(1-C and 3-C). Tsujisaka et al. found that a lipase that cannot hydrolyze the ester bond at β-position (C_2) hardly synthesizes ester bond at the same position, yielding mainly 1-monoglyceride and 1,3-diglyceride (4).

General Discussion

A new bioreactor with a hydrophobic microporous membrane was developed for continuous synthesis of glycerides by lipase in a nonemulsion system. The advantages of the new microporous membrane bioreactor are summarized as follows. First, the bioreactor does not require the making of an emulsion. This obviates the need for surfactant and stirring. Second, the oily product can be obtained in a pure state with no other phase. Third, the control of the water content is easier in this nonemulsion system than in the conventional emulsion system. Fourth, autoxidation of fatty acid is avoidable in the bioreactor simply by freeing the fed substrate from oxygen because the bioreactor has no free space to hold air inside when it is filled with reactants. Last, the flow of the fatty acid in this microporous membrane bioreactor is close to plug flow, whereas the emulsion system is always operated in perfect mixing. These advantages might be exploited to push forward to industrial application. Unit sets of the bioreactor could be piled up to make a bigger set, resulting in a large surface area in a compact system. Alternatively, one might be able to use hollow fiber modules as a more effective bioreactor configuration.

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☘Prooxident Activities of Chlorophylls and Their Decomposition Products on the Photooxidation of Methyl Linoleate

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ABSTRACT

Although chlorophylls in oils affect oxidative stabilities, little has been known about the prooxidant activity of the chlorophylls or their decomposition products. To evaluate the prooxidant activities of chlorophyll derivatives, chlorophyll (CHL) A and B, pheophytin (PHY) A and B and pheophorbide (PHO) A and B were added to methyl linoleate (ML). The sample oils were then subjected to photooxidation at 0 C and the peroxide value and absorbance at 234 nm were measured. Chlorophylls catalyzed oxidation of methyl linoleate at concentrations greater than 2.2×10^{-9} mol/g ML, and CHL B showed a stronger prooxidant activity, ca. twice that of CHL A under the same light intensity. PHY and PHO exhibited stronger prooxidant activities than CHL, the prooxidant activity of PHO being the strongest among them. Moreover, photosensitive activity was found in PHY as well as in CHL. These results suggest that particular attention should be paid to the decomposition products of CHL that affect the quality of vegetable oils.

INTRODUCTION

Since Coe (1) reported that the content of chlorophyll (CHL) is closely correlated with the extent of deterioration of oils, many reports have suggested that CHL is a prooxidant in fats and oils (2-5). CHL is known to act as a photosensitizer of oils, accompanying the generation of singlet oxygen when exposed to light (6-8). However, the prooxidant effects of CHL, especially the effect of its degradation products on the deterioration of oils, has never been systematically studied. In addition, what role the decomposition products of CHL play on the oxidative stabilities of oils has not been known.

The purpose of this study is to investigate the effects of magnesium chelated CHL and its degradation products, pheophytin (PHY) and pheophorbide (PHO), which are free of magnesium (Fig. 1), on the oxidative stability of oils, especially the prooxidant effects on methyl linoleate (ML).

MATERIAL AND METHODS

Materials

CHL A and B were prepared from acetone extracts of fresh spinach by DEAE-Sepharose CL-6B and Sepharose CL-6B (Pharmacia Fine Chemicals Piscataway, NJ) column

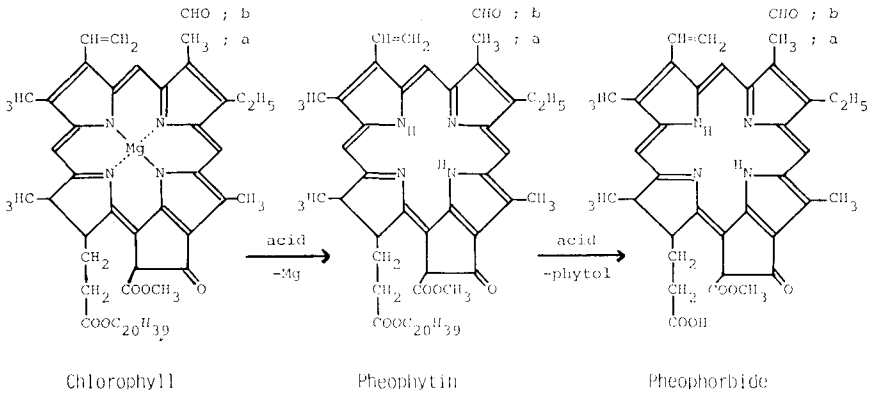

FIG. 1. The decomposition of chlorophyll.

