
Methyl oleate[b]			
None	6.6	15.1	23.2
CHL A	4.2	4.9	7.7
PHY A	6.5	11.7	16.2

[a]Addition level of CHL A and PHY A is 2.2 × 10^{-8} mol/g oil.
[b]Initial PV of methyl stearate and methyl oleate are 2.3 and 0.3 meq/kg, respectively.

appearance of antioxidant activities of CHL and PHY. Moreover, this observation is in accord with the result shown in Table I, that CHL showed a stronger antioxidant effect on the autoxidation of soybean oil which consisted of highly unsaturated fatty acids (linoleate + linolenate = 61%) as principal constituent fatty acids than on rapeseed oil (34%). CHL and PHY generally can exhibit very strong antioxidant activities when highly unsaturated fatty acids are used as a substrate. On the other hand, many vegetable oils contain some tocopherols as a natural antioxidant. Therefore, tocopherols are expected to affect the antioxidant activities of CHL and PHY. Tocopherol contents were α, 0.8; γ 52.4; δ, 0.6 μg/g oil in soybean oil and α, 22.0; β, 7.7; γ, 123.4, and δ, 0.8 μg/g oil in rapeseed oil, determined by high performance liquid chromatography (23). Rapeseed oil contains high levels of tocopherols, which tend to antagonize the action of lipophilic antioxidants (24). Effects of tocopherols on the antioxidant activities of CHL and PHY will be reported in another paper.

Although the effects of CHL and PHY on the oxidation of oils in the presence of light have been studied, their effects in the dark remain unclarified. Many investigators have considered CHL as a prooxidant, but a few have regarded CHL as an antioxidant. Imura reported the prooxidant effects of sacrophyl (sodium copper chloroporphyrin) and PHY on the autoxidation of linoleic acid (25). On the other hand, Matsushita et al. observed antioxidant activity in some porphyrin compounds such as PHY and chlorophyllin (14). Sato et al. reported the antioxidant effect of sodium copper chlorophyllin, a CHL derivative, on lipid peroxidation in rat liver homogenates (26-28). These observations are consistent with the results obtained in our experiments. We claim that CHL existing in vegetable oils can act as an antioxidant preventing oxidative deterioration if the vegetable oil containing CHL is not exposed to light and is stored at low temperature.

The mechanism for the antioxidant effects of CHL on the autoxidation of some oils in the dark has not been clarified in this paper. However, we suspect that CHL may act as a radical scavenger, trapping the peroxy radical and other free radicals, inhibiting the autoxidation of oils. We will make clear the mechanism for the antioxidant effect of CHL on the autoxidation of oils in the next paper (29).

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

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[Received December 12, 1984]