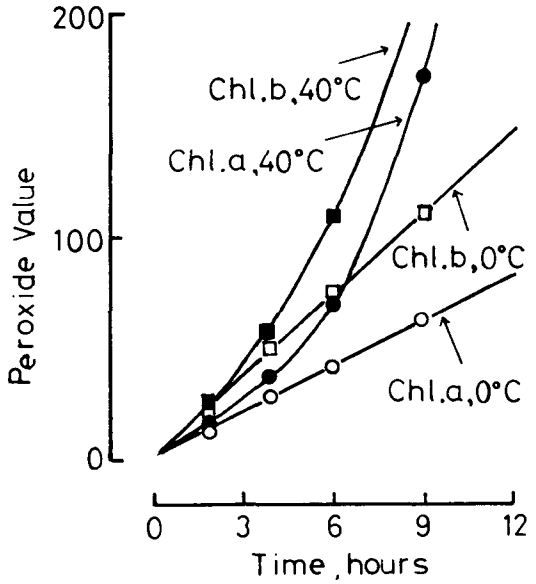

FIG. 2. Effect of temperature on chlorophyll-sensitized photooxidation of methyl linoleate.

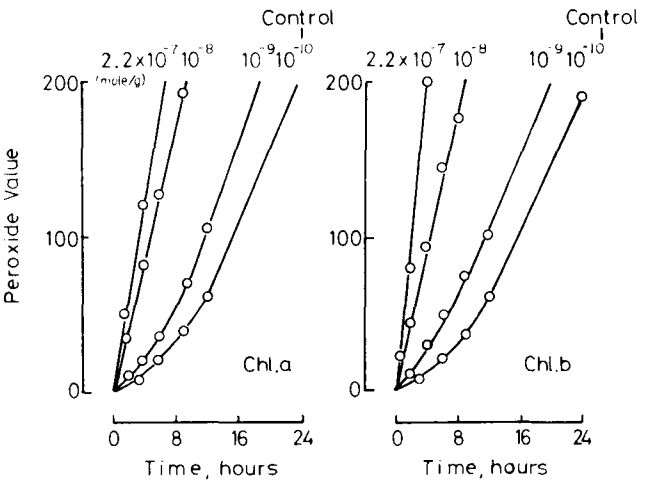

FIG. 3. Photooxidation of methyl linoleate with different amounts of chlorophylls.

chromatography according to the method of Omata and Murata (9).

A PHY mixture was prepared by adding petroleum ether and 10% sodium chloride aqueous solution to acetone extracts of spinach followed by treatment with oxalic acid solution. Separation of PHY A and B was carried out by sugar-corn starch (7:3) column chromatography (10).

CHL A and B were converted to PHO A and B by mixing the ether solution with 30% hydrochloric acid. The PHO was transferred into the ether layer by diluting the acid layer containing the reaction mixture (11).

The purity of all these compounds was confirmed by thin layer chromatography (TLC) and visible absorption spectrum. These compounds were then placed in an ether-n-hexane solution and their concentrations were photometrically determined (10-13).

The ML (99%) was prepared from mixed methyl esters of safflower-oil fatty acids by the method of urea adduct formation (14) and trace amounts of impurities, e.g., hydroperoxides, tocopheroles and CHL, were removed by vacuum distillation and activated carbon black-diatomaceous earth (1:1) column chromatography.

Photooxidation

The ML (1.00 g), with and without CHL A or B (2.2×10^{-7} to 10^{-10} mol), were put in a glass beaker (ϕ27 mm) and irradiated with transmitted light throughout the water layer from a 15 W fluorescent lamp at 0 C. Light intensity on the surface of the sample was 500 μW/cm^2 sec.

The peroxide value (POV) of the sample oil was measured according to the AOCS method (15). To determine the content of conjugated diene hydroperoxide (ϵ=26,000), 30 mg of the sample was diluted to 50 mL ethanol solution and its absorption at 234 nm was measured.

These experiments were done at 2 temperatures (0 C, 40 C) and 5 light intensities (0, 10, 50, 100 and 500 μW/cm^2 sec). The same procedures, using ML with PHY and PHO, were carried out.

RESULTS AND DISCUSSION

A comparison of the oxidative rate of ML with CHL A or B at 0 C and 40 C is shown in Figure 2. The oxidative rate is represented as the change in the POV of ML during the experiment. The photooxidation at 40 C was recognized as involving autoxidation caused by a chain reaction, as indicated by the existence of an induction period.

The POV changes of ML, with various concentration of CHL A or B 0 C, are shown in Figure 3. The addition of CHL A or B in amounts exceeding 2.2×10^{-9} mol catalyzed oxidation of ML, whereas a concentration of 2.2×10^{-10} mole had no effect on the photooxidation of ML. In ML containing CHL in concentrations greater than 2.2×10^{-8}

TABLE I

Molar Ratio of UV Absorbance (234 nm)/Peroxide in Oxidative Products of Methyl Linoleate (ML) with Chlorophyll (CHL) A or B

Concentration of CHL (mol/g ML)	UV/POV[a]	
	CHL A	CHL B
2.2×10^{-7}	0.53	0.51
10^{-8}	0.54	0.51
10^{-9}	0.66	0.65
10^{-10}	0.73	0.73
0	0.74	0.74

[a]Ratio of UV/POV in ML at POV 100.

TABLE II

Oxidative Rate of Methyl Linoleate (ML) with Different Amounts of Chlorophyll (CHL) A and B

Concentration of CHL (mol/g ML)	ΔPOV/Δt (10^{-6} mol/hr)		
	CHL A	CHL B	B/A
2.2×10^{-7}	13.9	23.4	1.7
10^{-8}	10.3	12.3	1.2
10^{-9}	4.4[a]	4.3[a]	1.0
10^{-10}	2.7[a]	2.7[a]	1.0
0	2.7[a]		–

[a]ΔPOV/Δt=100/2t; t is the oxidation time (hr) to reach to POV 100.

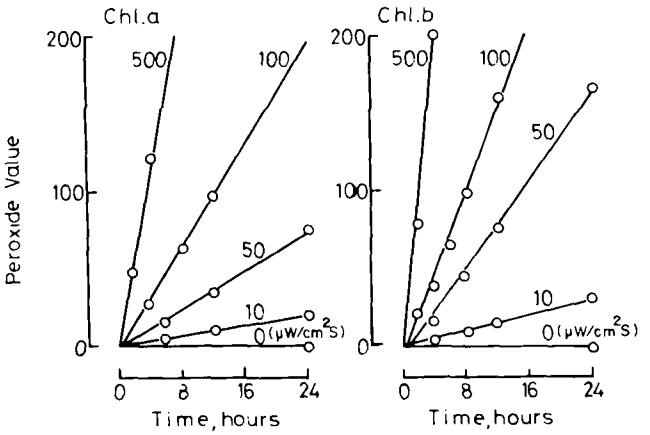

FIG. 4. Effect of light intensity on chlorophyll-sensitized photooxidation of methyl linoleate.

mol, only photosensitized oxidation was presumed to have occurred, because there was no induction period and a low UV absorption/POV ratio (0.5), indicating the formation of unconjugated diene hydroperoxide produced by singlet oxygen (Table I).

Table II shows the oxidative rate of ML with various concentrations of CHL A or B. The oxidative rate of ML with CHL of 2.2×10^{-7} and 10^{-8} mol/g ML is expressed as the amounts of increase in hydroperoxides per hr: ΔPOV/Δt (10^{-6} mol/hr). Because the photooxidation of ML containing CHL in concentrations of 2.2×10^{-9} and 10^{-10} mol/g ML was not the 0 order reaction as shown in Figure 2, oxidative rate is expressed as 100/2t (10^{-6} mol hydroperoxides/hr; t is the time in hours to reach to POV 100.) The result was that the prooxidant activity of CHL B was stronger than that of CHL A in concentrations greater than 2.2×10^{-8} mol/g ML: in fact the activity of the former was ca. twice that of the latter at 2.2×10^{-7} mol/g ML. The assumption was made that CHL A can not display the prooxidant effect on ML that CHL B does because CHL A was easily decomposed by the singlet oxygen generated. But the remarkable difference was not observed between prooxidant activity of CHL A and B at 2.2×10^{-9} and 10^{-10} mol/g ML.

TABLE III

Effect of Light Intensity on Oxidative Rate of Methyl Linoleate (ML) with Chlorophyll (CHL) A or B

Light intensity (μW/cm² sec)	ΔPOV/Δt (10^{-6} mol/hr)		
	CHL A[a]	CHL B[a]	B/A
500	13.9	23.4	1.7
100	2.5	4.9	2.0
50	1.4	2.2	1.6
10	0.3	0.7	2.3
0	<0.01		--

[a]Concentration: 2.2×10^{-7} mol/g ML.

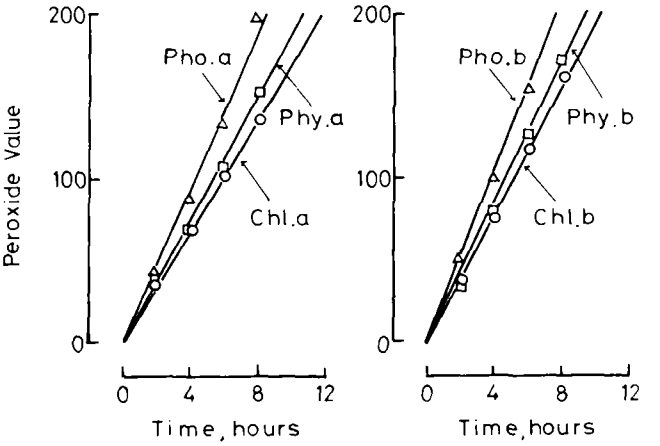

FIG. 5. Effects of chlorophylls, pheophytins and pheophorbides on photooxidation of methyl linoleate.

TABLE IV

Effects of Chlorophylls (CHL), Pheophytins (PHY) and Pheophorbides (PHO) on Oxidative Rate of Methyl Linoleate (ML)

Sample	ΔPOV/Δt (10^{-6} mol/hr)	
	2.2×10^{-8} mol/g ML	2.2×10^{-7} mol/g ML
Control	2.7[a]	--
CHL A	8.6	14.4
PHY A	9.2	14.1
PHO A	11.5	16.0
CHL B	9.8	16.2
PHY B	10.4	17.7
PHO B	12.7	17.5

[a]See legend in Table II.

In the next experiment, the oxidative rate of ML with CHL A was compared with that of ML containing CHL B at various light intensities (Fig. 4, Table III). As shown in Figure 4, no induction period was observed at any light intensity. Weak light levels of 10 μW/cm² sec caused photosensitized oxidation. In a comparison of the prooxidant activities of CHL A and B, CHL B showed a prooxidant effect twice as strong as that of CHL A, at the same light intensity: these results are similar to those in Table II.

Several researchers have compared the decomposition rate of CHL A and B (17-24). However, in this paper, we have indicated the different prooxidant activities of CHL A and B for ML as a model compound.

Next, the prooxidant activities of CHL were compared with those of PHY and PHO, as decomposition products of CHL, at the same concentrations (Fig. 5). No induction period was detected during the photooxidation of ML with PHY or PHO as in the case of CHL at 0 C. Furthermore, the presence of unconjugated diene hydroperoxide in the oxidation products of ML was investigated by determining the ratio of UV absorbance (234 nm) to POV. The ratio for oxidation products of ML with PHY A and B, and PHO A and B was 0.53, 0.54, 0.50 and 0.50, respectively, at 2.2×10^{-8} mol/g ML, indicating that photooxidation of ML with PHY or PHO was caused by singlet oxygen. The prooxidant activities of PHY and PHO were higher than those of CHL at 2.2×10^{-7} and 10^{-8} mol/g ML (Table IV). In particular, the prooxidant effect of PHO was the strongest among them, proving to be 1.3 times that of CHL at 2.2×10^{-8} mol/g ML. The stabilities of CHL and its decomposition products against photooxidation are presumed to be responsible for their different prooxidant activities. Comparing PHY A and B with PHO A and B, stronger prooxidant effects were observed in PHY B and PHO B than in CHL.

Some investigators (25,26) have reported on the photosensitive activity in PHY. While Jen and Mackinney (27) found no photosensitive activity in PHY, Rawls and Van Santen (6) suggested that PHY A caused the photosensitized oxidation of ML, followed by the formation of oxidation products similar to those of CHL A, which were detected on TLC.

Box and Boekenoogen (28) and Niewiadomski et al. (29) indicated that some crude plant oils contained much greater amounts of PHY than CHL. Therefore, a smaller quantity of PHY was also expected to be present in the refined edible plant oils. Actually, by fluorospectrometric analysis, we detected greater amounts of PHY than CHL in refined edible oils such as soybean, rapeseed, cottonseed, safflower, corn and palm oil (30,31).

From these results, the PHY content in oils much be noted as well as the CHL content, when considering the oxidative stability of edible oils. We assumed that no PHO is contained in commercial edible oils (31). However, the other decomposition products of CHL may contribute to the deterioration of oils.

Many more questions about the role of CHL and its analogs in the stability of edible oils and fats need to be explored. We will investigate these questions in the near future.

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